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High Pressure Processing of Food

Principles, Technology and Applications

Springer

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Preface

High-pressure processing (HPP) of food, one of the most successful nonthermal technologies, has been adopted by the food industry because of the number of advantages it offers to some conventional methods of preservation. The high demand for minimally processed foods, but at the same time very safe, makes this technology appropriate for a number of applications. HPP could replace or partially replace well-accepted technologies because it offers the opportunity for the development of new products and it might bring an alternative to address processing issues not yet resolved.

The possibility to use HPP to treat foods is not new, but applications at industrial scale are fairly recent. Some of the pioneering research on high pressure in food processing has been reported by Hite (1899) and Bridgman (1912). However, it took nearly 80 years for the food industry to embrace high-pressure processing as a viable food manufacturing technology for preserving a variety of value-added products of excellent quality and very safe from a microbiological point of view. HPP offers the possibility to have mildly processed, wholesome, convenient, fresh-tasting foods with minimal to no preservatives to satisfy health conscious lifestyles.

Pressure-treated jams and jellies were introduced in the Japanese market in early 1990, followed by introduction of HPP guacamole in the USA in 1997. Now high-pressure processing is a commercially viable technology for the pasteurization of products of diverse origin such as meat, seafood, beverages, dairy, fruits, and vegetables that are enjoyed by consumers all over the world.

This comprehensive book includes the basic principles to understand the technology behind high-pressure processing as well as its current and future applications within the food industry. The book has 31 chapters distributed in seven parts addressing topics such as process engineering characterization, industrial equipment, packaging, microbial safety, preservation of bioactive compounds, quality changes, and applications in the food industry.

The editors are very thankful to more than 80 authors for sharing their expertise, experience, and vision to come up with very valuable chapters to make the book an excellent reference for high-pressure processing of food for the years to come. The editors are aware of some overlaps between a few chapters, but this is inevitable in

a book of this magnitude. This, however, will also help to visualize basic concepts from different angles for the benefit of the readers in this rapidly evolving field. The gratitude is extended to all the reviewers who contributed their time and expertise to improve the chapters.

It is worth mentioning that from the pioneering efforts made by the Japanese to bring this technology to fruition, to what is taking place now, a significant number of developments took place. Nonthermal strategies to process foods caught the attention of a number of institutions, scientists, regulatory agencies, the food industry, and, of course, the consumers. Several technologies were scrutinized roughly at the same time, and it is quite apparent HPP is the one that, so far, has been receiving the most attention based on its potential to process a wide variety of food products and because it is amenable to be combined with other technologies, such as in the case of pressure-assisted thermal processing (PATS), which allows sterilization of low-acid foods.

The development of HPP technology and its adoption by the food industry were expedited by a number of factors such as the vision of some of the earlier researchers, investors who believed in long-term commercial viability of the technology, as well as worldwide coordinated research and technology transfer efforts among scientists and engineers from academia, equipment manufacturer, food processors, policy makers, and regulatory agencies. In 1997, the Institute of Food Technologists (IFT) Nonthermal Division and European Federation of Food Science and Technology (EFFoST) started to organize annual workshops on nonthermal processing in various European countries and a number of places in the USA. Later on, these workshops have been offered in other parts of the world such as Australia, China, and Brazil. These professional clusters facilitated the rapid growth of several nonthermal technologies, and, as mentioned before, HPP is one that has been receiving great attention. The team approach to develop these technologies has been an example of cooperation, fast development, and an unselfish manner to disseminate acquired knowledge by leading groups. The synergism between regulatory agencies, equipment manufacturers, consumer groups, scientists from research institutions, and food processors has been remarkable, maybe like never seen before. Such collaborative environment enabled fruitful technology partnership between equipment manufacturers and the food industry taking advantage of basic and applied research developed in a number of research institutions, mainly universities. Participation of regulatory authorities facilitated development of science-based regulations that are also harmonized across many countries. Academic researchers not only contributed to technology development and evaluation but also in the training of numerous postdoctoral fellows and graduate and undergraduate students as future leaders in the high-pressure industry. A number of academic institutions developed centers of excellence around nonthermal technologies facilitating interaction among all constituencies interested in exploring new alternatives to process the foods of the future. Universities also play a critical role in providing a pipeline of trained, scientifically sound, next-generation workforce with industrially relevant skills for sustained long-term success of this advanced food manufacturing industry.

We sincerely hope the book will be inspirational to entrepreneurs to continue bringing to the market new and exciting high-pressure-treated food products to enhance the health and well-being of a good number of consumers.

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Biography

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Gustavo V. Barbosa-Cánovas Professor of Food Engineering at Washington State University (WSU), received his B.S. in Mechanical Engineering at the University of Uruguay and his M.S. and Ph.D. in Food Engineering at the University of Massachusetts-Amherst while being a Fulbright Scholar. Quite recently he was awarded a Honoris Causa Doctorate at Polytechnic University of Cartagena, Spain. He has received several prestigious awards such as the IFT Nicholas Appert Award, IFT International Award and is an IFT, IFST, and IUFoST Fellow as well as Member of the Uruguayan Academy of Engineering and the Mexican Academy of Sciences. He received the Sahlin Award for Research, Scholarship and Arts at WSU (highest research award at this University) as well as a Fulbright Fellowship as expert in Food Engineering. In 2013, he received another relevant Fellowship granted by the Japan Society for the Promotion of Science (JSPS) to lecture in several prestigious Japanese universities.

Huub L.M. Lelieveld is President of the Global Harmonization Initiative, Past-President of the European Federation of Food Science and Technology and the European Hygienic Engineering and Design Group, a fellow of the International Academy of Food Science and Technology and of the Institute of Food Technologists. He also served on the Governing Council of the International Union of Food Science and Technology. At Unilever, he was responsible for hygienic processing and novel food processing technologies. He is editor of many books (on food safety, hygiene, and food technologies) authored many chapters and articles and presented hundreds of papers, globally. He has been awarded doctor *honoris causa* at the National University of Food Technologies in Kiev, Ukraine.

Part I
Introduction

Chapter 1
Fundamentals and Applications of High-Pressure Processing Technology

Sergio I. Martínez-Monteagudo and V.M. Balasubramaniam

Abstract High-pressure processing has been established as a commercially viable food preservation technology, where application of elevated pressure serves as the main lethal agent for pathogen reduction without compromising nutritional and organoleptic properties of the food. The rapid temperature increase during compression, and subsequent cooling upon decompression, is a unique benefit of high-pressure-based technologies to reduce product thermal exposure during treatment. A variety of pressure-pasteurized products (including juices, meat, seafood, and vegetable products) are commercially available worldwide. To date, FDA issued letters of no objection to two industrial petitions for preserving shelf-stable low-acid samples by pressure-assisted thermal process (PATP). This chapter summarizes the basic principles associated with preserving foods by the application of various pressure-based technologies and reviews relevant process and product parameters for product microbiological safety and quality. Various pressure-based unit operations have been reviewed. Application of pressure-based technologies in different commodity food processing has been discussed.

Keywords High-pressure processing • Pasteurization • Sterilization • Process uniformity • Microbial safety and quality

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1.1 Introduction

Modern consumer's health and wellness-oriented lifestyle prompted the food processors to consider introducing alternative food technologies for preserving fresh-like organoleptic and nutritional quality attributes in foods. Further, these technologies may only use minimal or no preservatives.

Among the alternative preservation technologies, advanced "volumetric-based" thermal technologies utilize rapid volumetric heating of food materials to overcome the limitations of conventional thermal processes. Ohmic heating, microwave heating, and radio-frequency heating are examples of advanced thermal technologies. While rapid volumetric heating reduces the nutrition and quality degradation, the processed products still need to be cooled by conventional conduction and convection heat transfer.

Nonthermal technologies describe those processing technologies where alternative form of source of energy is used as the main lethal agent. Nonthermal technologies are designed in such a way that the main lethal agent (high pressure, electric field, irradiation dose) with or without the combination of heat is used to inactivate pathogens and spoilage microorganisms. Nonthermal technologies have the potential to retain product nutrients and preserve "fresh-like" organoleptic quality attributes by reducing or minimizing thermal exposure during processing.

Among nonthermal technologies, the application of high pressure (400–600 MPa) at chilled, moderate, or elevated temperature has met consumers' rising demands, delivering a variety of innovative food products. Like thermal processing, the technology can be used for both liquid and solid foods and considered as one of the greener and cleaner processes as the preservation effect can be accomplished with minimal or no preservatives. The pressure-treated products were first introduced in Japanese and US market by the early to mid-1990s. Since then, a variety of pressure-pasteurized products are commercialized worldwide. The technology has been recognized by US Department of Agriculture (USDA) Food Safety Inspection Service as one of the intervention technology for inactivating variety of pathogens commonly found in the meat. In 2009, the Food and Drug Administration (FDA) issued no objection to an industrial petition for pressure-assisted thermal sterilized mashed potato product.

1.2 Historical Progress

The use of high pressure for the preservation of foods dates back to the late nineteenth century. Technological developments have allowed operating vessels at pressure levels in the range of hundred MPa, which opened a window to an unexplored region. Historical developments of high pressure for food processing are provided in Chap. 2. The industrial use of high pressure has its origins in mechanical and chemical engineering. Mechanical industry developed steam engines that withstand few MPa,

while chemical industry employed vessels in the range of 10–70 MPa for the synthetic production of ammonia. Alongside with such developments, other components including high-pressure pumps, compressors, tubes, fitting, sealing, and pressure vessels were also developed (Eggers 2012).

1.3 Process Engineering

1.3.1 Equipment

High-pressure pasteurization of foods is essentially a batch operation. The major components of a high-pressure equipment systems consist of pressure vessel coupled with top and bottom closures, yoke mechanism to secure pressure vessel while in operation, pressure intensifier and pump for generating the pressure, material-handling system for loading and unloading the food, and process control system for monitoring and recording various process variables (Ting 2011). Chapter 3 presents commercial-scale high-pressure equipment available to the food processors. Semicontinuous systems are being developed for pumpable foods in which three or more vessels are used. The vessels are connected in a way that when one vessel discharges the product, the second vessel pressurizes, while a third vessel gets loaded with food samples. Chapter 4 reviews the state of the art of semicontinuous or continuous high-pressure operations.

1.3.2 Typical High-Pressure Process

A typical operation of high-pressure processing resembles that of thermal retort system. The food to be treated is vacuum packaged in flexible polymeric package and subsequently loaded inside a cylindrical carrier basket. At least one interface of the package should be flexible enough to transmit pressure to the material. Typically high-barrier packaging material is used so that product quality attributes can be preserved during extended storage. The effect of high pressure on different packaging materials is reviewed in Chap. 5.

The carrier basket containing the product packages is then loaded into pressure vessel. The pressure vessel and its content are closed with the end closures. The pressure vessel is filled with pressure-transmitting fluid (typically water). The target process pressure is achieved through compression of pressure-transmitting fluid using the combined action of a pump and intensifier. The physical compression of high-moisture content foods typically reduces its volume by 15 % while under pressure. The product returns close to its initial volume upon decompression. During high pressure, the product is held for the desired time at the target pressure, the vessel depressurized quickly at the end of the treatment time, and the product is unloaded. Typical cycle time for the process is about 10 min.

During high-pressure treatment, compression of the product also transiently increases temperature of the foods (3°/100 MPa for water and high-moisture content foods; 8–9 °C/100 MPa for lipid-based foods). Upon decompression, the product temperature returns back close to its initial temperature value. The rapid volumetric temperature increase in the product during compression and subsequent cooling upon decompression is a unique advantage of high-prssure technologies for food pasteurization and sterilization. A modified operational high-pressure method consists of subjecting the product to compression-decompression cycles, a process known as pressure pulsing. Details of such method are given in Chap. 9.

1.3.3 Governing Principles

1.3.3.1 Isostatic Principle

The term hydrostatic refers to the equilibrium of fluids under the action of force or pressure. In general, all fluids yield to a force imposed on them and possess the ability to transport such force among themselves without friction. Isostatic principle considers that a force transported to the surface of a fluid is equally transmitted through the contact surface. In high-pressure applications where a packed food material is surrounded by pressuring fluid, the pressure effects are quasi-instantaneously and homogenously distributed within the food, regardless of geometry and size. Since air and water are compressed differently during application of pressure, the structure and shape of the foods containing air pockets (as in the case of marshmallows) may be altered upon pressure treatment, unless the food is perfectly elastic and consists of closed-cell foam from which air cannot escape (Balasubramaniam and Farkas 2008).

Hydrostatic pressure reduces the volume of the pressurized material without changing its shape. Mechanistically, pressure alters the distance between molecules having direct effect on distance-dependent interactions. For instance, van der Waals forces are one of those interactions strongly affected by pressure because their optimal working distance is altered by pressure, which disrupts the balance between attractive and repulsive forces. Other interactions affected by pressure due to their working distance are hydrogen bonding, electrostatic, and hydrophobic interactions (Martinez-Monteagudo and Saldaña 2014). Contrary, covalent bonds are unlikely to be affected by pressure because their working distance can be hardly reduced any further. Indeed, covalent bonds from primary structure of proteins were unaffected by pressure (up to 1500 MPa) (Mozhaev et al. 1994). The fact that high pressure does not alter covalent bonds has been the central hypothesis behind the preservation of activity of functional compounds. Chapter 22 reviews the effect of high-pressure treatments on the biological activity of bioactive compounds and nutritional content.

1.3.3.2 Le Chatelier's Principle

According to Le Chatelier's principle, a system in equilibrium will shift to new equilibrium to partially undo any induced change. This principle has a basis in the second law of thermodynamics, and it is valid for reversible processes. Le Chatelier's principle is often used to explain the effects of pressure and temperature on chemical, biological, and physical phenomena. Despite its various applications, Le Chatelier's principle is rather vague and ambiguous. In the 1920s, the term of affinity was developed based on the thermodynamic conjugate pairs, extensive and intensive variables. Basically, thermodynamic variables come in conjugate pairs: temperature and entropy, pressure and volume, chemical potential and moles. An interpretation of affinity is that such system held at fixed entropy, and volume will come to equilibrium by varying temperature and pressure. In the case of HPP, if pressure (extensive variable) changes, the equilibrium shifts in the direction that tends to reduce the change in the corresponding intensive variable (volume). Thus, any phenomenon (phase transition, change in molecular configuration, chemical reaction) accompanied by a decrease in volume is enhanced by pressure (Hamann 1957).

1.3.3.3 Principle of Microscopic Ordering

This principle postulates that an increase in pressure at constant temperature increases the degree of ordering of molecules of a given substance. According to this principle, pressure restricts rotational, vibrational, and translational motion, which will increase the molecular order. Interestingly, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny and Masson 1993).

1.3.3.4 Transition State Theory

Transition state theory states that if the molar volume of the intermediate state (activated complex) differs from that of its reacting components, the reaction velocity can increase or decrease by changing pressure, according to whether the intermediate state is less or more voluminous (Wentorf and De Vries 2001). This principle is used to explain the effect of pressure on chemical and biochemical reactions as well as physical processes (Chap. 17). For a given chemical reaction, the effect of pressure favors those reactions with negative reaction volumes and those reaction pathways with negative activation volumes (Martinez-Monteagudo and Saldaña 2014). Chapter 21 exemplifies the effect of pressure on the formation of volatiles in milk.

1.3.4 Importance of Considering Pressure-Thermal Effects

Pressure just like heat is an extensive thermodynamic variable. According to the second law of thermodynamics, the effects of temperature cannot be separated from the effects of pressure during compression. This relationship (pressure-temperature) has been exemplified through the phase diagram of water, where for every temperature there is a corresponding pressure. Thermal effects during pressure treatment can cause volume and energy changes. On the other hand, pressure primarily affects the volume of the product being processed. The combined net effect during a high-pressure processing may be synergistic, antagonistic, or additive (Gupta et al. 2011). Mathematically, the impact of pressure (p) and temperature (T) can be quantitatively related using Gibbs's definition of free energy G:

$$G \equiv H - TS \tag{1.1}$$

where S and H are the entropy and enthalpy, respectively. Further,

$$H \equiv U + pV \tag{1.2}$$

where U = internal energy and V = volume.

From above equations, it can be deduced that

$$d(\Delta G) = \Delta V dp - \Delta S dT \tag{1.3}$$

Therefore, reactions such as phase transitions or molecular reorientation depend on both temperature and pressure and cannot be treated separately.

1.3.5 Process Development Consideration

In order for high pressure to be an effective preservation technology, it requires that the entire food material is subjected to uniform processing conditions at which safety and quality are achieved. Variations in both temperature and pressure can contribute to the development of nonuniformity within a processed volume during a high-pressure processing experiment. A number of factors can influence heat transfer-related process nonuniformity within a pressure chamber. These include the design of the pressure equipment as well as the geometry and insulation characteristics of the pressure chamber.

During pressure treatment, the temperature of different food material increases transiently due to physical compression and returns back to its initial value upon decompression. The thermal exchange between the food, pressure-transmitting fluid, and the environment through the walls of the pressure chamber can influence the uniformity of a high-pressure process. This heat exchange can be further governed by the thermophysical properties of food, packaging material, and the pressure-transmitting fluid. The understanding of the extent process uniformity

during pressure treatment requires the knowledge of thermophysical properties. Chapter 6 discusses in situ measurements during high pressure.

For practical purpose, pressure treatment is assumed to be transmitted uniformly and quasi-instantaneously throughout the sample volume. However, pressure nonuniformity may exist in heterogeneous samples (e.g., large ham product containing bone). Researchers have reported that pressure at the geometric center of a large food such as ham is approximately 9 MPa less than 600 MPa delivered by the process system. Numerically, simulations showed that the nonuniformities may arise due to the effect of convective transport and heterogeneous heat transfer during high pressure. It has been hypothesized that a fluid flow develops which interacts with temperature changes affecting the uniformity of the treatment. Mathematical models of combined pressure-heat treatment are reviewed in Chap. 12. Other factors influencing process nonuniformity during HPP and PATP may include position of the sample within the high-pressure chamber and sample phase transition characteristics. Several approaches have been proposed to minimize thermal nonuniformity during pressure treatment. Temperature control of the product, package, pressure-transmitting fluid, and pressure vessel for each cycle during high-pressure processing is critical. Chapter 13 discusses approaches used to evaluate the process uniformity during HPP and PATP.

1.4 High-Pressure-Based Unit Processes

Application of elevated pressure (100–800 MPa) is a proven tool for a number of processing and unit operations including homogenization, extraction, freezing and thawing, blanching, pasteurization, extended shelf life (ESL) and commercial sterilization. Figure 1.1 organizes the current and promising industrial applications of high-pressure technologies based on pressure-temperature intensity.

1.4.1 Pressure-Assisted Freezing and Thawing

Pressure caused depression of the freezing point, a colligative property. Pressure levels of 200 MPa depress the freezing point of water from 0 °C to −21 °C. This colligative effect is reversible upon decompression. Pressure-assisted freezing and thawing could be potentially exploited to rapidly freeze and thaw high-moisture content foods. During pressure-assisted freezing (PAF), the sample is cooled under pressure up to its phase change temperature at applied pressure. The product is frozen under pressure by super cooling at faster ice-nucleation rate. This process helps in preserving the microstructure of food and biological materials. Pressure-assisted thawing (PAT) involves thawing a food material under constant pressure. The process can help in reducing the thawing time and the drip loss. Another potential

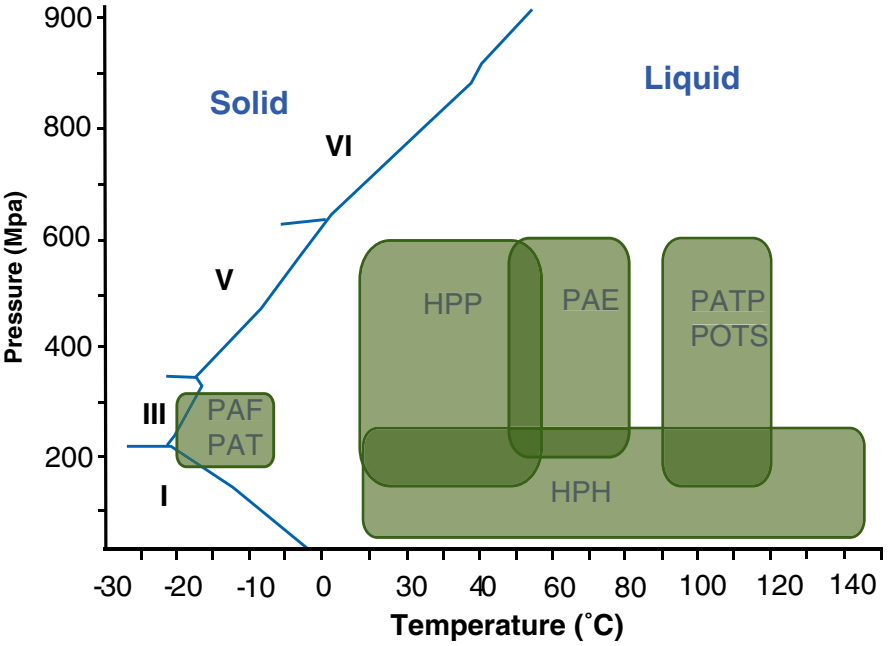

Fig. 1.1 Schematic diagram illustrating processing technologies conducted at high pressures (>100 MPa). *PAF* pressure-assisted freezing, *PAT* pressure-assisted thawing, *HPH* high-pressure homogenization, *HPP* high-pressure processing, *PAE* pressure-assisted extraction, *PATP* pressure-assisted thermal processing, *POTS* pressure-ohmic thermal sterilization (adapted from Balasubramaniam et al. 2015)

application is the storage at freezing conditions (up to −20 °C) without the transition from liquid to solid. Principles and potential applications of pressure-assisted freezing and thawing are discussed in Chap. 8.

1.4.2 High-Pressure Homogenization

This technology consists in forcing a pressurized fluid (up to ≈320 MPa) to pass through a minute orifice, homogenization chamber. High-pressure homogenization is based on the dissipation of kinetic energy due to the passage of a pressurized fluid through the tiny gap, which results in reduction of particle size and rise in fluid temperature. The rise in the fluid temperature is due to heat of compression (similar to HPP) as well as temperature increase due to homogenization effort. Unlike hydrostatic pressure, the temperature increase during homogenization, which is about 18 °C per 100 MPa, is not reversible. The magnitude of this temperature increase in part depends upon specific HPH valve geometry. Valve geometry can

also influence the characteristics of the final processed product. By adjusting initial fluid temperature, target pressure, and HPH valve design, it may be possible to achieve both pasteurization and sterilization treatment temperatures. Chapter 7 presents current developments in high-pressure homogenization of liquid foods.

1.4.3 Pressure-Assisted Extraction

Extraction of valuable compounds from biological matrix has been enhanced by high hydrostatic pressure. Extraction yields obtained by pressure mainly depend on the pressure, time, and type solvent used. Mixtures of organic solvents have been tested under pressure to assist the extraction of antioxidants and other bioactive compounds. Upon decompression, pressure can break weak chemical bonds of the extracting matrix, making some compounds available for extraction. The latest developments on pressure-assisted extraction are discussed in Chap. 10.

1.4.4 High-Pressure Processing

Pressure levels of 400–600 MPa at ambient or chilled conditions have been effective in inactivating variety of pathogenic and spoilage vegetative cells, yeast, mold, and viruses (Chaps. 14 and 15). The magnitude of microbial reduction during pressure treatment is also influenced by the composition of the food, its pH, and water activity. Even within a given microorganism, wide variation in pressure resistance of different strains is reported. Thus, food processor should work with competent expert to identify appropriate target microorganism for the process validation studies.

The term pasteurization not only applies for heat treatment, but recently, in 2004, the National Advisory Committee on Microbiological Criteria for Foods recommended to federal regulators to redefine pasteurization as any process treatment (not just heat treatment alone) or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage. It should be highlighted that like thermal pasteurization, pasteurized food products via HPP still require additional barriers (such as refrigerated storage) for product stability during subsequent handling and distribution. There can be significant variation among strains. Microbial cells in stationary growth conditions tend to have higher pressure resistance than exponential phase. Resistant to pressure treatment varied considerably among microorganisms. High-pressure processing conditions (400–600 MPa at ambient or chilled temperature) can be useful for pasteurizing a variety of liquid and solid foods including deli meats, salads, seafood, fruit juices, and vegetable products (Tonello 2011). High-pressure pasteurization units are available in both vertical and horizontal configurations with range of sizes (35 liter through 525 liter capacity; see Chap. 3).

1.4.5 Pressure-Assisted Thermal Processing

Pressure alone at or near ambient temperature has very limited or no effect on spore inactivation. Pressure-assisted thermal processing (PATP) is an emerging sterilization technology. During PATP, prepackaged products are then preheated to a certain initial temperature (T_i, 75–90 °C) from the knowledge of target PATP temperature (T_p, 90–120 °C), applied pressure (ΔP, 400–600 MPa), heat of compression (δ_m) values for different food and packaging materials, pressure-transmitting fluid, and their respective mass (M_f) fractions

$$T_p = T_i + \frac{\left(\sum_i (\delta_m \times M_f)\right)}{M}\left(\frac{\Delta P}{100}\right) \pm \Delta T_H \tag{1.4}$$

In Eq. (1.4), ΔT_H represents heat gain or loss by the product from the surroundings. The entire process results in a shorter treatment time (3–15 min) when compared to that of the conventional canning, which involves slow external heat transfer.

PATP was first developed to inactivate bacterial spores and achieve commercially sterility of low-acid foods. Chapters 11, 14, and 29 discuss the inactivation of bacterial spores by means of PATP. In 2009, FDA issued no objection to an industrial petition for sterilization of mashed potato by PATP (Chap. 29). The petition primarily considered thermal lethal effects and did not consider pressure lethal effects on ensuring commercial sterility of treated low-acid foods. In July 2015, FDA issued no objection to second industrial petition on pressure-enhanced sterilization (PES) process. The petition demonstrated the achievement of commercial sterility of a multicomponent complex particulate-bearing low-acid food using processing conditions of time and temperature that are lower than those used for a conventional thermal sterilization process.

Although there are no low-acid products preserved by PATP commercialized yet, this technology has the potential to deliver a variety of commercially sterilized or extended shelf-life (ESL) low-acid products, such as egg, and milk-based products, baby foods, vegetables, ready-to-eat foods, desserts, gravies, soups, and sauces. Further, PATP equipment is primarily restricted to pilot scale. Since PATP utilizes intensive pressure and heat, from the standpoint of materials science and engineering, the process demands significant stress on the vessel and seals, potentially limiting the equipment life. Pressure-temperature inactivation kinetics of bacterial spores has been the topic of discussion over the last decade. Unlike thermal treatment, the inactivation kinetics obtained by PATP are nonlinear, showing a shoulder at the beginning and a tail at the end of the treatment. If this issue is not addressed properly, the calculated lethality can lead to over-processing or under-processing conditions. Chapter 17 reviews the use of nonlinear kinetic models to describe combined pressure-heat microbial inactivation.

An interesting application of PATP is the inactivation of infectious agents like prions. The conventional inactivation methods for prions are pretty aggressive using elevated temperatures. Feasibility studies have proven that PATP can

inactivate prions with less severe conditions, and it has been suggested as an alternative to decontamination of prions. Chapter 16 describes the pressure inactivation of prions.

1.4.6 Pressure-Ohmic Thermal Processing

Pressure-ohmic thermal processing (POTP) is a novel technology involving sequential and simultaneous application of elevated pressure and ohmic heating to preserve shelf-stable low-acid foods or extended shelf life (ESL) food products. The technology synergistically combines the heat of compression effects of elevated pressures along with ohmic heating to minimize thermal exposure effects on product quality (Balasubramaniam et al. 2012). The rate of temperature rise in the food within the pressure vessel during POTP pressure holding can be adjusted by controlling of electrical heating under pressure; POTP design also avoids the requirement of preheating the pressure vessel, thereby reducing energy demand and thermal stress on the pressure equipment (Balasubramaniam et al. 2015). Other process combinations are presented in Chap. 11.

1.5 Food Chemistry and Quality

The motivation of using high pressure lies on chemical and physical effects induced by pressure. There are three important consequences of applying high hydrostatic pressure:

1. Changes in physical properties, such as melting point, solubility, density, viscosity, etc.
2. Effects on equilibrium processes, such as dissociation of weak acids, acid–base equilibria, ionization, etc.
3. Effects on rates of processes, such as delaying or accelerating the rate at which a particular reaction occurs.

Some quality attributes such as microbiological safety, functionality, instrumental quality, and nutritional attributes are the resultants of the way these three phenomena are affected by pressure. For instance, inactivation of microorganisms is a combination of changes in physical properties of membrane lipids, changes in the chemical equilibrium that modify the internal pH, and changes in the rate of specific physiological functions that cause irreversible or lethal damage on bacteria cells (Molina-Guitierrez et al. 2002). Table 1.1 summarizes various examples on how food quality attributes are dictated by the way pressure affects the physical, equilibrium, and rate processes. A rate process was considered when pressure increases or decreases the concentration of a particular compound.

Table 1.1 Examples on how food quality attributes are influenced by the effect of pressure on physical properties and equilibrium and rate processes

	Effect upon pressure treatment		
Quality attribute	Physical property	Equilibrium process	Rate process
Mechanically shucking of oysters	– Detaching the adductor muscle	– Releasing intramuscular components	– The extent of shucking depends on the pressure level
Improving of meat tenderness by pressure-heat treatment	– Pressure causes disaggregation of proteins	– Shifts the pH value that prevents reassociation of protein fragments	– Biochemical reactions are controlled by pressure
Starch gelatinization	– Changes in viscosity of starch suspension – Changes in rheological properties	– Shifts the chemical balance	– Retrogradation rate is controlled by pressure
Increasing cheese yield	– Disruption of casein micelles – Denaturation of whey proteins	– Alters mineral balance (colloidal and soluble calcium)	– Pressure controls the extent of protein-fat interactions
Improving texture in egg patties	– Increases viscosity of egg patties, protein aggregation	– Change in pH, which enhances hydrophobic interactions	– Pressure affects the rate of protein aggregation
Cell membrane damage in onions	– Induces membrane permeabilization	– Shifts the balance between extra- and intra-cellular pH	– Pressure controls diffusion of solutes within the cell

Another relevant aspect of high pressure is the effect on endogenous enzymes. These enzymes are known to have beneficial or detrimental effects on foods. The enzyme activity is highly influenced by high pressure. Inactivation of enzyme by combination of high pressure and temperature is a complex phenomenon. The primary structure of the enzyme is minimally affected by pressure, while the secondary structure suffers structural modifications only at very high pressures. The tertiary structure is greatly affected by pressure because pressure disrupts hydrophobic and electrostatic interactions. Consequently, water solvates the exposed charge groups, leading to a volume reduction that inactivates the enzymes. A discussion on the inactivation of enzymes due to high pressure is provided in Chap. 19.

Pressure unfolding of proteins has been studied for quite some time. The extent of unfolding depends on the type of protein and processing conditions. Protein unfolding due to pressure is partially irreversible, and it has great relevance in providing unique functional properties to the unfolded proteins. A summary of recent developments on the effects of high pressure on proteins is described in Chap. 18. Examination of pressure-temperature-treated proteins and carbohydrates revealed significant structural and morphological changes, which can be exploited in the development of texture, consistency, and other physical properties. Chapter 23 illustrates structural modifications induced by pressure-temperature treatments.

1.6 Food Industry Applications

A remarkable example application of pressure is the mechanically shucking of oysters. Pressure treatment released the abductor muscle from the shell with minimal drip loss values. Quality parameters in seafood affected by pressure are reviewed in Chap. 27. Combination of high pressure with moderate thermal treatment could improve meat tenderness when applied to pre-rigor meat. Chapter 26 deals with the effects of pressure on meat and meat products. Another interesting application of high pressure is the phase transition of starch suspensions. The gelatinization temperature decreased when suspension is exposed to high pressure. Effect of pressure on starch gelatinization is provided in Chap. 20. It has been demonstrated that moderate pressure treatments could increase cheese yield, ripening characteristics, and functionality possibly due to complex interactions between whey proteins and casein micelles. Applications of high-pressure processing of dairy foods are discussed in Chap. 24. Pressure in combination with thermal treatment has become a valuable alternative to retorting for the production of high-quality shelf-stable products. Indeed, pressure-temperature conditions approaching commercial sterilization produced shelf-stable scrambled egg patties with desired quality attributes such as texture, color, and flavor.

Chapter 28 discusses advantages of pressure-assisted thermal processing for the production of egg-based products. The potential benefits of HPP over thermal processing have been exemplified in a variety of vegetables products. The changes induced by pressure are less intense than those induced by thermal processing. Chapter 24 exemplifies the effects of pressure on vegetables.

1.6.1 Industrial Implementation of High-Pressure Technology

Since the 1990s, high-pressure-based technologies have received significant commercial interest. Products such as guacamole deli meat, juices, salads and oysters are now being marketed in the United States; jams, jellies, sauces, fish, meat products, sliced ham, salad dressing, rice cakes, and yogurt in Japan; and fruit juices in France and Portugal. Due to emerging nature of the technology, pressure-pasteurized products are slightly more expensive (5–10 cents/pound more than conventionally processed products). Accordingly, identification of suitable value products is critical for commercial success for pressure-treated products (Fig. 1.2).

As new HPP products are launched, consumer acceptance is a key factor for commercial success. Consumers are generally receptive toward HPP technology. In addition, HPP is perceived as environmental sustainable due to energy efficiency. However, misconceptions regarding the influence of HPP on food safety may represent significant barriers to consumer acceptance. Chapter 31 discusses consumer acceptance of pressure-treated products.

Developing techniques to validate HPP is not a trivial task. Unlike traditional thermal treatments, the minimum processing conditions that warrant microbiological safety of HPP-treated products especially for ESL and commercially sterile

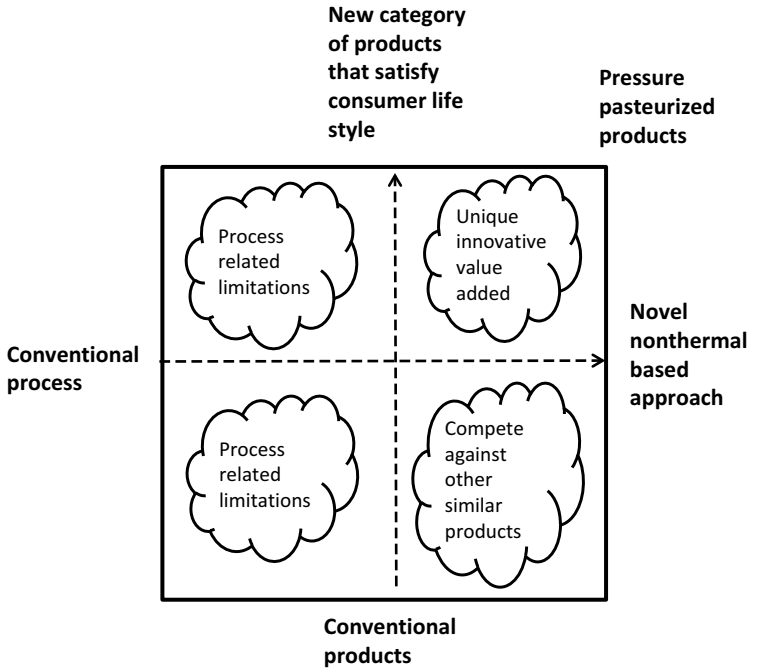

Fig. 1.2 Identification of suitable value-added products is critical for commercial success of the pressure-treated products

products, are still being developed. European regulation for novel foods and ingredients governs commercial introduction of pressure-pasteurized products. Details on EU high-pressure regulation are reviewed in Chap. 30. Future outlook and opportunities for pressure-based technologies are summarized in Chap. 32.

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Chapter 2
A Short History of Research and Development Efforts Leading to the Commercialization of High-Pressure Processing of Food

Daniel F. Farkas

Abstract The history of the development and commercialization of high hydrostatic pressure processing of foods includes groups in Japan, Europe, and the United States. This narrative focuses on early research and development commercialization efforts starting in 1984 in the Department of Food Science at the University of Delaware, Newark, Delaware. Research efforts expanded in 1990 as a joint program among the University of Delaware, the Department of Food Science and Technology at Oregon State University, and the US Army Combat Feeding Directorate at the Natick Soldier Research, Development and Engineering Center, Natick, Massachusetts. Activities of an industry-university High Pressure Consortium, managed by Marcia Walker and D. Farkas, at Oregon State University, helped commercialization by providing a focus for research, development, technology transfer, regulatory challenges, and solutions. The plan was to develop the use of high hydrostatic pressure into a profitable, new food processing technology that provided safe, fresh-tasting, convenient foods, with an extended shelf life.

Dr. Edmund Ting at Flow International recognized that breakthroughs in the design of high-pressure vessels and pump intensifiers would be needed. He pioneered unique equipment developments required by the food processing industry and helped clear the way for cost-effective commercial use of high pressure for food preservation.

The commercial use of high hydrostatic pressure for food preservation started in Japan in the late 1980s. Acid products, including yogurt and strawberry jam, were produced. These products resulted from work by a consortium of 25 companies brought together by Professor Rikimaru Hayashi to exploit the potential of high pressure as a nonthermal technology for pasteurizing foods with minimum heat damage.

Independently, Professors Dan Farkas, Dallas Hoover, and Dietrich Knorr initiated studies on the feasibility of high-pressure food processing starting in 1984 at

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the University of Delaware. Workers from the two programs met for the first time in June 1989 at a conference on engineering and food in Cologne, Germany, convened by Professors Walter Spiess and H. Schubert. In 1992 R. Hayashi and C. Balny edited a book (Hayashi and Balny, 1992) covering a joint conference drawing from Japanese and European research work on high-pressure food processing.

A symposium held at the University of Reading, England, on March 28–29, 1994, and the subsequent book, *High Pressure Processing of Foods*, serves as a milestone marking the end of the early history of research and development leading to the wide commercial use of high-pressure food processing. This symposium recognized that: "The scientific basis for the success of the process does involve many disciplines including both chemistry and microbiology. However, its commercial success, as with many food operations, has to be based on an effective integration with the engineering disciplines involved in the design and manufacture of equipment and plant capable of efficiently and safely applying such pressure to both solid and liquid food" (Ledward et al., High Pressure Processing of Foods, Nottingham University Press, Leicestershire Press, UK, 1995).

Keywords High-pressure processing • History • Research and development • Commercialization

2.1 Introduction

The use of high hydrostatic pressure to preserve foods is the first truly new method for food preservation since Nicolas Appert developed the use of heat to preserve foods in sealed glass bottles some 200 years ago. High pressure does not depend on heat, chemicals, reduced water activity, or reduced temperatures to control pathogens or spoilage microbes. Pressures in the range of 200–600 MPa have been found to unfold proteins so that they lose their biological functions as cell wall membranes and enzymes. In contrast to heat, high pressure does not break covalent bonds. High pressure is transferred instantly throughout a package of food independently of size, shape, or composition. These advantages give the food processor an almost ideal method to preserve food without heat and to conserve the product without any loss of original nutrients, flavors, pigments, or functionality.

The benefits of high-pressure food preservation (HPP) were recognized as early as 1894 through the research efforts of Burt Hite at the West Virginia State University Agricultural Experiment Station in Morgantown, West Virginia (Hite et al. 1914). Burt Hite worked with field gun engineers at the Harper's Ferry Arsenal to develop pressure vessels capable of operating at hydrostatic pressures over 680 MPa. His research showed that pressures between 200 and 680 MPa could inactivate yeasts, molds, and spoilage bacteria in foods. However, he was not able to sterilize whole cow's milk to allow room temperature storage. This was Hite's original objective. Hite, and his associates, predicted that fruit juices, in large containers, could be sterilized by high pressure. Work showed that there would be little change in their fresh flavor during room temperature storage.

The work of Hite and his associates did not attract the attention of food processors even when his 1914 paper was cited in W.V. Cruess' *Commercial Fruit and Vegetable Products* first published in 1924. A considerable number of research papers were published over the following years describing the effects of high pressure on microbes, starches, and various proteins including enzymes and enzyme activity (Johnson et al. 1954). However, food processors concluded that the use of high pressure for commercial food processing would be impractical. Pressure vessels, pumps, and instrumentation needed development. Food grade, low gas transmission, and flexible packaging were not available. The availability of frozen foods was increasing and their preservation and packaging technologies were easily adopted. Consumers perceived frozen foods to be close to fresh in quality, nutritional value, and convenience. Frozen foods became a source of convenient meals with the marketing of frozen "TV dinners" through the 1950s.

In this period the demand for polymer plastics drove the development of high-pressure pumps, seals, vessels, tubing, and instrumentation. The 1950s saw the development and increasing demand for jet engine turbine blades. Blades were made by fusing shaped, powdered metal, at high temperatures and pressures, in an argon gas atmosphere. The process required rugged pressure vessels, pumps, and instrumentation. The systems for hot and warm isostatic pressing of powered metal and ceramic parts, in effect, pioneered safe and reliable high-pressure equipment. Pressures in the range of 680 MPa became available in relatively large-volume vessels for routine manufacturing use.

2.2 Early Exploration of High Pressure for Food Processing

Macfarlane (1973) and Bouton et al. (1977), in Australia, published research work on the effect of high pressure, in the range of 140 MPa, on cuts of red meat pre and post rigor mortis. Commercial trials indicated that these pressures could help tenderize cuts of beef. The action of the pressure appeared to be through activation of in vivo proteolytic enzymes in the beef muscle. This was noted by Kennick et al. (1980). D.C. Wilson reported, at the 34th Annual Meeting of the Institute of Food Technologists in 1974, that high pressure could preserve fruits such as apricots packaged in flexible, hermetically sealed pouches.

Sale et al. (1970) published an excellent description of the effects of high pressure on spore survival. Spores of non-proteolytic *Clostridium botulinum* were shown to be extremely resistant to inactivation at pressures approaching 680 MPa. The high-pressure tolerance of bacterial spores indicated that food preservation by high pressure may be limited to pasteurization processes in the absence of hurdles that inhibit the germination and outgrowth of spores in low-acid foods. Foods with a pH value lower than 4.5 would be ideal products for high-pressure commercial sterilization. *Clostridium botulinum* spores in foods with a pH below 4.5 are prevented from producing toxin.

Still, in the early 1980s, a number of technical problems were perceived to prevent the commercial application of HPP to food preservation. These perceptions

were strong enough to discourage the application of available, off-the-shelf, commercial high-pressure equipment. In this period consumers were beginning to accept new preservation technologies such as modified atmosphere bagged salads with an extended shelf life. Preservation was achieved by modifying the gas atmosphere composition surrounding the vegetable mix during refrigerated storage and distribution. As more families included two working parents, the demand for convenient fresh-tasting foods strengthened.

2.3 Shifting Consumer Preferences and Perceptions

The food marketing scene in the early 1980s in the United States and in some countries in Europe began to shift in response to consumer demands for fresh-tasting, convenient, and safe refrigerated foods. Frozen foods were providing an increased selection of plated meals. The quality of frozen foods was improving dramatically. Companies were experimenting with cook-chill entrees that were microwave ready from the refrigerator rather than the freezer. Bagged salads were taking over the produce section of the supermarket. Controlled atmosphere packaging was being used to inhibit molds and yeasts in conjunction with reduced water activity. Reduce water activity could control bacterial spore germination and outgrowth. Extended room temperature shelf life of selected fresh-like foods was becoming a reality.

Ionizing radiation for food pasteurization and sterilization was available from the early 1950s as one of the early commercial "cold sterilization" technologies. Ionizing radiation sterilization of drugs and medical devices was embraced by the pharmaceutical, plastics, and packaging industries starting in the 1950s. However, commercial application of this technology for food pasteurization and sterilization was slowed by concerns that ionizing radiation could break covalent bonds. It was thought that the myriad compounds found in foods could form carcinogens or other harmful products during irradiation. Additionally, foods high in sulfur-containing amino acids, such as certain meats and milk, could produce strong off-odors and flavors when irradiated. Negative public perception of the safety of irradiated foods, and the classification of irradiation treatments as chemical additives, effectively blocked the general early acceptance of this technology for food treatment (Baily et al. 1957).

2.4 The Race to Develop Nonthermal Food Preservation Technologies

New technologies for "cold sterilization" of liquid and solid foods were needed to fill the gap left by low consumer interest in ionizing radiation. The race was on to develop viable pasteurization and sterilization technologies that required minimum

amounts of heat. These technologies would not depend entirely on heat and could be called minimum-thermal preservation methods. A simple example was the successful use of membranes to remove microbes in beer by filtration. While this technology had been demonstrated in Europe in the nineteenth century using porous ceramic filters, newly developed polymer membranes greatly facilitated expanded commercial applications. Some other nonthermal preservation technologies of interest included pulsed light, pulsed electric fields, and sonic energy. Each of these technologies was found to have advantages and disadvantages when considered for commercial food preservation. For example, particulate foods could not be treated effectively in some cases.

The drawbacks that slowed the commercialization of ionizing radiation for food preservation highlighted the challenges that had to be overcome for a new food preservation technology to be accepted. Acceptance had to be by consumers, food processors, equipment manufactures, and regulatory agencies. Regulatory agencies would need across-the-board assurance of the safety of the food products produced, the processes used, packaging, and equipment.

Equipment manufactures would have to deliver systems that would operate during multiple shifts with very low down time and have ease of maintenance and repair. Equipment would have to have the productivity that would allow both the food processor and the equipment manufacture a reasonable return on their investment. Additionally systems would need low operating costs and would be compatible with environments found in a typical food processing plant.

2.5 Research at the University of Delaware to Meet the Requirements for Successful Commercialization of High-Pressure Food Preservation

In the early 1980s Dallas Hoover, Dietrich Knorr, and Daniel Farkas, faculty members in the newly formed Department of Food Science at the University of Delaware, decided to explore the commercial application of high pressure. A perceived attraction was the availability of cost-effective, off-the-shelf, commercial, warm isostatic pressing equipment. As an example, the lab-scale units manufactured by Autoclave Engineers in Erie, Pennsylvania, were portable, fully instrumented, and very user-friendly. Pressure chamber volumes were large enough to accommodate up to one or more liters of packaged food samples. Despite the findings of Sale and coworkers, that high pressure had little effect on spore inactivation, the incentives for using high pressure, even for pasteurizing foods, appeared to far surpass actual and perceived drawbacks.

Literature research indicated that high pressures, in the range of 340–680 MPa, would not break covalent bonds, but would disrupt hydrogen bonds. The preservation of covalent bonds in biological materials, such as foods, meant that flavors, pigments, and nutritionally important compounds would be conserved when treated

with high pressure. The disruption of hydrogen bonds could result in the loss of enzyme and membrane activity of proteins. This loss of activity could result in the death of bacteria, yeast, molds, and parasites.

The effects of high-pressure treatment were instantaneous and uniform throughout a mass of food. Effects were not limited by heat, mass, or momentum transfer. Pressure applied to a 5-g packet would result in the same treatment as a 50-L bulk bag.

The major advantage of high-pressure food treatment, from a regulatory point of view, is that the process does not break covalent bonds. The effects of high-pressure would be similar to freezing or homogenization. Existing microbial safety, processing, labeling, and packaging regulations could apply to high-pressure treated foods. Simply put, the process would be benign and consumer friendly. The consumer could not differentiate treated from untreated products.

The decision to go forward with commercial high-pressure food preservation studies was driven by the favorable factors noted. Research would be needed to place the following essential developments on a sound scientific basis.

- Demonstrate that published research findings indicating that pressures in the range of 200–680 MPa could indeed inactivate six log cycles of bacterial pathogens, spoilage microbes, yeasts, molds, parasites, virus, and insect eggs. Inactivation would need to take place in foods with a range of pH and water activity values and in a wide range of food compositions.
- Determine that package shape and size does not influence inactivation kinetics in various sizes of cylindrical pressure vessels.
- Show that high-pressure processing (HPP) can actually operate at or near room temperature given that compression heating of water can cause significant product temperature increase.
- Demonstrate that foods treated by high pressure for a time sufficient for pasteurization show no change in chemical composition.
- Demonstrate that laboratory and pilot plant size equipment can yield experimental data that can be scaled and applied directly to industrial processes.
- Demonstrate that existing flexible packaging films and laminates can be used to package foods for high-pressure preservation.
- Demonstrate that high-pressure processing systems can be designed to be entirely compatible with existing food processing equipment; processing lines; and plant operating requirements such as ease of installation, cleaning, maintenance, and repair.

Additional process details such as the effect of high pressure on packaging materials; the operation of high-pressure vessels and supporting equipment in a food processing environment; the projected high treatment cost per package; the lack of information on the effects of high pressure on food enzymes; and the batch nature of the process would have to be addressed in subsequent laboratory and pilot plant studies.

2.6 Developing Support for High-Pressure Food Preservation Research, Development, and Technology Transfer

On October 24, 1984, two faculty members from the University of Delaware's Food Science Department in Newark, Delaware, traveled to Autoclave Engineers' headquarters in Erie, Pennsylvania. The objective of the visit was threefold: to discuss the use of Autoclave Engineers' laboratory-/pilot plant-scale cold isostatic pressing equipment for food preservation; to inquire if a unit could be loaned to the Department to conduct feasibility tests; and to convince the management of Autoclave Engineers that if the tests were successful, a lot more high pressure equipment possibly could be sold to the food industry than could ever be sold to the metal working industries. Autoclave Engineers was a leader in the manufacturing of small, reliable, high-pressure vessels and supporting systems. They were also an established supplier of replacement valves, fittings, high-pressure tubing, and accessories for commercial isostatic high-pressure users.

The isostatic press equipment industry is tied closely to the cyclic market for parts requiring fabrication in hot or warm isostatic presses. During good times companies expand and purchased capital equipment. During a recession, orders decrease. The isostatic pressing process itself requires long process cycle times for heating, cooling, and loading and unloading. Equipment may operate six cycles per day. This equates to about 2000 cycles per year. Orders for wear and replacement parts are tied to the number of cycles per year.

During the morning's conversation with company sales and development personal, and after the Autoclave Engineers' people had a good laugh about squeezing tomatoes in one of their presses, the potential for year-round equipment and parts sales to hundreds of potential food processors began to come into focus. The idea of an equipment loan took root based on the potential production tonnages of products such as fruit juices.

The Department of Food Science began work in earnest with the commissioning of a 2-in. inside diameter by 22-in.-long Autoclave Engineers CIP 2-22-60 isostatic press. The high-pressure chamber featured a pin closure and mono-block construction. The complete, self-contained system with pump, intensifier, pressure vessel, pin closure, instrumentation, and controls had a maximum working pressure of 400 MPa. The front and back of the unit are shown in Fig. 2.1. This unit is still in use in the high-pressure research program of Professor Brian E. Farkas. Professor Farkas was the first undergraduate student to use the equipment at the University of Delaware in conjunction with high-pressure research directed by Professor Dietrich Knorr.

As predicted the advantage of the off-the-shelf Autoclave Engineers' system for high-pressure food research was the ease and simplicity of loading, compression, decompression, and unloading samples. The equipment was used, with a minimum of instruction, by undergraduate and graduate students, faculty, and research associates. Sufficient samples could be prepared in the 1-L pressure chamber for sensory tests, long-term storage shelf life tests, and multiple microbial samples. The

Fig. 2.1 Autoclave Engineers CIP 2-22-60 isostatic press at Oregon State University

convenience and simplicity of the system allowed high-pressure research to be undertaken by microbiologists, sensory scientists, nutritionists, food chemists, and food engineers.

2.7 Early Research Observations

In honor of Nicolas Appert, cranberries and orange-cranberry sauce were the first products treated. These were foods first preserved with heat by Appert. Treatment of dry spices showed the resistance of spores to pressure, but more importantly demonstrated the need for high water activity for the efficacy of high pressure as a pasteurization technology. A water activity close to one was needed to optimize the inactivation rates of vegetative bacteria, yeasts, and molds in spices.

One question that was answered in early experiments was the effect of high pressure on different strains of microbial pathogens. Early experiments on the sensitivity of heat-resistant and heat-sensitive *Salmonella* strains showed that reverse results were observed with pressure (Metrick et al. 1989). The heat-resistant strain was sensitive to pressure and the heat-sensitive strain was quite resistant to high-pressure inactivation.

Professor Hoover was able to study the effect of pressure magnitude and holding time on the ability of microbes to recover from sublethal pressure treatments. Storage time, temperature, and enrichment of recovery media were found to play a role in the rate of recovery of microbes exposed to sublethal treatments. The need for storage studies of pressure-treated foods was underlined since products could

show no growth just after treatment, but would show growth after one or more weeks of storage (Pandya et al. 1995).

Professor Knorr was able to show that certain compounds, such as chitosan, could increase the rate of microbial inactivation during HPP treatment. He investigated the role of food composition on inactivation rates of microbes during pressure treatment. The "protective effect" of foods was quickly noted since the rate of inactivation of food pathogens and spoilage microbes was found to be less than in buffer solutions (Papineau et al. 1991).

2.8 Building a Scientific Foundation and a Technology Transfer Program for the Commercial Use of High-Pressure Food Preservation

As laboratory research data accumulated showing the favorable effects of high pressure on food quality, shelf life, packaging, and pathways of microbial spoilage, it became clear that several areas of development were needed to facilitate technology transfer and to educate the food processing industry. The unique nature of high-pressure food processing, in contrast to heat processing, would require education of regulatory agencies, equipment suppliers, consumers, and marketers. Needed developments were:

- Knowledge was needed to help regulatory agencies understand the technology and develop regulations for the use of high-pressure processing (HPP) in any desired food processing application.
- Knowledge was needed by HPP equipment makers, in the form of performance specifications, to meet the operating requirements and returns on investments of food processors. Projections were for pressure systems to operate at six cycles per hour during 20-h daily shifts. Production could be 250 days per year. A total of 30,000 cycles per year was projected. This projection was many times the existing use level of available isostatic pressing equipment.
- Knowledge was needed by food processors on the effect of HPP on enzyme activity, package-product interactions, and appropriate packaging materials and package shapes to optimize the fill (volumetric efficiency) of high-pressure vessels. Vessel diameters above 10 in. (250 mm) were favored.
- Knowledge was needed by food processors on how to build and operate efficient high-pressure food processing lines, their staffing, maintenance, and repair needs, space needs, and utility requirements.
- Also needed were HPP pilot-scale food processing lines to allow food processors to prepare sample products for storage studies, home use tests, and consumer focus studies. Processors needed to evaluate the actual pressure, time, and temperature requirements to preserve products prepared under commercial processing conditions using good manufacturing practices. This information would help regulatory agencies write science-based regulations.

2.9 Formation of a High-Pressure Food Processing Consortium

In 1990 Dr. Farkas moved to Oregon State University's Department of Food Science and Technology. The Department had a food processing pilot plant that food processors could use to manufacture small batches of new products for market tests. The decision was made for the Department to sponsor a university-industry-government High Pressure Consortium to help coordinate and facilitate the transfer of research data to interested food processors, equipment makers, regulatory agencies, consumers, marketers, and other researchers. This information also could help to identify operating and regulatory needs that could be made into relevant research projects.

Hayashi (1989) had described the successful use of an industry-university consortium in Japan to help apply HPP research to the development of commercial high-pressure pasteurized products. Consortium meetings in Japan resulted in the publication of three important summaries of ongoing research and development work (Kyoto, San-Ei Publishing Company 1989, 1990, 1991).

The Oregon State University HPP Consortium was based on the successful Fruit Juice Quality Advisory Committee established by Professor Ronald Wrolstad in 1987 in the Food Science and Technology Department. The High Pressure Food Processing Consortium was supported through a small yearly dues contribution from industrial members. Dr. Marcia Walker managed the Consortium and arranged the twice-each-year meetings. The January meeting was held at the Northwest Food Processors Association annual meeting in Portland, Oregon. The June meeting was held in conjunction with the annual Institute of Food Technologists national meeting at various major cities in the United States.

Consortium meetings were open to all personal from companies, government agencies, and universities, from any country in the world. The only requirement for participation was an interest in high-pressure food research, equipment development, government regulations, packaging, microbial safety, spoilage issues, shelf life studies, and related research. Industrial members were not required to make a presentation at each meeting, but were encouraged to ask questions that would highlight needed research. All other participants were encouraged to present summaries of their current research activities. Domestic and international university faculty and students, and government researchers, from around the world, were encouraged to expand on their fields of research. High-pressure equipment suppliers were encouraged to describe the latest developments in their high-pressure food processing systems and any new applications.

The Consortium provided a forum for the then newly created National Center for Food Safety that was established in Chicago as a joint program between the Illinois Institute of Technology and the Food and Drug Administration. The missions of the Center were to help Food and Drug personal develop science-based regulations and to keep FDA workers abreast of rapidly developing new and novel food preservation and processing technologies (Lechowich 1993).

The US Army Natick Laboratories in Natick, Massachusetts, has played a lead role in the development of new food processing technologies in the United States for over 50 years. The Army Quartermaster Corps pioneered the application of ionizing radiation to food preservation starting in the 1950s (Baily et al. 1957).

As research results confirmed the commercial potential for high-pressure food preservation, it was suggested that the Army Food Ration Development Program at Natick be contacted to determine their interest in high-pressure food processing. A seminar "Application of Ultra-High Pressure to Microbial Inactivation" was scheduled for July 28, 1989. Drs. Dallas Hoover and Daniel Farkas were presenters.

Dr. C. Patrick Dunne recognized the potential benefits of fresh-tasting foods with minimal heat damage and suggested a possible joint research and development effort. The food development program at the Natick Labs covered food ration needs from space feeding to assault rations. Single serve and group feeding requirements in arctic, dessert, and jungle feeding situations were included. Soon military-sponsored research on high-pressure food processing joined programs on ionizing radiation, freeze-drying, microwave heating, pulsed electric field pasteurization, and pulsed light treatments. The US Army Natick Combat Feeding Directorate coordinated product, process, and packaging studies. These studies, along with other developments, would result in military and civilian food quality breakthroughs. Commercialization of high-pressure processed foods in the United States would follow.

2.10 High-Pressure Research on Food and Food Components in Europe

High-pressure food processing research spread across Europe. Research support from the European Union and industry resulted in active programs in Germany, France, the United Kingdom, and Italy. Several countries were encouraged to come together to submit joint proposals for funding by the European Union. In this period, Professor Dietrich Knorr accepted a faculty position at the Technical University of Berlin. Professor C.J. Cheftel (1992) carried out high-pressure research on meat preservation. Mark Hendrickx at the Catholic University, Leuven, Belgian, began a lengthy, key study on the effect of high pressure on the activity of food enzymes. Starch and protein denaturation by high pressure was described by Professor K. Heremans (1982). Professor Bernard Tauscher studied the effect of high pressure on vitamins and other nutrients. Professor Margaret Patterson and coworkers (1995) developed an active high-pressure food research program at Queen's University in Belfast. Much early work is summarized in *High-Pressure and Biotechnology* (1992), edited by C. Balny, R. Hayashi, K. Heremans, and P. Masson, John Libby/INSERM. European and Japanese researchers supplied important research findings on the chemical and biological effects of high pressure in foods and food compounds.

In 1995, *High-Pressure Processing of Foods*, edited by D.A. Ledward, D.E. Johnson, R.G. Earnshaw, and A.P.M. Hasting, was published. The book was based on the proceedings of a symposium held at the University of Reading, in England, March 28–29, 1994. This book served to mark the end of the exploratory phase of research and development on the use of high pressure for food preservation. The book organized basic research on the effect of high pressure on food quality and safety and focused this research on the development needs of the industry. As a result international research and development efforts began to focus on solving equipment and food plant manufacturing problems.

2.11 Development of Dedicated High-Pressure Food Processing Equipment

The rapid expansion of high-pressure food research around the world provided the basic science needed for the commercialization and regulation of the technology. As predicted, in the United States, the minimum chemical changes in foods treated by high pressure resulted in regulations based on existing good manufacturing practices. Food processors were required to demonstrate that HPP-treated foods were safe to consume by insuring a six-log reduction of pathogens likely to inhabit the product. Use-by dates were required to insure the quality and safety of refrigerated and shelf-stable products.

A large amount of research data describing the inactivation kinetics of a wide range of microbes was being generated in laboratories in Europe and the United States. Researchers began comparing the shapes and rates of inactivation curves published by workers using identical microbes, pressures, and time values. At a Nonthermal Processing meeting, several members presented data showing discrepancies in the rate of inactivation of identical microbial samples among reporting laboratories. There was an immediate interest in determining the source of the discrepancies. A group was formed and charged with finding the cause of the discrepancies and proposing methods for their prevention.

The ability to produce accurate inactivation data for pathogens and spoilage microbes using high pressure is fundamental to the commercial success of the technology. Accurate and precise inactivation data is central to regulatory acceptance of a process, to proper equipment design specifications, to HACCP programs, and to the cost and efficiency of the process.

The study group analyzed the variables that could affect the lethality of a high-pressure treatment and concluded that the source of lethality variability rested in the design and operation of the high-pressure chamber and associated equipment used in any lethality study. While high-pressure processing is considered a nonthermal process, when water or high-moisture foods are compressed, there is a parallel increase in sample temperature due to compression heating. A thermocouple located in the center of the sample may show a higher temperature than a thermocouple

located in the sample in contact or near the pressure vessel wall. These temperature discrepancies may be transient and may depend on holding time at pressure or on the rate of compression and decompression. High-pressure systems that used oil instead of water could generate perhaps three times the temperature increase over those systems using pure water. Similarly fatty foods would increase in temperature much more than low-fat products.

Dr. V.M. (Bala) Balasubramaniam, Food Engineering faculty member at the National Center for Food Safety and Technology, Illinois Institute of Technology's Moffett campus, undertook the preparation of a protocol that could help researchers produce lethality rate data that did not depend on the type of high-pressure equipment used. This information was a set of guidelines for measuring the lethality of high-pressure treatments (Balasubramaniam et al. 2004). Subsequently Dr. Balasubramaniam proceeded to do basic studies on compression heating rates of foods and food components at various pressure levels (Rasanayagam et al. 2003). After moving to The Ohio State University in 2002, Dr. Balasubramaniam carried out additional studies on heat capacities, thermal conductivities, and other physical properties of foods under pressure (see Chap. 6).

In the mid-1990s, food processors desiring to use high pressure were still hampered by the costs of high-pressure treatment (Demetrakakes 1996). Equipment manufacturers were challenged to design, fabricate, assemble, and install high-pressure food processing equipment that would treat retail size packages of food at costs in the range of five to ten US cents per pound rather than 30 or more cents per pound.

The development of cost-effective, dedicated, high-pressure food processing equipment required a shift in the thinking of the companies' manufacturing pressure vessels, pumps, intensifiers, valves, high-pressure tubing, seals, and instrumentation. While the science was available for designing pressure vessels with cycle lives of well over 100,000 cycles at pressures in the range of 680 MPa, the requirements for the use of water for compression dictated stainless steel liners for these pressure vessels. Pressure relief valves operating 120 times or more per day required special designs to protect the valve from rapid erosion by decompressing water. Erosion and wear were one set of challenges that needed to be overcome. The need for reduced capital costs, cost-effective operation, and ease of maintenance and repair seemed to present an impossible challenge.

Several high-pressure vessel manufactures showed an early interest in the treatment of foods using high pressure. These included Uhde in Germany; ABB Pressure Systems (Quintus) in Sweden; Engineered Pressure Systems, a division of National Forge, in Belgium and the United States; ACB GEC, in France; and Stansted Fluid Power, in the United Kingdom. High-pressure equipment companies in Japan that had manufactured production systems for Japanese food companies did not appear to be interested in developing food processing pressure vessels operating above 400 MPa. Research had established that treatment pressures just under 610 MPa allowed hold times of 3 min to achieve a six-log reduction of most vegetative microbial pathogens and many types of spoilage microbes. Since bringing the treatment vessel to pressure was a function of intensifier horsepower and vessel volume, in

principle ten cycles per hour, with a 3-min hold, could be possible. The vessel needed to be loaded, sealed, compressed, held at pressure for 3 min, decompressed, and unloaded in no more than 6 min. A more feasible six cycles per hour appeared to be possible with a 3-min hold at 600 MPa. Dr. Ed Ting, Vice President of Research, at Flow International Corp., in Kent, Washington, observed that without dependable, cost-effective equipment, the commercial development of high-pressure food processing would remain a technical curiosity.

Dr. C.P. Dunne, in 1995, at the Army Combat Feeding Directorate, Natick Labs, proposed funding a design contract. The contract had the objective of challenging the pressure vessel industry to design a high-pressure food processing system that would meet the production needs of both the food processors and equipment suppliers. System goals would be high production rates, ease of integration into existing food processing lines, reliability, ease of maintenance and repair, and low operating costs. A team of pressure vessel experts was assembled to evaluate submitted design proposals. This design competition was a first step in developing an economically feasible high-pressure food processing industry in the United States and later in Europe. Design proposals were submitted by most of the builders of high-pressure processing systems.

The design contract was awarded to Dr. Joseph Kapp of Elmhurst Systems. Elmhurst Systems was a high-pressure-equipment consulting firm. His design proposed the use of multi-wall pressure vessels made from used 155-mm field guns available at the Army Watervliet Arsenal. The gun barrels would be sawed to length and machined to form an outer layer and an inner layer held in compression by shrinking the outer layer on the inner layer. A third inner layer of stainless steel would be inserted in such a manner that the residual compression pressure at its inner surface would be in excess of 680 MPa.

The ASME pressure vessel code, in the United States, required a design that would allow the vessel to leak before breaking if its maximum operating pressure was exceeded or if the inner stainless steel liner wall was damaged through routine use. The strength and thickness of the steel in each layer was specified to achieve a theoretical vessel cycle life well over 100,000 cycles. The design operating pressure was just under 610 MPa. The vessel volume was 30 L, and the inside diameter was in the range of 150 mm (6 in.).

The design proposal showed a pair of 30 L vessels alternately driven by a single pump/intensifier and mounted on frames that would allow each vessel to move from a 45° angle, for automatic gravity driven loading (Fig. 2.2) to the vertical position inside the yoke, for compression, holding, and decompression. Unloading was achieved automatically as the vessel was returned to the 45° angle starting position and the bottom closure was automatically opened. After closing the bottom closure, the vessel was ready to receive water and packages by automatic gravity feed to begin the next cycle.

Two special design features were used to fully automate the system. As the filled vessel tilted toward the vertical position, the top closure plug was automatically placed in the end of the pressure vessel just as the vessel swung inside the yoke. Clearance for the top and bottom closures was achieved by fully inserting them in the vessel.

Fig. 2.2 Prototype Elmhurst Research tilting HPP system showing pressure vessel ready to receive packages from an automated feed chute. After loading, the vessel is moved to the vertical position inside the yoke. A 10-L pilot-scale Elmhurst unit is located in the Department of Food Science of Rutgers University in New Brunswick, NJ

In order to deliver compression water to the vessel through the top closure, the design called for a high-pressure tube to be inserted into a hole drilled through the yoke above the vertically positioned pressure vessel. The high-pressure water line was held in place using a special seal in the top closure. The water delivery tube was held in place during compression, holding, and decompression by the insertion apparatus. After decompression the tube was retracted to allow tilting the pressure vessel for product discharge and reloading.

Elmhurst Systems built a commercial food processing system incorporating the design features described. The pair of vessels remained in commercial operation logging many tens of thousands of cycles.

One major advantage of the Elmhurst design was the movement of the packaged foods through the vessel. This insured that treated product would be handled separately from untreated product. The through flow design is now standard in commercial horizontal units.

Dr. Edmund Ting was Vice President of Research at Flow International Corp. in Kent, Washington. Flow pioneered the use of high-pressure water jets for stone, metal, plastic, and food cutting. He became interested in high-pressure food processing as an expanded market for their high-volume, high-pressure, reliable, rugged, pump/intensifier systems. Under Dr. Ting's direction, with microbial study help from Dr. Errol Raghubeer, Flow was able to develop a semi-continuous high-pressure processor for liquid and semiliquid foods. The system consisted of three 20-L vessels. Each vessel operated on a four-stroke cycle and

discharged pasteurized product to an aseptic holding tank. The treated product was filed aseptically in presterilized packages. Results from testing of salsa in a pilot-scale, semicontinuous unit were published by Raghubeer et al. (2000).

In operation untreated product was pumped into the pressure vessel through the intake valve, against a moving free piston, to fill the vessel. High-pressure water was then pumped into the vessel behind the free piston to cause the free piston to compress the product. The product was held at the desired pressure for the necessary time. A discharge valve, leading to the treated product holding tank, was opened, and the treated product was discharged from the vessel by low water pressure on the backside of the free piston. The piston pushed the product through the discharge valve. The pressure vessel was then ready for the next cycle. The fully automated system could achieve over six cycles per hour on all three vessels.

In Europe GCE demonstrated both the feasibility of a semicontinuous system for treating liquids and a horizontal, through flow, high-pressure vessel system for batch treating packaged foods. The horizontal batch system solved two operating problems encountered in the use of existing vertical high-pressure vessel systems in food plant operations. First, the horizontal system removed the need for a high ceiling production area to house large-volume, high-pressure, vertical vessels. Second, high-volume, larger-diameter vessels could be built in longer lengths to meet the package sizes and greater production capacities desired by food marketing people. Horizontal vessels with an internal diameter of 250 mm or larger could be built with the lengths needed to accommodate vessel capacities well over 350 L. Increased production per hour could be achieved, not so much through increased cycle rates per hour but by increased capacity per cycle and increased volumetric efficiency. Larger diameter vessels allowed better packing of desired consumer package shapes. Volumetric efficiencies of 75 % became possible.

Very quickly horizontal, wire wound vessels, 300 L and larger, became the standard for the high-pressure food processing industry. Despite some operating drawbacks, these systems have brought capital and operating costs toward single-digit pennies per package. Flow purchased the Swedish company ABB Pressure Systems and marketed large, horizontal, wire wound food processing systems under the Avure brand. Dr. Raghubeer was able to use these systems to demonstrate the consistent ability of this equipment to pasteurize a wide range of food products. His work helped to achieve acceptance of high-pressure processing by both the Food and Drug Administration and the USDA meat inspection agency in the United States.

The lessons learned from the commercialization of high-pressure food processing in the United States underscore the value of open communications among academic and government research laboratories, regulatory agencies, equipment manufacturers, and food processors and their trade associations. As with the development of heat preservation by Appert in France, military support, in the United States, through the vision of Dr. C. Patrick Dunne, played an important role in moving high-pressure processing technology to successful commercial food processing use.

In 1995 Food Engineering Magazine published an article: “Avomex to Pioneer High Pressure Food Process in the U.S.” The goal of full commercialization had

been attained in the United States. The first product was guacamole made from fresh ripe avocados. This refrigerated product with an extended shelf life is still produced by Avomex's successor company Fresherized Foods and several other companies in high volumes. A number of other different high-pressure pasteurized products, including seafood, fruits, and fruit juices and ready-to-eat meat products, are now marketed worldwide with a volume of sales in the United States estimated at over $2 billion annually.

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Part II
Process Equipment and Packaing

Chapter 3
High-Pressure Processing Equipment for the Food Industry

V.M. Balasubramaniam, Gustavo V. Barbosa-Cánovas, and Huub L.M. Lelieveld

Abstract High-pressure processing has been adopted by a number of food processors, and the demand for adequate equipment in size and throughput is continuously growing. This chapter provides information on the basic components of industrial-scale high-pressure equipment and how they work. This may help to select appropriate high-pressure systems to match industrial needs in a cost-effective manner where safety of the operations is paramount. This chapter includes write-ups from the most relevant companies manufacturing pilot plant and industrial high-pressure systems for processing foods. Major components of the systems are presented, specifications are summarized, and modes of operations are described. The contribution from manufacturers makes the presentation of a number of models very accurate and facilitates highlighting those that are the most promising. At the same time, the equipment manufacturers are key to recommend the selection of the right piece of equipment for a given application and the identification of pros and cons of each model.

Keywords Industrial equipment • Pasteurization • Sterilization • Pressure vessel • Throughput • Package

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3.1 Introduction

Industrial high pressure food processing is typically carried out in batch processing mode. Batch systems can process prepackaged liquid and solid foods. Though no continuous equipment is available for commercial practice, the importance and benefits of developing continuous or semicontinuous systems are highlighted in Chap. 4. It is worth mentioning that high-pressure homogenization systems, as described in Chap. 7, work in a continuous fashion but at lower pressures than conventional batch high-pressure units.

The pressure vessels can be oriented either in vertical or horizontal configuration. Moderate-sized vessels can also be configured vertically and to tilt to load or unload products. Available ceiling height, dimensions of the food processing plant, and the specific product to be processed may be factors for deciding pressure vessel configuration. For example, vertically configured equipment may offer advantages for shellfish shucking.

In vertically configured vessels, a carrier basket containing the prepackaged food is loaded into the pressure vessel through the top opening of the pressure chamber using either a simple manual chain hoist mechanism or an automated robotic system. Careful attention must be paid to separate processed products from raw products when utilizing vertically configured pressure vessels to prevent raw (untreated) product from inadvertently bypassing the pressure treatment. Horizontally configured pressure vessels facilitate the circulation of the product through the production floor by having distinct loading and unloading zones located at each end of the pressure chamber.

3.2 Components

Batch equipment typically consists of the following components (Traff and Bergman 1992; Mertens and Deplace 1993; van den Berg et al. 2001; Ting 2011):

(a) Pressure vessel
(b) Closures
(c) Yoke
(d) Pressure pump
(e) Process control system

3.2.1 Pressure Vessel

Typical commercial pressure vessel range from 35 to 525 L in volume. Pilot-scale systems may be available from 1 to 30 L capacity. Cylindrical pressure vessels are typically constructed using one of the following three approaches (Ting 2011):

(a) Monoblock: The single forged monolithic chamber is often constructed of low-alloy steel of high tensile strength. In comparison with other designs, they are less expensive to fabricate and operate at moderate pressures (less than 400 MPa), and they have small vessel diameters (less than 15 cm). Over-pressurization of monoblock vessels may promote plastic deformation of the inside wall of the pressure chamber leading to crack formation.
(b) Multiwall vessel: The chambers are constructed from a series of concentric cylinders that are shrunk fit on each other to form a multiwall chamber.
(c) Wire-wound vessels: These vessels are formed by layering high-strength wire under tension on a thin wall core of a pressure vessel. Most of the industrial-scale vessels are fabricated using wire-wound technology. This enables the manufacturer to make large diameter pressure vessels operating at higher than usual pressures (typically 600 MPa). The industrial-scale wire-wound high-pressure vessels typically utilize a few hundred kilometers of wire. The technology also enables the pressure vessel to "leak" before breakage, thereby avoiding catastrophic failures. Wire-wound and pre-stressed technology vessels can be operated more than hundreds of thousands of cycles by periodically replacing the core cylinder.

The compression energy within a high-pressure vessel can be estimated using the following relationship:

$$E_{\text{comp}} = \frac{\beta}{2} P^2 V_0 \tag{3.1}$$

where E_{comp} is the compressed energy stored within the pressure vessel of a specified volume (V_0) and operated at a target pressure (P). β is the compressibility of the pressure-transmitting medium. For example, a 100 L vessel pressurized to 400 MPa at room temperature may contain 1920 kJ of compression energy. This is lower than the energy in a retort containing 1 kg of saturated steam at 120 °C (2700 kJ) (Deplace 1995). The temperature of the pressure chamber, carrier basket, and pressure-transmitting fluid of the laboratory-scale equipment often can be controlled by heating or cooling through a jacket surrounding the pressure vessel. For industrial-scale high-pressure equipment used in food pasteurization applications, the temperature of the pressure chamber is not controlled but equilibrates with ambient temperature. The equipment is generally placed in a chilled/refrigerated room. The temperature of the pressure-transmitting fluid tank can be controlled by heating or cooling through a plate exchanger or a serpentine.

3.2.2 Closures

Both ends of the pressure chambers are sealed by means of two closures (also known as plugs). When designing closures, sealing system, mechanical safety of pressure vessel, and efficiency of material handling via loading and unloading should be considered.

For high-pressure vessels, the high-pressure seals used on the end plugs can be a major source of maintenance cost. Seals are subject to frequent wear, especially at elevated pressure conditions. They should be replaced periodically as per the recommendation of the equipment vendor.

3.2.3 Yoke

The yoke retains high axial forces that act on the sealing plugs of the pressure vessel. The yoke may be made by laminating a number of steel plates or wire-wound steel frame (or yokes and columns).

3.2.4 Pumping System

A pumping system is used to bring the pressure vessel, packaged product, and pressure-transmitting fluid from atmospheric pressure to the target processing pressure. This can be achieved by direct or indirect pressurization methods. The commercial-scale pressure vessels utilize the indirect pressurization approach through external electro-hydraulic intensifiers located in one or several cabinets (called HPP pumps). Typically, the prepackaged food is loaded in the pressure vessel by placing it inside a carrier basket. The pressure vessel is then closed with the yoke mounted on it. Then the vessel is filled with pressure transfer medium (typically water) using a low-pressure pump. Subsequently, the chamber is pressurized to target pressure via external compression using robust HPP pumps.

3.2.5 Pressure-Transmitting Fluid

The pressure vessel of small units, such as those utilized at laboratories or pilot plants (often fabricated using non-stainless steel material), typically uses oil, or water with MPG glycol mixture as the pressure-transmitting fluid to avoid corrosion issues and also to provide greater process temperature range for the pressure-transmitting fluid, which is sometimes needed for systems operating below 4 °C and above 95 °C. Industrial-scale high-pressure food processing systems use only pure water as the pressure-transmitting fluid. Difference in choice of pressure-transmitting fluids (and their heat of compression properties) between laboratory- and industrial-scale equipment will influence the magnitude of heat transfer among the pressure-transmitting fluid, food product, and the environment (see Chap. 6). Thus, adequate caution must be exercised for process scale-up.

3.2.6 Process Control System

A computer is commonly used to control the pressure vessel operations as well as to keep electronic records of the processes to meet GMP standards for a food factory. Typical variables monitored by this computer system are processing pressure, temperature, and holding time. For certain processes, the sensors should be calibrated using the National Institute of Standards and Technology (NIST) procedure. At the same time, some critical processing operations require sensor redundancy (Ting 2011). For example, when high pressure is identified as a critical control point in an HACCP program, potential pressure transducer failure or drift needs to be anticipated and addressed. Other considerations to include as part of the control system are the extent of plant automation for loading and unloading the carrier baskets, drying, and wrapping treated packages prior to distribution.

3.3 Cost

Typical investment costs of high-pressure equipment may range from 0.6 to 4 million American dollars depending upon options chosen. The capital cost of a high-pressure equipment system may be distributed as follows:

(a) High-pressure vessel, closures, and yoke	50–60 %
(b) Pumping system	30–35 %
(c) Process control and allied instrumentation	10–15 %

Capital cost (75–80 %) is the major cost for installing a commercial high-pressure processing plant. In comparison to equipment cost, operating costs are modest: labor (5–10 %), maintenance (5–10 %), utilities (2–4 %), and space (1–2 %) (Farkas 2010).

3.4 Industrial-Scale High-Pressure Equipment

Table 3.1 lists major industrial-scale high-pressure equipment manufacturers. In the following sections, we summarize the specifications and more relevant features of commercial scale as well as pilot-scale high-pressure equipment available from various equipment manufacturers who responded to the editors' request for information.

The editors acknowledge that the equipment vendor list (Table 3.1) is not comprehensive. Listing specifications of the equipment vendor does not imply that the editors endorse the equipment vendors listed over others. In addition to learning from the material provided in this chapter, the reader is encouraged to do independent research prior to selecting specific equipment for industrial processing of food products.

Table 3.1 Suppliers of industrial-scale high-pressure equipment

Manufacturer	Address	Website
Avure Technologies	2601 South Verity Parkway, Middletown, OH 45044, USA	http://www.avure.com
Hiperbaric	Polígono Industrial Villalonquéjar. C/ Condado de Treviño, 6-09001 Burgos, Spain	http://www.hiperbaric.com
KOBELCO	2-4, Wakinohama-Kaigandori 2-chome, Chuo-ku, Kobe, Hyogo 651-8585, Japan	http://www.kobelco.co.jp
MULTIVAC	MULTIVAC Sepp Haggenmueller GmbH & Co. KG, Bahnhofstr. 4, D-87787 Wolfertschwenden, Germany	http://www.multivac.com
Resato	Postbus 232, 8440 AE Heerenveen, The Netherlands	www.resato.com
BaoTou KeFa High Pressure Technology Co., Ltd	No. 38, Campus Road, Baotou Rare-Earth Hi-tech Zone Baotou, Inner Mongolia 014030, China	http://www.btkf.com/enindex.htm
EPSI Inc.	EPSI, Inc., 165 Ferry Road, Haverhill, MA 01835, USA	http://epsi-highpressure.com/
Harwood Engineering	Harwood Engineering, Inc., 455 South Street, Walpole, MA 02081-2799, USA	http://www.harwoodeng.com/
Stansted Fluid Power	Unit 5/New Horizon Business Centre/ Barrows Rd, Harlow CM19 5FN, UK	http://www.stanstedfluidpower.com/
Toyo Koatsu Co. Ltd	Toyo Koatsu Co. 2-1-22, Kusunokicho, Nishiku, Hiroshima 733-0002, Japan	http://www.toyokoatsu.co.jp/toyo-e2/index.html
Unipress	Institute of High Pressure Physics, ul. Sokolowska 29/37, 01-142 Warsaw, Poland	www.unipress.waw.pl
Wenzhou Binyi Machinery Co., Ltd	Zhengzhai Tianzhu Village Road, Yongzhong Street, Longwan District, Wenzhou, Zhejiang 325025, China	http://www.wzbinyi.com/en/

3.5 Avure Technologies Industrial-Scale High-Pressure Equipment

Jerry Toops, Avure Technologies, Middletown, Ohio

Avure Technologies (www.avure.com) traces its high-pressure processing history back to the 1950s with former owner ASEA's work in the development of the industrial diamond. This early work includes industrial processes with pressures ranging from 100 to 800 MPa (approximately 15,000 to 120,000 psi). The ability to safely and efficiently employ such high pressures has enabled Avure Technologies to become among the leading high-pressure equipment manufacturers for food and beverage processing worldwide. Avure's 60+ years of expertise relating to the safe

Fig. 3.1 Practical uses of high-pressure pasteurization

containment and utilization of high pressure have made it a global leader in every market they occupy.

Avure's programs in high-pressure processing (HPP) of food, beverages, and other emerging applications (Fig. 3.1) began in the 1990s to extend the shelf life of avocado-based products in support of Avomex (now Fresherized Foods). This work, spearheaded by the vision of Don Bowden of Avomex, took HPP from a laboratory curiosity into the widely accepted nonthermal pasteurization and shelf life enhancement technology (see Chaps. 1, 14). According to industry estimates, high-pressure technology produces nearly 10 billion US dollars per annum of products.

Avure has developed a wide range of equipment sizes and designs to meet the demands of the ever-increasing number of products for which HPP is desirable (Fig. 3.2). Avure has developed equipment specifically designed for:

- Research applications and product development application
- Specialty companies and low-volume production companies
- Seafood and shucking applications
- High-volume production
- Large-throughput and larger-sized products (525 L)

Fig. 3.2 Commercial-scale high-pressure equipment system (horizontal configuration)

In addition, a specialty HPP system for research and development of commercially sterile shelf-stable products has been delivered to the Institute for Food Safety and Health (IFSH), Chicago, IL. The system has the capability of operating at 700 MPa (120,000 psi) at elevated temperatures for pressure-assisted thermal sterilization (PATS) of shelf-stable low-acid foods (see Chaps. 14, 29). Avure's commercial food processing equipment features horizontal and vertical processing solutions to fit facility and processing requirements and ease of product loading.

The heart of any HPP system is the pressure containment system. Avure Technologies designs and builds its systems using wire-winding and prestress technology that provides a combination of long cycle life, maximum safety, and low vessel weight when compared to other pressure vessel designs. The wire-wound vessel is designed a to leak before burst mode of failure, which makes it the safest high-pressure containment system available. Avure also wire-winds and pre-stresses its frames. Avure's HPP systems are designed in accordance with both the ASME Section VIII, Div. 3 pressure vessel codes and meet the EU directives and CE mark as well as other national high pressure and food regulations.

As of mid 2015, Avure delivered more than 260 HPP systems with many multiple system owners. In this same time frame, Avure placed over 160 HPP systems into commercial applications. These presses have an available producible volume of over 2.8 billion pounds per year (1.3 billion kg). These HPP equipment owners represent 30 different countries and a variety of applications, including ready-to-eat meats and meals, guacamole, juices, deli salads, seafood, salad dressings, and hummus, to name a few (Fig. 3.3). Avure also supplies equipment to the growing toll or contract HPP processing industry, which has rapidly expanded as HPP has become a mainstream process and as smaller and emerging companies seek access to this exciting technology.

All Avure HPP systems feature a combination of the largest pressure cylinder diameter, combined with high-throughput pumping systems to allow for the highest product throughput per cylinder volume in all system sizes. Larger-diameter HPP systems allow for the greatest load efficiencies and therefore allow for the lowest unit cost to produce. In addition larger-vessel-diameter pressure vessels provide the greatest load efficiencies for larger products and bulkier packages.

Fig. 3.3 Selected examples of pressure-treated products

Avure HPP systems are designed with the end user in mind. The AV-10 vessel was designed for small- to medium-sized businesses, but with a large-diameter cylinder and rapid cycling to allow plenty of room for growth. The AV-30 unit is Avure's most popular production HPP system because of its high throughput (Table 3.2). The AV-60 size vessel utilizes a number of new design concepts to speed up cycling, lower operation, and maintenance costs and produce more than 8200 pounds (3700 kg)/h even for products requiring a 3-min hold in the processing cycle. Avure has recently introduced a new series of expandable and upgradeable HPP machines. The new "X" series of presses allows producers to grow their business and have the Avure HPP machine grow with them.

While Avure systems are designed to maximize uptime, when something does break down, Avure has trained and experienced service technicians in the major HPP food markets to get the equipment backup and operating as quickly as possible. Remote diagnostics and 24-h service hotlines make solving problems, wherever they occur, quick and easy.

Avure can offer many additional items and auxiliary equipment needed to install and run a successful HPP operation. Items like chillers, load baskets, and product handling equipment are either Avure designed or supplied through partnerships with some of the world's leading food equipment companies so that each customer gets a solution best suited to its products, volume, and budget.

Table 3.2 Avure Technologies HPP machine portfolio

HPP system model	Internal vessel diameter	Cycles/h (3-min hold)	Estimated annual throughput
AV-10	12″/306 mm	10	10,000,000 lb 4,600,000 kg
AV-30	15.3″/386 mm	8.4	33,000,000 lb 15,000,000 kg
AV-40X	18.7″/471 mm	6.8	43,000,000 lb 19,500,000 lb
AV-50X	18.5″/471 mm	9	57,000,000 lb 25,800,000 kg
AV-60x	18.5″/471 mm	10	63,500,000 lb 28,800,000 kg
AV-70X	18.5″/471 mm	11	70,000,000 lb 31,700,000 kg
AV-S	18.7″/475 mm	8.7	23,800,000 lb 10,800,000 kg

Although equipment is the heart of HPP, successful entry into the market, as well as longevity in producing HPP products, is equally dependent on understanding the science of HPP. That is why Avure, along with its equipment solutions, offers assistance to its customers with expert support including:

- Food science and technology
- Pathogen and shelf life testing
- Product formulation
- Development of HPP production parameters
- Packaging requirements
- Facility design and HAACP planning
- Product promotion

This knowledge can shorten capital expenditure payback, time to market entry, as well as successful launch and market penetration.

Avure HPP systems are designed and manufactured in Middletown, Ohio, USA. Avure also operates an HPP high-pressure application laboratory staffed by world-renowned experts in HPP technology and food safety. At the same time, Avure also collaborates with a number of HPP certified laboratories around the world. Avure employs HPP business development managers throughout North America and in various European and Asian countries, along with agent representatives in additional locations. These individuals and companies, and a Netherlands-based logistics center, serve the aftermarket needs of Avure clients throughout Europe and more than 30 countries where Avure equipment resides.

3.6 Hiperbaric Equipment for Successful Commercial High-Pressure Processing

Carole Tonello-Samson, Hiperbaric, Calle Condado de Treviño, 6-09001 Burgos, Spain

3.6.1 Hiperbaric Company

Hiperbaric is exclusively dedicated, since 1999 to the design, manufacture, and marketing of HPP industrial equipment for the food industries. Its machinery, consisting of more than 140 industrial units (total vessel volume of more than 33,000 L) at the end of 2014, is operating in all five continents, serving more than 100 different customers processing meat, dairy, fruit, vegetable, and seafood products (see also Chaps. 24 to 28 for more information about various high pressure applications in the preservation of different food material). Hiperbaric has more than 50 % of the HPP machines currently in production in the world, with North America as its main market, with 60 % of total Hiperbaric vessel volume installed in the USA and Canada.

3.6.2 Design and Materials

From its conception, each machine is designed to meet the needs of the food industry. The fluid used to transmit the pressure is additive-free non-toxic water, which dries off without leaving any waste on machine components or food packaging materials. To be successful every system component is optimized for high-pressure conditions and to operate in a food industry environment. Particularly, pressure intensifiers, machine enclosures, and all materials in contact with processing water and food products are made of food-grade stainless steel. Equipment cleaning is fast and simple with an automated clean-in-place (CIP) cycle programmed into the control system.

3.6.3 Quality

Hiperbaric manufactures its equipment in compliance with the most demanding sanitary and safety directives, rulings, and standards. Its machinery can be installed in any country, as it fulfills all of the requirements of the Pressure Equipment Directive 97/23/CE enforced in Europe (Anonymous 2015), those of the U3 Certificate of Authorization according to ASME (American Society of Mechanical Engineers 2015) Code VIII, Div. 3 Boiler and Pressure Vessel Code (for the USA, Canada, Australia), and those of GOST-R, mandatory in Russia and several other countries. Hiperbaric is one of the few companies in the world having these three certifications.

3.6.4 Horizontal Design

Horizontal design by Hiperbaric is the current standard (94 % of the machines installed in the last 3 years were horizontal) because it provides clear advantages:

- Improves product traceability as input and output locations are at different sides of the equipment.
- Being physically separated and avoiding the risk of mixture between processed and non-processed products.
- Increases production, as ergonomics eases loading and unloading of products, speeding up the process and avoiding unnecessary use of cranes inside the factory.
- Allows easier maintenance operations in any part of the equipment and facilitates cleaning of the area.
- Reduces equipment height, thus facilitating installation that helps the equipment fit into any food production line.

For these reasons, Hiperbaric builds only horizontal models (vessel in horizontal configuration). Its vessel size range is the world's largest with six different sizes of industrial machines. This includes vessel volumes of 55, 120, 135, 300, 420, and 525 L, working at a maximum pressure of 600 MPa at chilled (or room) temperature (see Hiperbaric 525 model in Fig. 3.4 and characteristics in Table 3.3).

3.6.5 Reliable and Safe

Equipment reliability and safety have been two important key considerations in technical design. The design of the "multiple wall" vessel ("wire-winding" technology) guarantees its reliability in equipment operation. This way, the "leak before burst"

Fig. 3.4 Hiperbaric 525 equipment: vessel volume of 525 L, integrated design with five high-pressure pumps including double intensifiers on the top of the vessel, carriers loading and unloading lines, carrier return line, and operator panel. Maximum working pressure: 600 MPa (87,000 psi)

Table 3.3 Main characteristics and processing cost of the Hiperbaric equipment portfolio

Model	Hiperbaric 55	Hiperbaric 120	Hiperbaric 135	Hiperbaric 300	Hiperbaric 420	Hiperbaric 525
Vessel volume in L (US gallon)	55 (14.5)	120 (32)	135 (36)	300 (79)	420 (111)	525 (139)
Vessel diameter in cm (in)	20 (7.9)	20 (7.9)	30 (11.8)	30 (11.8)	38 (15)	38 (15)
Footprint m^2 (ft^2)	22 (237)	37 (398)	39 (420)	61 (657)	56 (601)	63 (679)
Number of cycles/h	9.7	9.1	8.7	8.1	9.0	9.0
Production[a] in kg/h (lb/h)	321 (707)	658 (1450)	708 (1559)	1459 (3216)	2257 (4972)	2821 (6215)
Processing cost in €/kg (US$/lb)	0.140 (0.064)	0.102 (0.046)	0.117 (0.053)	0.079 (0.036)	0.074 (0.034)	0.071 (0.032)

[a]Calculations for HPP during 3 min at 600 MPa (87,000 psi), considering a vessel filling efficiency of 60 %, including amortization in 5 years, 300 working days/year, 16 h/day, wear parts, and utilities. Exchange rate: €1 = US$1.15

mode of failure is guaranteed for the vessel. Safety features include alarm systems for monitoring over-pressurization of the chamber and the intensifiers, alignment (between vessel, plugs, and yoke), elongation of the yoke, interlocked doors, and a safety programmable logic controller (PLC) for communication. A patented low-pressure filling system of the vessel uses a circuit that is separated from the high-pressure one without the presence of valves subjected to high pressure and minimizes wear and tear on main parts for closing, compared to traditional systems of oversized high-pressure valves.

An industrial-grade SCADA (supervisory control and data acquisition) computer control system registers all data (batches, process parameters, user ID, errors, maintenance data, etc.). This maximizes traceability and meets the quality requirement of the food industry. It is possible to access the inputs/outputs of the machine from any part of the world via a modem in the electrical cabinet. It may be also possible to perform equipment maintenance, diagnosis, and support via remote control.

3.6.6 High-Pressure Pumps

All Hiperbaric high-pressure pumps are made of stainless steel and work on a single stage from 0 to 600 MPa. Each high-pressure pump is equipped with a hand valve which enables it to be disconnected for maintenance work while the other high-pressure pumps continue working; this prevents unnecessary equipment downtime.

3.6.7 Hiperbaric Integrated Design

The Hiperbaric integrated design, with high-pressure pumps on the top of the vessel, minimizes the distance between intensifiers and chamber. This arrangement has three main advantages:

- It reduces the pressure drop occurring along high-pressure pipes, minimizing the work of the intensifiers and mechanical fatigue.
- It shortens the length of high-pressure pipes between the intensifiers and the vessel, reducing wear-and-tear cost and increasing reliability. High-pressure piping must be changed after a specified number of cycles.
- It saves space and makes Hiperbaric machines far more compact than any other commercial equipment (e.g., the 525 L machine occupies 40 % less space than required by other high-pressure equipment manufacturer).

3.6.8 Automation

Automatic carrier loading/unloading systems are part of each Hiperbaric machine. Additional investment in special equipment for handling of products in the carriers, like a crane, is not necessary. After unloading, the return line for empty carriers back to the loading point makes the process even easier.

Hiperbaric is the only HPP manufacturer that has in production fully automatic HPP lines (with several machines working in parallel) without the need for manual labor. In such lines, automatic devices load the products into the carriers. Carriers are then loaded on the loading line of Hiperbaric equipment (Fig. 3.5) using automated-guided vehicles (AGV). Carriers are automatically moved into the high-pressure chamber at the start of the cycle. At the end of the high-pressure cycle,

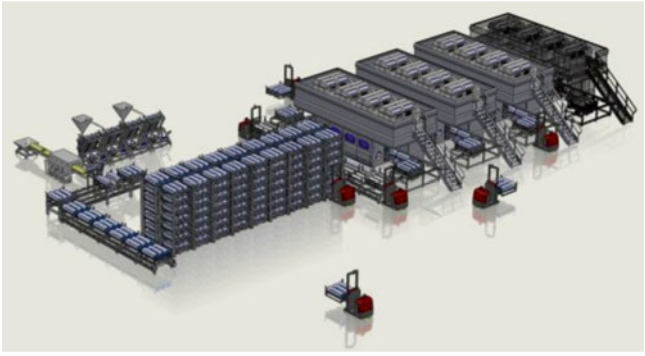

Fig. 3.5 Fully automated HPP lines with Hiperbaric 420s. Carriers loaded and unloaded, thanks to automated-guided vehicles (AGV)

carriers exiting from the vessel are unloaded. Depending on subsequent operation steps, the carriers can be stored, or they are directly brought to an automatic station for emptying the carrier content onto a belt. This belt then brings the product to a drying and packaging station.

3.6.9 Production Capabilities and Costs

Regular increase of production capabilities through increased vessel size has been a main driving force in the implementation of high-pressure processes in the food industry. The largest machine commercially available working at 600 MPa consisted of a vessel of 215 L volume in 2001, but in 2014 it had a 525 L vessel. During the same period, the maximum number of cycles achieved using an HPP machine (at 600 MPa with a 3-min holding time) also increased from 6 cycles/h to 10 cycles/h. This has been achieved by reducing machine operational time (carrier loading/unloading, low-pressure water filling, chamber moving into the yoke) as well as shortening the pressure come-up time (time to reach processing pressure). Come-up time reduction was possible through increasing high-pressure pumping power and improving the efficiency of the intensifiers. The machine operation generally takes from 1 to 2 min/cycle. The standard pressure come-up time is currently from 2 to 3 min, depending on the installed pumping power and vessel volume.

At present, the cost for an HPP machine is in the range of €500,000–€2,500,000 (US$ 575,000–2,875,000) depending on the volume of the vessel. Table 3.3 presents processing cost per kg or pound of the product for a 3-min holding time at 600 MPa processing pressure, with a vessel filling efficiency of 60 % (60 kg or 60 L of product processed/100 L of vessel). This estimate considered amortization, energy cost, and maintenance cost, but did not include manual labor cost, which may be variable depending upon the country and the extent of automation used. The cost ranged from €7.1 cents/kg (US$3.2 cents/lb) to €14 cents/kg (US$6.4 cents/lb) depending on machine size. Since equipment amortization is the main component of the processing cost, the larger the machine, the lower the processing cost. It is further worth noting that equipment price is not directly proportional to its volume. For example, a Hiperbaric 525 costs about five times more than a Hiperbaric 55, but it produces ten times more: about 3000 kg/h (6500 lb/h) against 300 kg/h (650 lb/h) for the Hiperbaric 55.

HPP is a volumetric process and the products are packaged prior to treatment. Thus, packaging has a significant impact on processing costs. The cost varies in direct proportion with the amount of products that fit in the vessel at each cycle measured by the vessel's filling efficiency. As illustrated previously, processing cost is €7.1 cents/kg (US$3.2 cents/lb) for a Hiperbaric 525 if we assume 60 % vessel filling efficiency. The cost would double (€14.2 cents/kg or US$6.4 cents/lb) if the same product is to be packaged in a given vessel with 30 % filling efficiency. Thus, it is important to select and/or optimize the packaging to maximize vessel filling efficiency.

Table 3.4 HPP vessel filling efficiency versus packaging type

Packaging type	HPP vessel filling efficiency (%)
Bag-in-box big pouches	75–85
Vacuum-sealed small pouches/Doypacks	60–65
Hexagonal bottles	50–55
Square bottles	45–50
Round bottles	40–45
MAP pouches	40–55
Vacuum-packed trays—skin packaging	35–55
Cups	35–40
MAP trays	25–40

Table 3.4 provides some examples of vessel volume efficiency for different types of packages. Packaging with the lowest vessel volume efficiency (25–40 %) is that using MAP trays. This is because the MAP packages include gas, which also takes away vessel space. Further, since compressibility of the gas is much higher than many high-moisture-content foods, additional time and energy is required for compression. Further, the trays do not fit perfectly into the cylindrical-shaped carrier basket and void space is left between trays. Vacuum-packaged big pouches can provide maximum volume efficiency (75–85 %), since such packages are free from headspace gas, have flexible shape, and reduce volume. It is possible to package the product using bottles; bottle shape can influence vessel filling efficiency. Hexagonal bottles have less void space between bottles and have a higher filling efficiency (50–55 %) than square (45–50 %) or round bottles (40–45 %).

3.6.10 Market for HPP Industrial Equipment

At the end of 2014, there were around 270 high-pressure machines currently in commercial production (this count does not include pilot plant- or laboratory-scale machines), representing a total vessel volume of about 55,000 L (14,500 gallons). Commercial-scale pressure equipment is found in approximately 200 companies all over the world. Global HPP food production can be estimated at more than 500 million kg (or more than 1000 million lb). The main markets are North America (more than 40 % of global HPP equipment is installed in the USA), the European Union, Japan, Korea, Australia, and New Zealand. HPP equipment can be also found in Peru, Chile, Brazil, Dominican Republic, South Africa, Thailand, Taiwan, China, and Russia.

Most of the equipment installed around the world is dedicated to the processing of fruit- and vegetable-based products (40 %). This high percentage is due to the avocado industry that has been one of the main drives for this innovative technology since the 1990s. Nevertheless, since 2012, the majority of HPP lines installed in this

sector is used for processing juices, smoothies, and cleanses (detox juices). The beverage sector use about 15 % of global HPP equipment.

Since the US Department of Agriculture recommended HPP as a suitable method for controlling *Listeria monocytogenes* in ready-to-eat meat and poultry products, this technology has gained acceptance from consumers and companies; about 25 % of the global HPP equipment is used to process meat products.

At the end of 2014, more than 25 companies worldwide (toll processors or contract manufacturers) were offering HPP services to other companies to treat various products. Companies pay toll processors per cycle (or kg or lb). In recent years, the toll processors, who have about 15 % of all HPP machines, have been mainly serving the juice industry. Some other HPP food processors provide HPP service while manufacturing their own HPP products. This enables effective utilization of their extra production capacity, and thus the amortization of their investment is much faster.

Shucking meat from crustaceans (lobsters, crabs) and opening bivalves (oysters, mussels, clams) are another application of high-pressure technology in the food industry. The seafood processing industry employs 12 % of the machines installed around the world. Shucking meat from mollusks or crustaceans is not the only application for seafood products; the manufacture of ready-to-eat products is another main application for HPP in this sector. The reminder of the machines can be found in companies processing dairy, egg products, etc.

3.7 MULTIVAC Industrial-Scale High-Pressure Equipment

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MULTIVAC was established in 1961 and employs more than 4400 people worldwide, with around 1600 employees based at its headquarters in Wolfertschwenden, Germany. With over 70 subsidiaries, the family-owned company has a presence on every continent (http://www.multivac.com). Advisors and service technicians use their expertise and experience to assist food processors to ensure the maximum production of all installed MULTIVAC machines.

In addition to being an HPP equipment provider, MULTIVAC is one of the world's leading suppliers of packaging solutions; it is a global market leader in thermo formers and manufacturer of an extensive range of various packaging machines, labelers, and quality control systems, as well as automated turnkey lines.

MULTIVAC has pooled its HPP, packaging, product, and application expertise in both its HPP division and its worldwide in-house application and innovation centers, in order to offer top-class and tailored equipment solutions. MULTIVAC's products cover vast areas of the food industry—including meat, poultry and fish, bakery and dairy products, fresh fruit, vegetables, and ready meals. All solutions are designed specifically to meet the needs of customers in the catering, wholesale, and retail sectors.

MULTIVAC HPP represents the completion of MULTIVAC's extensive packaging and product handling portfolio. Among a new durable state-of-the-art HPP machine generation, an innovative concept for HPP treatment of MAP packs was developed, and several patents were granted. An integrated HPP packaging solution was thereby established, consisting of three pillars:

- Packaging technology
- Packaging design
- High-pressure process management

3.7.1 MULTIVAC HPP Machine Portfolio

MULTIVAC HPP products range from small 2 and 4 L units for basic R&D up to commercial HPP units tailored for higher-throughput expectations. Low production capacities for start-ups or small batch production can be realized with MULTIVAC HPP 055 (Table 3.5). This machine size enables average throughput capacity in the range of 170–250 kg/h. Depending on process parameters, product and package characteristics, and finally the number of packages that fit in the transport basket, different production volumes can be obtained.

In general, optimization of throughput capacities can be attained with larger vessel diameters. Therefore, MULTIVAC HPP 160 and HPP 350 were designed with an inner diameter of 380 mm, which enables more efficient processing of large and voluminous bulk products. As a result, medium throughput capacities of up to 1800 kg per hour could be reached.

The MULTIVAC HPP 700 Tandem provides optimal solutions in terms of efficient processing and throughput (Fig. 3.6). The MULTIVAC HPP 700 Tandem consists of two HPP 350 machines operated by one central pressure-building system that alternates between the two vessels. This machine layout makes the MULTIVAC HPP 700 Tandem unit up to 25 % more efficient compared to two stand-alone units. Due to the tandem operation mode, a quasi-continuous treatment of products, which are continuously supplied in-line from the packaging machine, is possible. Furthermore, this tandem concept enables production in dual-operation mode, whereby different recipes/products can be processed at the same time.

The redundant machine concept results in very efficient processing with reduced machine downtime. In addition, individual control of the vessels allows for their independent maintenance. Service and repair due to wear of the machine, replacing spare parts, and periodic inspections can be carried out without losing complete production capacities. The MULTIVAC HPP 700 is equipped with six high-pressure pumps, each containing two pressure intensifiers. As part of the redundant maintenance concept, every pump can be disconnected in order to conduct the recommended maintenance work program with the least negative impact on overall machine performance and availability.

Table 3.5 MULTIVAC HPP machine portfolio

	HPP 055	HPP 160	HPP 350	HPP 700
Vessel volume	55 L	160 L	350 L	2×350 L
Inner diameter	200 mm	380 mm	380 mm	380 mm
Vessel length	1730 mm	1430 mm	3060 mm	3060 mm
Machine dimensions (*length*, *width*, *height*)	7.7 m×2.9 m×2.1 m	7.7 m×3.95 m×3.32 m	11.2 m×3.95 m×3.32 m	11.2 m×11 m×3.32 m
Weight	~17 t	~53 t	~65 t	~2×65 t
Number of pumps	1 (1 intensifier)	2 (4 intensifiers)	3 (6 intensifiers)	6 (12 intensifiers)
Cycle time for *vacuum products* (*without product specific pressure holding time*)	~4.8 min	~4.4 min	~4.8 min	~3.6 min
Throughput capacity per hour for *vacuum products*[a]	170–250 kg	700–950 kg	1400–1800 kg	3400–4000 kg

[a]The exact throughput will vary depending on process and product requirements. Listed numbers show the average range for vacuum products, treated at 600 MPa and 3-min holding time

Fig. 3.6 MULTIVAC HPP machine models: MULTIVAC HPP 055 (*upper left*), MULTIVAC HPP 160 (*upper right*), and two combined MULTIVAC HPP 350 units resulting into the MULTIVAC HPP 700 Tandem solution [© MULTIVAC]

3.7.2 *Design and Processing of HPP Packs*

Despite many global success stories, HPP cannot be considered as a standardized process for every application. Special focus should be directed toward the optimal selection of packaging and HPP processing parameters.

Design and development of suitable packages for high-pressure processing is required to ensure package integrity, safety, and quality of the food product and operational efficiency through the HPP step (see Chap. 5 for discussion on packages for high-pressure treatment). Some key considerations during package design for HPP revolve around the barrier characteristics of the package material such as water vapor permeability, oxygen transmission, use of modified atmosphere, or light transmission. Additional considerations such as film characteristics, printing, and labeling should be predefined. Several other convenience features as well as an innovative design contribute to a unique and successful package that is both presentable and market-worthy.

The treatment of modified atmosphere packages (MAP) at high-pressure levels demands a high level of expertise in terms of packaging, process, and product know-how. Thus, package shape and format need to be tailored in accordance with product characteristics as well as with the individual package filling. Inappropriate design of gas packages may result in the deformation and potential damage of the pack and subsequently the product. In addition, delamination effects may occur in a multilayered film which negatively compromise the protective barrier properties (Fleckenstein et al. 2014). Nonetheless, despite defining a suitable packaging concept, uncontrolled rapid decompression of pressure during an HPP cycle may negatively affect the product and film surfaces/properties (Figs. 3.7 and 3.8). This is especially observed in products such as sliced or deli meats, as "white spots" on meat surface, also described as localized decompression failures, that alter the marketability or aesthetic appeal of the HPP-treated products.

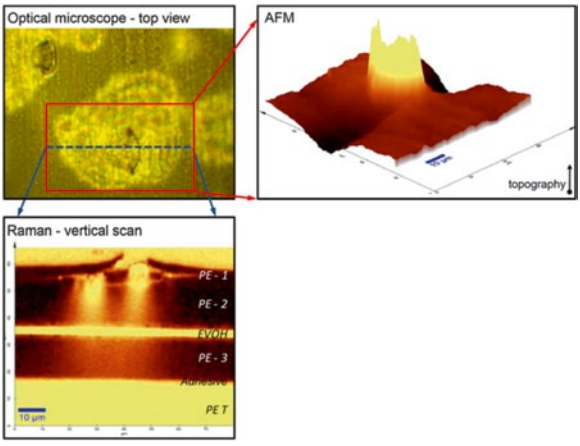

Fig. 3.7 Analysis of a local decompression failure by optical microscope (*upper left*), Raman microscopy (*lower left*), and atomic force microscopy (*upper right*) (Richter 2011)

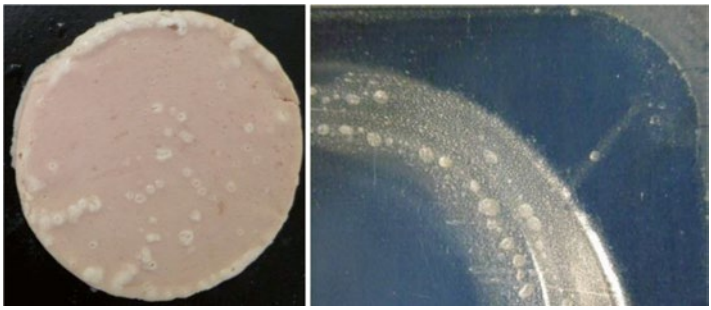

Fig. 3.8 Localized decompression failures after inappropriate process control in product. Lyoner-type sausage (*left*) and packaging film tray (*right*), respectively (© MULTIVAC)

To overcome those deficiencies with MAP packages during HPP, MULTIVAC has developed a patented (*EP2308325*, *US2011007341*) "soft decompression" process that assists in the control of decompression during HPP.

3.8 Stansted Fluid Power Laboratory-Scale High-Pressure Equipment

Mark Freeman, Stansted Fluid Power, Harlow, Essex, UK

Stansted Fluid Power Ltd (SFP) is a UK-based company supplying and supporting quality high-pressure systems globally (http://www.stanstedfluidpower.com/). Established in 1970, SFP has evolved from a range of standard high-pressure products while continuing to develop new and novel high-pressure systems for evolving technologies with systems operating at 1400 MPa.

SFP's range of high-pressure equipment includes pumping systems for liquids and gases, valves, pressure vessels, telemetry lead throughs, fittings, and tubing. Diverse applications include systems used at the laboratory, pilot plant, and production scales, in areas such as nuclear power, pharmaceuticals, food, and biotechnology.

From the 1970s SFP have been manufacturing isostatic compaction and other high hydrostatic pressure systems, these comprise of pressure vessels, decompression valves, and temperature control systems (Figs. 3.9 and 3.10). Pressures used in these systems are generally in the 50–400 MPa range, but also include special applications to 1000 Mpa and this core technology has been used to develop the SFP range for food and bioscience applications.

While working with high-pressure homogenization for cell rupture since 1971, SFP's involvement in the modern era of high-pressure bioscience for food applications began in 1992 in the first European conference on high-pressure bioscience in France. Shortly following this conference, SFP developed and sold their first system specifically designed for food research (Fig. 3.11). This system was supplied in 1993 to Queen's University in Belfast, UK, and is still in service, having the

Fig. 3.9 Multi-configuration laboratory isostatic pressing system

Fig. 3.10 SFP high-pressure valves

Fig. 3.11 Five liter HPP systems

capability to work at 900 MPa, and is the first of some 70 systems worldwide SFP that was installed specifically in the food and bioscience areas. Although commercial scale has been adopted with great success, SFP, in line with overall corporate philosophy, concentrates on the development and supply of high performance research and development scale equipment.

3.8.1 High Hydrostatic Pressure Systems for Research and Development

While technical requirements for production systems have become focused around operation at 600 MPa, generally at ambient temperature, the requirements and ambitions of research scientists working in HHP for food and beverage applications have required the design and development of systems to work at performance levels well in advance of those required for industrial production (Table 3.6).

For equipment development at the pilot plant level, the design emulates performance, such as pressure cycles, of large capacity industrial units.

The majority of systems are offered with pressure ratings of 800/900 MPa, providing pressure capability beyond the majority of current production requirements.

For most HHP processes, pressure may be considered the predominant parameter; however, for many researchers, control over other key cycle parameters is essential. SFP HHP systems provide, in addition to control over the cycle pressure, temperature control, pressurization, and decompression ramp rate control. To accommodate applications in pressure shift freezing (PSF) operation down to −20 °C is permissible, while at the other end of the temperature range, operation up to +130 °C with initial cycle start temperatures of up to +110 °C allows researchers working on pressure-assisted thermal sterilization (PATS) to fully develop their process cycles. In addition, for those systems where the primary pressurization pumping system is external to the pressure vessel, a heat exchanger is provided allowing injected fluid (typically up to 20 % of the pressure vessel volume) to be temperature conditioned to the pressure vessel temperature while in transmission to the pressure vessel. Control over pressurization and decompression rates allows users to investigate the effects of these parameters and also mimic those of production scale equipment.

Control systems and data logging options range from simple manual systems to full PC-based SCADA (supervisory computer and data acquisition) systems, which allow users to simply recall data from previous tests, to recall and repeat parameters, and to record and store pressure and temperature information.

In addition to the range of standard systems, SFP has continued to evolve customized technical solutions in conjunction with clients to meet new and challenging technical requirements. Examples include multiple vessel systems (Fig. 3.12), optical systems, very high pressurization ramp rates (e.g., 0–1400 MPa <5 s), specialized telemetry, and sampling systems.

With a broad range of systems from 5 ml to more than 10 L capacity, and with pressures ranging from 200 to 1400 MPa accompanied by telemetry features, temperature,

Table 3.6 Stansted Fluid Power HPP machine portfolio

Model	MWP		bar	Dia ∅ (mm)	Length (mm)	Volume (ml)	Min. temp. (°C)	Max. temp. (°C)	Signal feeds
	MPa	psi							
S-FL-085-09-W	900	130,500	9000	17	150	30	−20	+90	1
S-FL-850-09-W	900	130,500	9000	37	300	300	−20	+90	3
S-FL-850-14-E	1400	203,000	14,000	22	100	35	+5	+90	1
S-FL-065-220-09-W	900	130,500	9000	65	220	675	−20	+110	3
S-IL-070-550-09-W	900	130,500	9000	70	550	2000	−20	+110	3
S-IL-085-550-06-W	600	87,000	6000	85	550	2800	−20	+110	3
S-IL-100-250-09-W	900	130,500	9000	100	250	2000	−20	+110	4
S-IL-120-250-06-W	600	87,000	6000	120	250	2800	−20	+110	4
S-IL-110- 625-08-W	800	116,000	8000	110	625	5000	−20	+110	4
S-IL-150-300-07-W	700	101,500	7000	150	300	5000	−20	+90	3

Fig. 3.12 Multi-vessel system 8 vessels

and pressure control, SFP system design does not adhere to a single design concept, but rather utilizes a combination of proven designs to manufacture systems with optimized performance, reliable operation, and safe and simple operation.

SFP pressure vessels are constructed with a number of prestressed materials depending on the size of the equipment and its specifications. Prestressing is a technique to improve longevity of the pressure vessel when coupled with other design strategies already mentioned.

3.8.2 Homogenizer Systems for Research, Development, and Pilot and Production Scale

In addition to an extensive range of systems for HHP applications, SFP also designs, manufactures, and supplies a range of high and ultrahigh-pressure homogenizers for chemical, pharmaceutical, cosmetic, and food applications (Fig. 3.13). The range extends from small-volume bench-top systems able to work with volumes as low as 1 ml through bench-top, pilot- and large-scale production with pressures up to 400 MPa and with operating temperatures to over 140 °C.

SFP's philosophy for UHUP, as with HHP, is to ensure that high-pressure pumping elements run slowly to ensure best life and reliability. Principal systems feature electro-hydraulically driven high-pressure intensifier pumps, typically cycling between 2 and 12 each times per minute.

For high-pressure homogenization, while the pumping system is a key element for reliability of the system as a whole, the homogenizing head (valve) will determine for many applications the effectiveness of the treatment. SFP offers a choice of both variable geometry "piston gap"-type valves and fixed geometry, high shear devices, which can be fitted individually and in combination, allowing optimal

Fig. 3.13 Laboratory and production high-pressure homogenizer

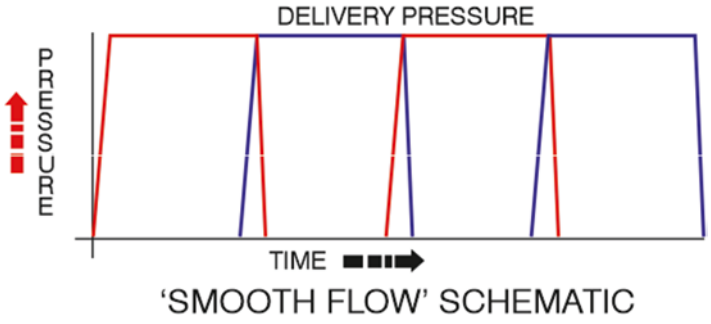

Fig. 3.14 Pressure time schematic for twin synchronized intensifier system

choice for each application. Some applications, such as oil in water emulsions, will be much more effective with a micro channel-based high shear device, while other applications such as sterilization benefit from specially profiled geometry in a reverse flow piston gap-type valve (Fig. 3.14).

3.9 Final Remarks

High-pressure processing offers unique opportunities to food processors to develop minimally processed healthy foods with consumer-desired sensory properties and extended shelf life. The food industry now has nearly two decades of successful

operational experience with industrial-scale high-pressure equipment. The commercial-scale equipment operates reliably over millions of cycles to deliver pressure-treated foods. At the same time, equipment cost and operating cost have been reduced as a result of innovative concepts introduced by various equipment manufacturers. More research and development efforts are needed to make the technology commercially viable for more commodities as well as shelf-stable products.

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Chapter 4
Continuous High-Pressure Processing to Extend Product Shelf Life

Huub L.M. Lelieveld and Hans Hoogland

Abstract The majority of commercial high-pressure pasteurisation operations is essentially conducted as a batch process, wherein prepacked product is subjected to pressure treatment for a specific time. However, the food manufacturing industry has been looking for opportunities to introduce continuous flow processes. This chapter reviews some of the recent efforts in the development of semi-continuous and continuous high-pressure equipment.

Keywords Continuous high-pressure processing • Semi-continuous operation • Energy efficiency

4.1 Introduction

Batch wise high-pressure processing is presumably expensive, as the intended process itself may take only minutes, the entire operation takes a much longer time. It involves vessel preparation, filling with product and pressure transfer fluid, pressure built up, pressure holding and depressurisation, including subsequent unload of the vessel. The true utilisation of the high-pressure vessel is therefore rather low. For other processes, a solution might be sought in increasing the scale of the operation such that the cost of "manual" loading and unloading of the vessel could be carried by large product batches. However, in the case of high-pressure batch processes, the size of the vessel is limited by the strength of construction materials. The larger the surface of the high-pressure container walls, the larger the forces at the prevailing pressure. Furthermore, the machinability and availability of the large high-grade steel parts become of an issue upon size increase.

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Next to the economic limitations, a high-pressure batch process is limited in active fast temperature control of the vessel content, in vessel mixing operation and sequential dosing of ingredients. All constraints are clearly driven by the constructional limitations of a high-pressure vessel, for which the aim of reliably withstanding high-pressure regimes is already a very complex design challenge. A solution might be found in a continuous high-pressure process.

Repetition of a large number of the operation steps carried out during batch wise processing can be avoided after implementation of continuous high-pressure processing, which would contribute to cost-effectiveness and scalability of the process. Furthermore, it would contribute to active temperature control and mixing operations within the vessel.

The simplest continuous high-pressure process uses a high-pressure homogeniser, where the homogenising valve is adjusted to the desired pressure. The product is subjected to a high pressure for an adjustable time, depending on the volume of the pipe between the high-pressure pump and the homogenising valve. At the homogenising valve, the product is also subjected to high shear, cavitation and impingement, resulting in a significant rise in temperature. Due to aforementioned effects, this method is suitable for liquids, such as milk and fruit juices, but not for particulate products. As discussed by Frederico Harte (see Chap. 7), the effect of high-pressure homogenisation on the inactivation of microorganisms occurs most probably due to the temperature effect.

Several companies, such as ACB Pressure System-Alstom, Kobe Steel and Avure (formerly Flow International), developed semi-continuous processes, the principle of which is shown in Fig. 4.1.

The liquid product is delivered by a low-pressure transport pump into the pressure vessel, followed by closure of the supply valve. Required pressure level in the vessel is reached using a high-pressure pump. The floating piston separates the pressure transfer medium from the product. After treatment, the product is transferred to a surge vessel that feeds a filling machine. Microbes in all parts of the machinery that are pressurised will be subjected to the same pressure treatment, and hence, susceptible microorganisms, which are relevant to the processed product, are being inactivated. However, decontamination of the machinery before the process starts, from the low-pressure side of the outlet valve up to and including the filling machine, should be taken care of. For conditions that are set to inactivate vegetative microorganisms, this may be done by using hot water at the adequate temperature and time. Self-evidently, when the process is set to sterilise the product, by applying pressure at elevated temperature (see Chap. 29), the line needs to be sterilised prior to use, e.g. by using hot water or steam. To obtain sufficient capacity, given the relatively small size of the pressure vessels, a number of vessels may be connected together in such a way that while one vessel is being filled, another one is pressurised and a third one is being emptied, as shown schematically in Fig. 4.2, maximising the time the high-pressure pump is used. Pressurising requires energy that does not have to be lost. During depressurisation of the vessel, pressure transfer fluid may be released into the next vessel, and energy required for pressure build up could be partially recovered.

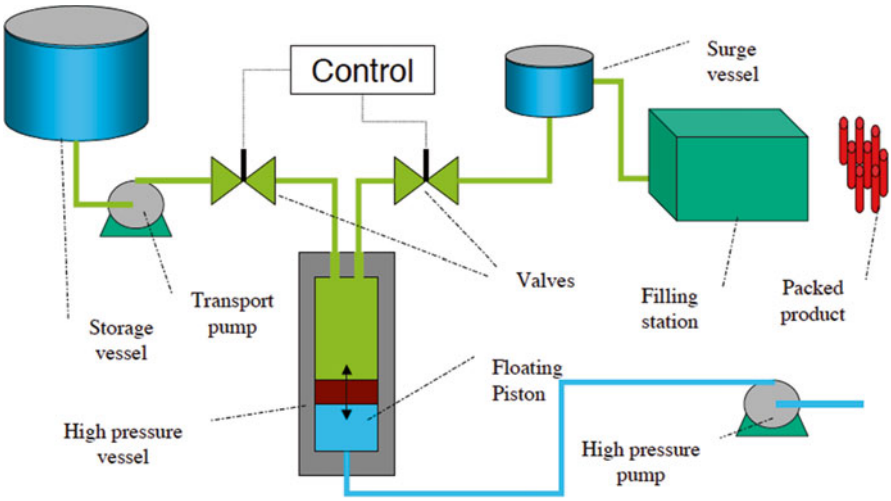

Fig. 4.1 Schematic representation of a semi-continuous high-pressure processing system

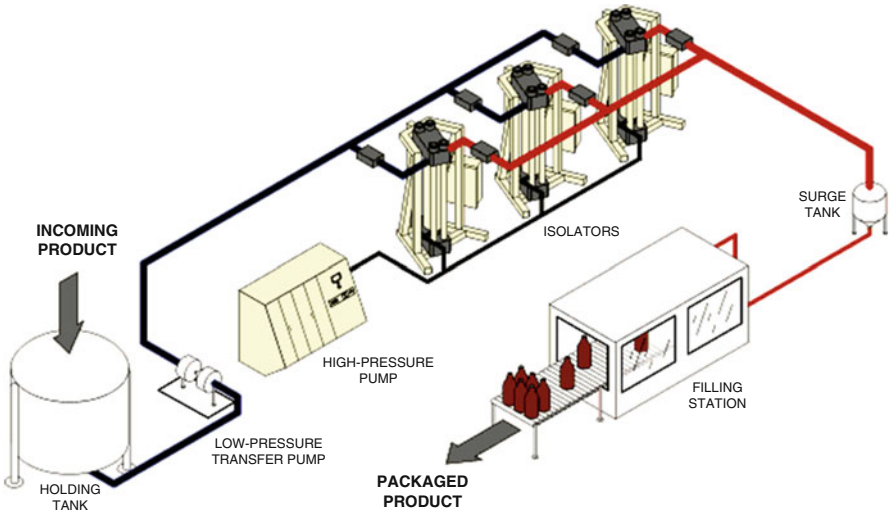

Fig. 4.2 Schematic representation of a multiple-vessel system to obtain a semi-continuous flow of high-pressure-treated product (source: Balasubramaniam, 2003)

In 1997, Unilever patented a fully continuous process, suitable for high-pressure processing of liquids, without the need for a homogenising valve (Agterof et al. 1997). The technique is based on the fact that viscosity of many liquids (except water) is strongly influenced by pressure. The exponential dependence of viscosity on pressure known in the literature as "Barus formula" suggests that a small difference in the composition of a liquid might cause big differences of the viscosity at high pressure

(Rostocki et al. 2008). The increase in viscosity to a greater extent can be reached, e.g. with eugenol (a liquid that can be extracted from, e.g. clove oil), of which the viscosity at 12,000 kg/cm^2 (1.176 GPa) is more than 10^7 times higher than at atmospheric pressure (Bridgman 1926). In the patented system, the product is conducted in a steady flow through an open-end tube, while the pressure difference between the entrance and the exit of the tube is maintained using a high-pressure pump, at pressure levels of over 100 MPa. The interesting aspect of this technology is that after pressurising the system at start-up to pasteurise the first product and tubing, the valve at the exit of the pipeline can be fully opened, and therefore, the product leaving the system is not exposed to extreme shear and the accompanying local temperature rise. Instead, the energy is dissipated by viscous forces over the length of the tube which can be cooled to maintain a fixed temperature. The temperature rise caused by the pressurisation of the liquid by the pump (adiabatic heating) may also be removed by (partial) cooling of the pipeline. However, since the temperature increase assists the inactivation of microorganisms under pressure, cooling might not be necessary. The drawback of the system is that the conditions need to be experimentally established for every product, since the viscosity is strongly influenced by the composition of the product and temperature, which is likely to change while flowing through the system. Alternatively, the pressure may be controlled by controlling the temperature of the liquid, and in such way, the pressure drops over the capillary. Table 4.1 gives an impression of the differences between viscosities of products and temperature.

Figure 4.3 shows the outline for a continuous high-pressure process line. The length L of the pipeline depends on the viscosity of the product to be processed and the diameter of the pipe. If the given length would be too short for the desired residence time, an additional chamber may be installed between the high-pressure pump and the pipe.

Table 4.1 The viscosity of some products at atmospheric pressure, at various temperatures

Product	Temperature (°C)	Viscosity (mPa s, which equals cp)	Source
Milk	25	3	Elert (2013)
Sour cream	25	100,000	Elert (2013)
Ketchup	20	50,000	Elert (2013)
Molasses	20	5000	Elert (2013)
Vegetable shortening	20	1,200,000	Elert (2013)
Sucrose solutions (%)			
20	20	1.967	Perry (1941)
	50	0.974	Perry (1941)
40	20	6.223	Perry (1941)
	50	2.506	Perry (1941)
60	20	56.7	Perry (1941)
	50	14.06	Perry (1941)
Castor oil	20	986	Weast (1973)
	40	231	Weast (1973)
Olive oil	20	84.0	Weast (1973)
	40	36.3	Weast (1973)

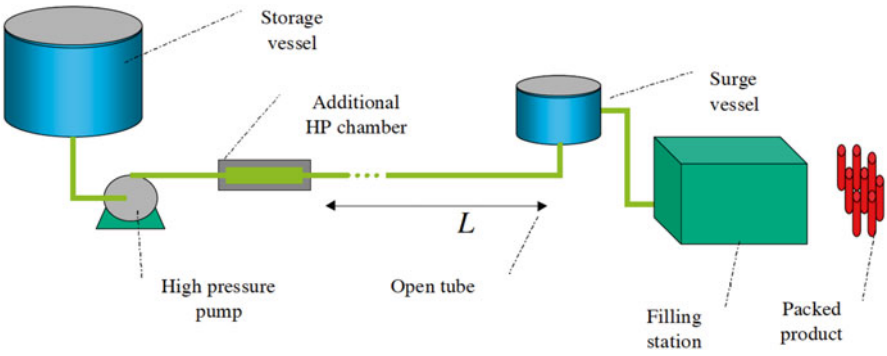

Fig. 4.3 Fully continuous high-pressure processing system (source: van den Berg et al., 2001)

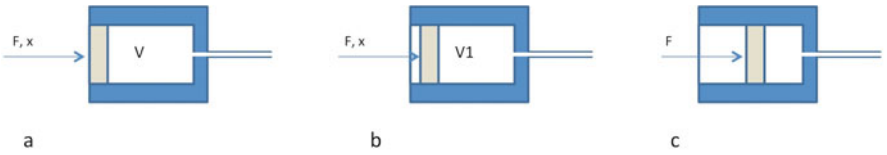

Fig. 4.4 The compression steps in continuous HP processing (a) start position of the plunger (b) plunger reached the position were the process pressure has been achieved (c) the product is pushed out from the cylinder into the tubing at the desired pressure

Complete inactivation of *Saccharomyces cerevisiae* (spiked at 1000 cells/mL) was achieved in the small-scale experiment described in the patent, which involved a pipe of 25 m length, with an inner diameter of 1 mm, an inlet pressure of 300 MPa, a temperature (at the inlet) of 21 °C and a residence time of 60 s.

Another benefit of continuous HP is in thin-wall tubing when compared to the wall thickness of HP vessels. The temperature control of large industrial vessels is due to thick walls very slow and complicated, especially when the product needs to undergo a dynamic temperature profile. In a continuous HP system with limited tube wall thickness, the temperature can be easily controlled by applying a tube in tube construction. This will allow the dynamic control of a product temperature, for example, enabling a sudden cooling or heating during the depressurisation of the product.

Additionally, continuous high-pressure processing would open up new avenues such as the combination of two ingredient streams and subsequent mixing by in-line mixers, processes that are impossible to be realised in present high-pressure batch process.

Unfortunately, continuous HP requires significantly more energy than batch processing as the product not only needs to be pressurised but also needs to be relocated out of the compressor, as illustrated in Fig. 4.4.

The energy needed for the "compression" step and the "push-out" step has been estimated by the use of data from Bridgman (1926) and by the use of physical properties of water as available through NIST (NIST/ASME Steam properties standard reference

Table 4.2 Energy required for the subsequent stages for water with a starting temperature of 20 °C

p (MPa)	e_comp (kJ/kg)	e_push (kJ/kg)	e_tot (kJ/kg)
300	12	273	285
500	26	437	463
700	45	593	638

10 software, 2013) which maintains a database on fluid properties containing values for the enthalpy, internal energy of water in a wide range of pressures and temperatures. Results are shown in Table 4.2 with e_comp, e_push the energy required for "compression" and "push" stage, respectively, at the indicated pressures (*p*).

The results in Table 4.2 do not take into account the efficiency of the process but indicate the minimum amount of energy needed from a physical point of view. The work needed during the "push out" is between 13 and 22 times higher than for the "compression", depending on the required pressure. An indication of the required power for a continuous high-pressure system can be calculated by multiplying the value of w-tot with the mass flow of the process, e.g. a process of 3600 kg/h (1 kg/s) requires at least 463 kW when running at 500 MPa.

The decision to utilise a continuous system should therefore be based on the possibly lower investment cost.

Although more data are lacking, based on the simplicity of the process and its advantages over the semi-continuous process such as scalability and the possibility to control temperature and mixing, it seems recommendable for manufacturers of high-pressure processing equipment to develop a commercial system for the food industry and for researchers to explore this new operating window for high-pressure research.

Acknowledgement We thank Ana Balasa for carefully checking the article and helpful comments.

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Chapter 5
High Pressure Effects on Packaging Materials

Huseyin Ayvaz, V.M. Balasubramaniam, and Tatiana Koutchma

Abstract During high pressure processing, the prepackaged food material is pasteurized or sterilized by subjecting it to combined pressure-thermal treatment of different intensity. This chapter summarizes the impact of pressure-thermal treatment on moisture and gas barrier properties, seal and mechanical strength, as well as migration characteristics of the packaging polymer. Packaging also play important role in preserving quality of pressure treated foods during extended storage life. Some of the recommended future research needs in the development of novel packaging material for high pressure processing are summarized.

Keywords Package • Polymer • Pressure • Temperature • Barrier properties • Migration

5.1 Introduction

Packaging is a vital aspect for any food preservation method to be successful since it protects the food products from adverse environmental conditions (Ozen and Floros 2001). It helps to preserve the internal properties of foods, including flavor components, by acting as a barrier between internal and external environments.

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During the high pressure pasteurization, the food material is prepackaged in flexible container and then pressure treated at ambient or chilled conditions (see Chap. 3). Package also protects the food material from contamination of the external pressure transmitting fluid and other contaminants. Pressure treatment can potentially alter various moisture and gas barrier properties of the packaging polymer. Understanding how different polymer properties altered by the pressure treatment can help to select suitable packaging material that can withstand pressure treatment. During semicontinuous operations, food products are processed in bulk and are not packaged before the application of high pressure (see Chap. 4). Therefore, package is not exposed to high pressure, and food product is packaged aseptically after processing to ensure shelf-stability.

Pressure treatment can also alter heat compression and other thermo physical properties of packaging polymers (see Chap. 6). For instance, it has been shown that polypropylene (PP) and polyethylene (PE) undergo compression heating greater than water under both high pressure at low and mild temperatures of 10 and 50 °C and pressure treatment at high temperature (90 °C) conditions (Schauwecker et al. 2002; Knoerzer et al. 2010). In particular, the temperature increase with pressure was not linear, and the relative increase with respect to water depended on the pressure range selected as well as the initial temperature. Assuming minimum 5 % of the HP vessel is filled with packaging material, changes in thermophysical properties of packaging materials may affect temperature distribution within the pressure vessel.

When high pressure and high temperature combinations are used to achieve sterilization (see Chaps. 11, 14, 29), prepackaged food products need to be preheated to a target initial temperature before the high pressure is applied. This process is generally known as pressure-assisted thermal processing (PATP). Reasonably harsh preheating process encountered during PATP has the potential to affect the properties of the packaging materials used, including changes in their structure and alterations of their mechanical and barrier properties (Koutchma et al. 2010). Moreover, these changes in the package may result in product quality loss during extended storage. That is why the packaging aspect of high pressure processing is very essential for the successful application of the technology. Researchers have investigated the effect of high pressure processing on packaging materials and food-packaging interactions (Bull et al. 2010).

5.2 Packaging Requirements for High Pressure Processing

Pressure pasteurized products are packaged using flexible, polymeric based pouches, jars, trays and bottles. Rigid packaging materials made from metal and glass are not suitablable as they could be deformed by elevated pressure treatment.

The following are some key considerations when selecting packaging material for high pressure processing:

5.2.1 Flexibility

A packaging material needs to be flexible enough to withstand compression forces so that it can avoid irreversible deformations and maintain its physical integrity. This statement is supported by understanding of volume change during high pressure processing. Typically, high-barrier flexible pouches made of polymers or copolymers with at least one flexible side can be used for processing solid or liquid food products by batch high pressure systems. It can be possible to retain the quality and freshness of the product throughout the shelf life if only a compatible type of packaging material is selected (Sorrentino et al. 2007). When an inelastic packaging material is used, severe deformation is expected to occur during the process (Guillard et al. 2010). Metal cans collapse permanently and glass bottles are likely to break. Thus, packaging containers made from metals and glass material may not be suitable packaging material for high pressure processing (HPP). Additionally, packages which are made of paperboard are not suitable either, since they can degrade under pressure (Caner et al. 2004).

5.2.2 Sealing Strength

Sealing is an important point for flexible pouches, and seal strength should be maintained during processing in order to avoid product leaks or infiltration of pressurization medium into the containers (Koutchma et al. 2010).

5.2.3 Robustness

Barrier, mechanical, and mass transfer properties (sorption and migration) of the package must be resistant to changes occurring during the process. During the process, the volume of the package and the food material decrease due to the pressure applied. The decrease in volume is expected to be temporary, and once the pressure is released, the package needs to regain its original conditions. This is possible only if the thermomechanical stress generated during combined pressure-heat treatment is within the limit that the package can tolerate and return to its original condition (Caner et al. 2004). Containers should also maintain their aesthetic qualities and any other built-in features included in their design, in order to ensure consumer acceptance and overall product convenience (Koutchma et al. 2010; Lambert et al. 2000).

5.2.4 Vacuum Packaging

Vacuum packaging is very important for a uniform treatment because air trapped after sealing the package has higher compressibility than food products and this could potentially lead to a nonuniform treatment and package deformation (Lopez-Rubio et al. 2005). Additionally, excessive amount of gases can increase the come-up time of the processing and cause unnecessary physical stress on the pouch (Schauwecker et al. 2002). Vacuum packaging can also help avoiding oxygen-related reactions including lipid oxidation during both processing and storage. Another advantage of vacuum packaging is that it can improve the loading factor and more packaged product can be processed at one time in the limited volume of the pressure vessel since the amount of air in the package is minimized. Package size and shape are also critical in terms of maximizing the number of packages which can be fitted in the chamber. Therefore, proper package design can contribute to the economical processing.

5.2.5 Barrier Properties

Packaging materials need to have adequate barrier properties to maintain the quality of food products throughout shelf life. In this regard, water and oxygen permeabilities are two of the most important considerations (Galotto et al. 2008). Foods that are sensitive to change in moisture can spoil quickly and lose their characteristics by either absorbing or losing water. Similarly, foods that are sensitive to oxygen changes can become rancid (Yoo et al. 2009). Developing packaging materials with improved water, oxygen, and light impermeability is essential, and choosing the correct type of polymer (or polymer blend) to be used as packaging material is key. Efforts are also underway to utilize modified atmosphere packaging (MAP) during high pressure processing (see Chap. 3.7).

5.2.6 Heat Transfer

Heat transfer properties of packaging materials need to be well characterized and enable fast preheating (Koutchma et al. 2010) prior to PATP. The preheating step can cause undesirable quality changes in the food product and enhance the negative developments in the properties of the package materials during subsequent high pressure application and storage. Therefore, it should be done as quickly as possible. Koutchma et al. (2010) evaluated the impact of packaging materials on heat transfer during the preheating stage and compared four plastic-laminated and two aluminum-laminated pouches. The authors reported that foil-laminated materials reached the targeted preheating temperature faster, providing shorter preheating

time than polymeric materials even though foil-laminated materials were thicker than the plastic-laminated materials tested. They attributed faster preheating of foil-laminated pouches to higher thermal conductivity of aluminum layer in the pouch.

5.2.7 Transparency

Consumers in recent years are more attracted towards transparent packages, which provides better product visibility (Lange and Wyser 2003). According to Lange and Wyser (2003), it is important to have good barrier polymers which can also provide transparency. Metal based containers such those made from aluminum lack transparency and thus consumer may not able to see package product content.

5.3 Commonly Used Packaging Materials in High Pressure Applications

Packaging materials used in the food industry can vary drastically from each other depending on specific properties of the polymers used, such as: functional groups in the structure, chain backbone, cross-linking, orientation, melting point, density, thickness of the polymer, and transparency (Valentas et al. 1997). Most of the flexible packaging materials used in the food industry show reasonably good compatibility with high pressure pasteurization when there is no severe heat involved (see Chap. 1, 3). Single and combinations of PET, PE, PP, and EVOH films are some of the commonly used packaging materials for high pressure processing applications in the food industry (Juliano et al. 2010). Additionally, co-extruded films with polymeric barrier layers, adhesive laminated films on a polymer base, or inorganic layer such as aluminum foil (a few micrometer thick) or more recently vacuum deposited coating (nanometer levels thick) are also used (Richter et al. 2010).

For pressure-assisted thermal treatment, where the packaging material is exposed to harsher pressure-thermal conditions, most of the packaging materials used by the food industry may not survive. Nylon, EVOH, PET, PP, aluminum oxide coating, and Al or metalized layer are some of the barrier materials which can survive the PATP conditions (Koutchma et al. 2010). High-barrier materials are needed in order to prevent the interaction of the internal atmosphere of the packaged food with the ambient environment so that the packaged food can retain the properties during the shelf life. A common technique used for creating a barrier layer is lamination or co-extrusion with a good barrier polymer. Polyvinylidene chloride (PVDC), ethylene-vinyl alcohol (EVOH), polyvinyl alcohol (PVAl), and polyamide (PA) are common high-barrier polymers. Good oxygen barrier polymers are generally effective when they are dry. Therefore, they are usually sandwiched between good water-vapor barrier films so that their oxygen barrier properties can be still retained (Lange and Wyser 2003). Another way of improving the barrier properties

of a package is blending. By mixing a high-barrier material with a regular film, tortuosity, which is the length that a diffusing compound such as oxygen or water vapor needs to travel, can be extended, and therefore a better barrier property is provided (Lange and Wyser 2003).

5.4 Effect of High Pressure Processing on the Properties of Packaging Materials

In the last decade, numerous research efforts assessed the effect of pressure on the mechanical, barrier, structural, and sealing properties of commonly used mono or multilayer packaging films (Guillard et al. 2010). Typically these studies utilized milder treatment conditions (pressure levels up to 800 MPa; temperature up to 60 °C) (Koutchma et al. 2010). Very limited studies reported the impact of pressure-assisted thermal processing (where temperature levels are above 100 °C) on the properties of the packaging materials (Caner et al. 2000; Schauwecker et al. 2002; Bull et al. 2010; Juliano et al. 2010). Similarly, the changes in properties of pressure-assisted thermally processed packaging materials during extended storage and how these changes in packages influence product quality during storage have been poorly understood (Ayvaz et al. 2012).

In general, two different phenomena occur in the packaging materials exposed to high pressure processing: reversible and irreversible changes (Fig. 5.1). Reversible changes are commonly seen in the packaging materials and occur as a result of compression, particularly on the amorphous regions in the packaging material structure. This generally results in a decrease in permeability values of the polymer, and the packaging material remains intact, which even could be considered as advantageous. As of irreversible changes due to high pressure, visible damage and considerable increase in the permeability values can be observed. In other words, functionality and aesthetic appearance of the packaging material can be negatively affected. One of the possible explanations for the irreversible damage is that the gases are absorbed within the structure of the packaging material under pressure and quickly released upon decompression (Richter et al. 2010).

5.4.1 Gas Barrier Properties

Polymers have repeating functional groups in their structures, and these groups contribute to the formation of packed and highly ordered crystalline regions. As opposed to amorphous regions (void spaces) in the structure, atmospheric gases as well as organic compounds cannot penetrate through crystalline regions. Therefore, high crystallinity in a polymer can result in better barrier to oxygen, water vapor, carbon dioxide, and organic compounds. Additionally, increase in crystallinity in a polymer improves its strength and stiffness (Schauwecker et al. 2002). For instance, EVOH is known as a high oxygen barrier, and this property is

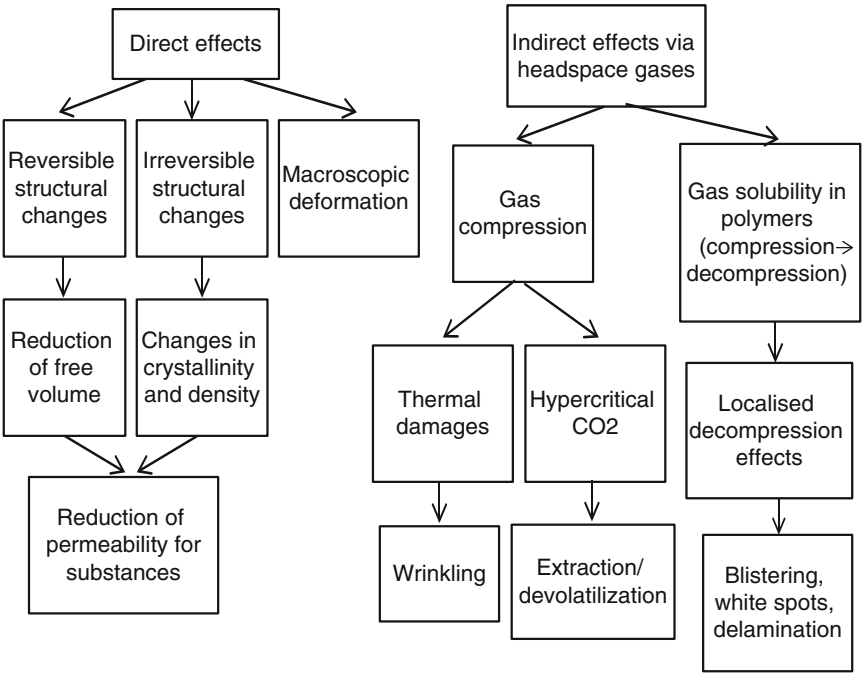

Fig. 5.1 Summary of the effects of high pressure on polymeric packaging materials (adapted from Richter et al. 2010)

a result of hydroxyl groups in its structure, which tend to make a high degree of hydrogen bonds and reduce the free volume between the chains in the polymer. This results in higher barrier to gas exchange. However, these hydroxyl groups also make the copolymers sensitive to water. That is why the barrier properties of EVOH are weakened in high relative humidity (Lopez-Rubio et al. 2005). To avoid this problem, EVOH is sandwiched between other high water-vapor barrier packaging materials in its applications. Additionally, there is a tendency for highly crystalline polymers to be more brittle which can be considered as a disadvantage (Kovarskii 1994). Some factors including the presence of nucleating materials, the rate of cooling, the molecular weight of the polymer, and the degree of agitation and alignment during the process can affect the crystallinity of a polymer or the size of an individual crystal (Jenkins and Harrington 1991).

5.4.2 Oxygen Permeability

The effect of combined pressure and heat treatment on the oxygen barrier properties of the packaging films differs greatly. Even though there are numerous research studies available about high pressure processing and oxygen transmission rates (OTRs)

of packaging materials, there is very limited research focusing on pressure-assisted thermal processing.

Earlier researchers documented that in general, high pressure processing did not increase the oxygen permeability of many tested packaging materials. Surprisingly, high pressure processing even decreased the oxygen permeation rates of some of the packaging films significantly. Masuda et al. (1992) reported no significant changes in barrier properties of tested OPP/EVOH/PE and PVDC-coated OPP/CPP at 400 and 600 MPa and maximum process temperature (T_{max}) of 40 °C for 10 min. On the other hand, Mertens (1993) applied 400 MPa and 60 °C for 30 min on LLDPE/EVA/EVOH/EVA/LLDPE and observed 15 % decrease in OTR. Similarly, Kovarskii (1994) evaluated the effect of HPP on PET and reported 70 and 25 % decreases in OTR and WVTR, respectively. Fradin et al. (1998) reported a slight reduction in the oxygen permeabilities of low-density polyethylene (LDPE)/ethylene-vinyl acetate (EVA)/ethylene-vinyl alcohol/EVA/LDPE and polyethylene terephthalate (PET)/aluminum (Al)/polypropylene (PP) after high pressure thawing of a model food at 200 MPa and 15–45 min holding time. Le-Bail et al. (2006) tested LDPE, which is the packaging material commonly used for HPP applications. They processed at 200, 400, and 600 MPa and 10 °C for 10 min and found that barrier properties were not significantly affected and even slightly improved (1 and 2 % decreases in OTR and WVTR, respectively). This could be due to high pressure compression that increased the stiffness due to volume reduction, which caused restrictions in macromolecular mobility and increase in microscopic ordering. In other words, increase in pressure improves the ordering of molecules of a material. Similarly, Lopez-Rubio et al. (2005) reported 2 and 7 % improvements in OTRs of PP/EVOH26/PP and PP/EVOH48/PP pouches in the pressure range of 400–800 MPa and 40 °C applied for 5–10 min, which was attributed to the increase in crystallinity of the structure caused by high pressure compression.

Some studies have indicated that there can be losses in barrier properties of flexible packaging materials. It was reported that metalized films were damaged by high pressure treatment and their barrier properties were impaired. Caner et al. (2000) processed water-filled PET/SiO_x/LDPE, PET/Al_2O_3/LDPE, PET/PVDC/Nylon/PE, Met-PET/EVA/LLDPE, PP/Nylon/PP, and PET/EVA/PET at 600 and 800 MPa and 45 °C for 5, 10, and 20 min. They found no statistical difference in barrier properties except for the Met-PET film, in which they observed that the oxygen permeation and water-vapor transmission rates (WVTRs) were almost doubled (95 % increase) and tripled (150 % increase), respectively. Lambert et al. (2000) processed different packaging materials and reported significant increase in OTR of PA 20 µm/adhesive 10 µm/PE 70 µm, which is similar to the result of a research conducted by Moueffak et al. (1997), who also found a 60 % increase in OTR of PE/PA/PE film after HPP (550 MPa at 55 and 85 °C for 20 min). In these films, the thickness of the polyamides was very low compared to the thickness of polyethylene layer, which minimized the contribution of the good gas barrier property of the polyamides. Galotto et al. (2008) reported a significant increase in OTR of metalized pouches due to the damage in the metalized surface coating. It could be explained by lower compression rate of metals

which can accelerate delamination of the packaging structures or discontinuities in the metal coating caused by the low mechanical resistance of the thin aluminum layer (Lopez-Rubio et al. 2005). Schauwecker et al. (2002) found that there were visible signs of delamination between the polypropylene (PP) and aluminum (Al) layers in the meals-ready-to-eat (MRE) pouches processed at ≥200 MPa and 90 °C for 10 min.

During PATP, the packaged products need to be preheated to a target initial temperature (typically 75-90 °C) prior to pressure-thermal treatment. Therefore, the preheating step can also be an important factor affecting the OTRs of the packaging films. Koutchma et al. (2010) applied PATP treatment (688 MPa and 121 °C) on scrambled egg patties packaged in four plastic-laminated materials (Nylon/co-extruded ethylene-vinyl alcohol, Nylon/polypropylene, polyethylene terephthalate/aluminum oxide/casted PP, and PET/polyethylene) and two aluminum foil-laminated pouches (PET/aluminum and Nylon/Al/PP). They observed an increase in oxygen permeability of the packaging materials and claimed that it was due to thermal damage occurred during the preheating of the packaged egg patties since the OTR values increased following preheating. According to the researchers, preheating caused 369 times increase in OTR of PET/AlOx/CPP pouch. Even though the PE pouch lost 40 % of its oxygen barrier property after preheating, the subsequent PATP treatment at 688 MPa and 121 °C led to recovery of the oxygen barrier property up to 200 %. They also reported that the magnitude of the increase in oxygen permeability in PATP-treated pouches was significantly less than that observed in traditional thermal treatment (121 °C for 3 min in steam retort). There were significant increases in the OTR values of the packaging materials tested, following the high pressure application which did not cause any further increase in OTRs except for PP. The reason for the further increase of OTR of PP was the structural damage occurred in PP pouches. Oxygen permeability of the foil-laminated pouches experienced less loss in oxygen barrier property compared to their thermally treated counterparts. Bull et al. (2010) processed 11 commercially available packaging materials used for thermal sterilization at 600 MPa and 110 °C for 5–10 min and reported that barrier properties of vapor-deposited oxide and Nylon containing films were negatively affected whereas barrier properties of aluminum foil and PVDC-MA containing films survived the PATP treatment well. They observed that regardless of the temperature of the product inside the pouch or the length of holding time of the process, high-barrier laminates with both SiOx and AlOx coatings were severely damaged by the process and lost their barrier properties. This observation was in agreement with that reported by Galotto et al. (2008) and Galotto et al. (2010), who found a complete destruction of the SiOx vapor-deposited barrier layer in PPSiOx, processed at 400 MPa and 60 °C for 30 min. Galotto et al. (2010) evaluated the effect of high pressure treatment (400 MPa and 20 and 60 °C for 30 min) on OTRs of four different flexible packaging materials (PE/EVOH/PE, metalized polyester/polyethylene, PET/PE, and PPSiOx). They observed significant increases in OTRs of packaging films tested except PET/PE film, which experienced almost no change. They reported delamination in metalized polyester/polyethylene. Additionally, water sensitivity and swelling of EVOH in PE/EVOH/PE caused partial loss of their barrier properties, resulting in significant

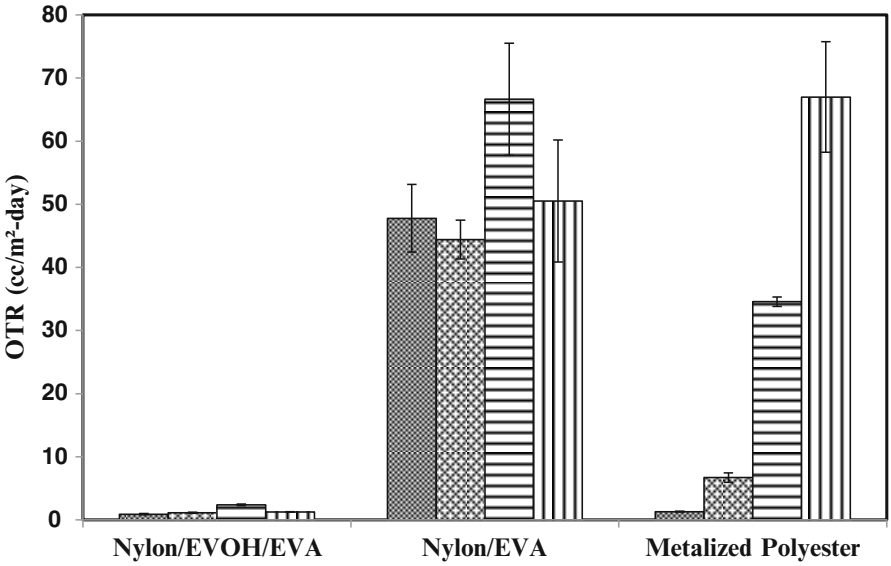

Fig. 5.2 Oxygen transmission rates of control and PATP-treated films stored for 4 weeks at 25 and 37 °C ( ), untreated control; ( ), preheated only; ( ), PATP and stored at 25 °C for 4 weeks; ( ), PATP and stored at 37 °C for 4 weeks (reprinted from Ayvaz et al. 2012, with permission from Elsevier Ltd.)

increase in OTR. Similarly, according to Bull et al. (2010), aluminum barrier layer showed great compatibility with the PATP processing and OTRs of the materials tested experienced the lowest oxygen barrier before and after PATP. This result was not consistent with findings reported by Ayvaz et al. (2012) who processed the Met-PET/PE pouch at 600 MPa and 110 °C for 10 min and observed dramatic increase in oxygen permeability values (Fig. 5.2). Ayvaz et al. (2012) processed baby carrots in three different pouches (Nylon/EVOH/EVA, Nylon/EVA, and Met-PET/PE) at 600 MPa and 110 °C for 10 min and stored the pouches in the dark at 25 and 37 °C for 4 weeks. As shown in Fig. 5.2, the authors observed significant increase in OTR values for Nylon/EVA and Met-PET/PE while high-barrier packaging material Nylon/EVOH/EVA had minimal impact. Moreover, even preheating the Met-PET/PE pouch without any pressure application caused significant increase in OTR of Met-PET/PE. They also reported that change in barrier properties of packaging materials (particularly change in oxygen transmission rates) accelerated the color change and β-carotene degradation of baby carrots processed during extended storage. In other words, packaging type had significant effect in color and β-carotene content of pressure-assisted thermally processed carrots.

5.4.3 Water-Vapor Permeability

Effect of HPP on WVTRs of the packaging materials shows similarity to the changes in OTRs. Several research studies showed that high pressure processing at ambient or chilled conditions did not cause any significant changes in WVTRs of the tested packaging materials. Additionally, high pressure processing even decreased the WVTRs of some of the packaging films remarkably. Masuda et al. (1992) reported no significant changes in water-vapor transmission rates (WVTRs) of the polyvinylidene chloride (PVDC)-coated oriented polypropylene (OPP) and polyethylene terephthalate (PET)/aluminum (Al)/cast polypropylene (CPP) by high pressure in the range of 400–600 MPa at process temperatures of up to 40 °C. Similarly, Halim et al. (2009) processed several co-extruded packaging materials at 800 MPa and 70 °C for 10 min. They reported no significant differences in WVTRs of any tested co-extruded films including Nylon 6/EVOH caused by processing. Mertens (1993) applied 400 MPa pressure at 60 °C on LLDPE/EVA/EVOH/EVA/LLDPE and PET/Al/PP for 30 min and observed 5 and 25 % reduction in WVTRs, respectively. Fradin et al. (1998) processed LDPE/EVA/EVOH/EVA/LDPE and PET/Al/PP laminates at 200 MPa for 15 and 45 min holding time and determined a slight decrease in water-vapor permeability.

Caner et al. (2000) evaluated the effect of HPP (up to 800 MPa and maximum 50 °C) on eight high-barrier laminated films and observed up to 150 % increase in water transmission for PETmet/EVA/LLDPE. Similarly, Galotto et al. (2010) evaluated the effect of high pressure treatment (400 MPa and 20 and 60 °C for 30 min) on WVTRs of four different flexible packaging materials (PE/EVOH/PE, metalized polyester/polyethylene, PET/PE, and PPSiOx). They observed significant increases in WVTRs of packaging films tested except PET/PE film, which experienced almost no change. They reported delamination in metalized polyester/polyethylene and water sensitivity, and swelling of EVOH in PE/EVOH/PE caused partial loss of their barrier properties, resulting in significant increases in WVTRs. Additionally, PPSiOx that was seriously damaged by a drastic increase in WVTR was observed. Bull et al. (2010) studied the effects of PATP (600 MPa and 110 °C for 5–10 min) on 11 commercially available packaging materials and reported that the foil laminates gave the lowest WVTRs of all the films examined. Additionally, although the laminates containing AlOx and SiOx had low WVTRs before processing, they were damaged by the PATP treatment, and the packaging films almost completely lost their barrier properties. Ayvaz et al. (2012) evaluated the effect of PATP (600 MPa and 110 °C for 10 min) and storage conditions (4 weeks at 25 and 37 °C) on Nylon/EVOH/EVA, Nylon/EVA, and Met-PET/PE and reported a significant increase in WVTR of the Met-PET/PE pouch by preheating (15 min in boiling water). Similarly, Caner et al. (2000) also reported that WVTR of Met-PET was significantly affected by pressure application. According to Ayvaz et al. (2012), Nylon/EVOH/EVA and Nylon/EVA, were not affected significantly in terms of WVTRs. Additionally, PATP treatment followed by 4-week storage at either 25 or 37 °C did not further increase the WVTRs of any pouches significantly.

5.4.4 Mechanical Strength

Mertens et al. (1993) reported no changes in mechanical properties, including tensile strength and elongation of LLDPE/EVA/EVOH/EVA/LLDPE and PET/Al foil/PP films by high pressure application at 400 MPa and 60 °C for 30 min. Similarly, according to the research conducted by Caner et al. (2003), no major impact of pressure alone was found in tensile strength, elongation, and modulus of elasticity for the tested packaging films (PET/SiO_x/LDPE, PET/Al_2O_3/LDPE, PET/PVDC/Nylon/HDPE/PP, PE/Nylon/EVOH/PE, PE/Nylon/PE, metallized PET/EVA/LLDPE, PP/Nylon/PP, and PET/PVDC/EVA). Lopez-Rubio et al. (2005) observed no damage on the structure of EVOH processed at 400–800 MPa and process temperatures of 40 and 75 °C for 5–10 min and reported some improvements in the crystallinity of EVOH. Similarly, Le-Bail et al. (2006) also conducted a research on the effect of HPP (200, 400, and 600 MPa and 10 °C for 10 min) on the mechanical properties of PA-PE, PET/BOA/PE, PET/PVDC/PE, PA/SY, LDPE, and EVA/PE and reported no significant effects as a result of HPP.

However, there can be some increase in the tensile strength of the packages after high pressure processing irrespective of the pressure level, the initial rigidity, and the thickness of the package. This shows that the package becomes more rigid and less flexible (Lambert et al. 2000). For instance, Lambert et al. (2000) reported that processing at 200, 350, and 500 MPa and 20 °C for 30 min resulted in an increase of tensile strength and rigidity for five out of six polymers tested (PA/EVOH/PE, PA/LDPE, PA/LDPE, PET/PVDC/LDPE, PE/PE, PA/PP/LDPE). Only PA/PP/PE film experienced a decrease in strength at 200 and 500 MPa.

5.4.5 Migration and Sorption

In general, three different mass transfer processes need to be emphasized.

- First, the *permeation* of the gases present in the environment such as oxygen, water vapor, and so forth. These gases can permeate from the environment into the packaged food.
- Second, the *migration* or movement of the low molecular weight substances including solvents, plasticizers, antioxidants, and monomers from the packaging material into the food. The migrated substances also have the potential to create some by-products through degradation reactions. This migration needs to be controlled since the substances may be toxic for humans and the environment. There are global and specific migrations and they can be measured. In order to measure the global migration, weight differences between the evaporated extracts of the processed and unprocessed samples are determined, whereas further instrumental analysis is required for specific migration measurement (Schauwecker et al. 2002).

- The third and the last mass transfer which can occur is the scalping of low molecular weight substances such as aroma compounds from food. Scalping of aroma compounds can alter the flavor and compromise the quality of food (Guillard et al. 2010; Mauricio-Iglesias et al. 2011).

Mertens (1993) used olive oil as food stimulant and measured the global migration for PET/Al/PP and LLDPE/EVA/EVOH/EVA/LLDPE at atmospheric and high pressure (400 MPa). The results showed a decrease in migration of olive oil under 400 MPa for PET laminate, while a considerable increase was observed under high pressure for LLDPE laminate. Pastorelli (1997) processed PET/Al/PP and PE/EVOH/PE at 400 MPa and 25 °C for 30 min and observed no significant overall migration or by-products of substances from the packages. Similarly, Lambert et al. (2000) tested different packaging materials including PA/EVOH/PE, PA/LDPE, PET/PVDC/LDPE, PE/PE, and PA/PP/LDPE after processing at 200–500 MPa and room temperature for 30 min. Authors reported insignificant amount of overall migration. Galotto et al. (2010) evaluated the effect of high pressure treatment (400 MPa and 20 and 60 °C for 30 min) on total migration of four different flexible packaging materials (PE/EVOH/PE, metallized polyester/polyethylene, PET/PE, and PPSiOx) into distilled water and olive oil. They found that total migration from high pressure-treated packages was lower than that of controls due to more compressed structure and higher degree of crystallinity caused by high pressure treatment. On the other hand, total migration from high pressure-treated packaging materials into olive oil was higher than that of controls. The authors mentioned that oil could be absorbed by packaging material and acted as a plasticizer causing changes in the structures of packaging materials such as swelling, pinholes, and delamination. Additionally, total migration values were lower at higher processing temperature (60 °C as opposed 20 °C) due to recrystallization and therefore higher crystallinity at high pressure and high temperature. In terms of specific migration, Caner and Harte (2005) reported no considerable changes caused by Irganox 1076 migration from polypropylene flexible structures by HPP at 800 MPa and 60 °C for 10 min.

Pressure-transmitting fluids such as glycol, castor oil, and silicone oil are used in HPP systems for the purpose of transmitting the pressure to the packaged product. Therefore, the fluid gets in contact with the package. Schauwecker et al. (2002) evaluated the migration of 1,2-propanediol (PG) into EVOH and MRE pouches treated by HPP (400, 600, and 827 MPa at 30, 50, and 70 °C for 10 min) and observed no detectable migration. It could be due to the high pressure induced reduction of the size of the micro-channels within the pouches.

Very limited studies investigated the impact of pressure treatment on aroma barrier properties of packaging material. Kuebel et al. (1996) found that HPP had no significant effect on the sorption of p-cymene and acetophenone into tested packaging materials (LDPE/HDPE, HDPE, and PET/Al/LDPE). In situ measurement of p-cymene, which had a very low polarity during HPP (50 MPa and 25 min) in LDPE/HDPE/LDPE and PET/Al/LDPE, was conducted by Götz and Weisser (2002). Similar to some other works in the area, they reported a slight decrease in the permeation rate of the p-cymene versus increasing pressure levels during processing due to the structural change in the packaging materials used.

Caner et al. (2004) treated model food containing D-limonene with HPP (800 MPa and 60 °C for 10 min) in PP and PE/Nylon/EVOH/PE films and observed no significant change in the sorption of D-limonene by the process. On the other hand, the Met-PET/EVA/LLDPE experienced less D-limonene sorption after the treatment.

It appears that during compression by high pressure, the polymer matrix loses its capacity to absorb compounds from food or the surrounding media due to a decreased free volume. As the pressure is released, the polymer quickly recovers its original dimensions, and thus sorption and diffusion processes proceed as expected at normal atmospheric pressure. Therefore, permeation and sorption values are statistically the same as for the non-compressed samples.

Rivas-Canedo et al. (2009) evaluated the effect of high pressure processing (400 MPa and 12 °C for 10 min) and packaging on volatile profile of minced beef and chicken breast. In terms of packaging, they compared wrapping the food with aluminum foil and subsequent vacuum packaging in multilayer package (LDPE/EVA layers/VDC (vinylidene chloride)) to only vacuum packaging in multilayer package with no initial aluminum wrap. They reported that upon high pressure processing, volatile profile of minced beef and chicken breast changed significantly and migration was the main phenomenon. Migration increased with increasing fat content since packaging material was more lipophilic, and therefore minced beef which had higher fat than chicken experienced more migration. According to the authors, wrapping the food with aluminum foil helped maintaining flavor profile better. Mauricio-Iglesias et al. (2011) studied the effect of high pressure pasteurization (800 MPa and 40 °C) and pressure-assisted thermal processing (800 MPa and 115 °C) on scalping of four aroma compounds (2-hexanone, ethyl butanoate, ethyl hexanoate, D-limonene) by LDPE and PLA (polylactate). They reported that tested aroma compounds were quite stable after pasteurization treatment whereas significant losses of aroma compounds were observed after sterilization treatment. They concluded that both LDPE and PLA were suitable for high pressure pasteurization but not for sterilization conditions. Especially for PLA, temperature becomes very critical if the temperature of the processing exceeds the glass transition temperature of PLA.

5.4.6 Packaging Integrity

Any damage or alteration in the packaging materials can potentially cause the loss of hermeticity, and therefore the quality, safety, and shelf life of the product can be adversely affected. According to Fradin et al. (1998), the presence of air and the volume of headspace could be a reason for delamination in HPP-treated packages. The authors conducted an experiment in which they processed vacuum-packaged pouches filled with water at 200 MPa and 25 °C for 45 min. They observed no delamination for any pouches without headspace. However, delamination was found in the regions close to the air bubbles in the material (on the top end of the pouches, close to seams) in the presence of less than 10 % headspace. Headspace in the

packaged product should be controlled in order to avoid any potential delamination, seal damage, flexing, cracking, and deformation (Schauwecker et al. 2002).

Götz and Weisser (2002) applied 500 MPa pressure for 5 min on PA/PE film at room temperature and observed delamination in the package. They explained it as a result of elasticity difference between the layers similar to the findings reported by Caner et al. (2003) who utilized scanning electron microscopy and ultrasonic imaging and also attributed the delamination in the pressure-treated (600 and 800 MPa, 45 °C for 5–20 min) Met-PET/EVA/LLDPE to the differences in elasticity and compressibility of the metallized and polymeric layers. Schauwecker et al. (2002) tested PE/Nylon/Al/PP and Nylon/EVOH/PE laminates filled with water after processing at 200 and 690 MPa and approximately 100 °C for 10 min. They observed that delamination occurred between PP and aluminum layer at processes above 90 °C while no delamination was observed when temperature levels decreased to 85 °C or processing was conducted at atmospheric pressure. Galotto et al. (2008) processed four different pouches at 400 MPa and 60 °C for 30 min and observed delamination and wrinkling except in PE containing pouches (PE/EVOH/PE and Met-PET/PE). On the other hand, Bull et al. (2010) processed eleven commercially available packaging materials used for thermal sterilization at 600 MPa and 110 °C for 5–10 min and also reported delamination and deformation. The authors explained the effect as a result of solubilization of the gases into the packaging film during compression and quick release of the gases upon decompression. They further explained that the concentration of the air in the headspace and in the gap of the laminate from manufacturing becomes denser due to the high pressure applied and dissolves into the polymer layers more easily. Then, once the depressurization step is reached, the dissolved gases are released quickly forcing the layers to separate. This resulted in delamination. Moreover, authors proposed that degree of solubility of the gases located in the material may be a factor affecting the magnitude of delamination. Similar to this, Fairclough and Conti (2009) also mentioned that upon depressurization of solubilized gases in the PP films processed at 695 MPa and 86 °C for 10 min, opaque areas and bubbles are formed.

Lopez-Rubio et al. (2005) processed EVOH-based packaging materials at 800 MPa and 75.8 °C as well as in the retort at 121 °C. They found that as opposed to the severe damage occurred in thermally treated packaging materials, high pressure processed packages were minimally damaged. Similarly, regarding the overall package integrity, Koutchma et al. (2010) reported that the pouches (four plastic-laminated materials and two aluminum foil-laminated pouches) processed at 688 MPa and 121 °C showed no leakage or severe damage except some blisters and ruptures formed on the outer walls of the metal layers. Good understanding of the delamination and reasons behind it such as headspace volume can be useful to avoid this problem and develop better packaging materials suitable for high pressure applications (Bull et al. 2010).

In several research studies, further analyses were conducted to better evaluate the damage on the surface or in the structure of packaging material. Caner et al. (2003), Galotto et al. (2008), and Ayvaz et al. (2012) utilized scanning electron microscopy (SEM) to locate damage on the surface of high pressure processed

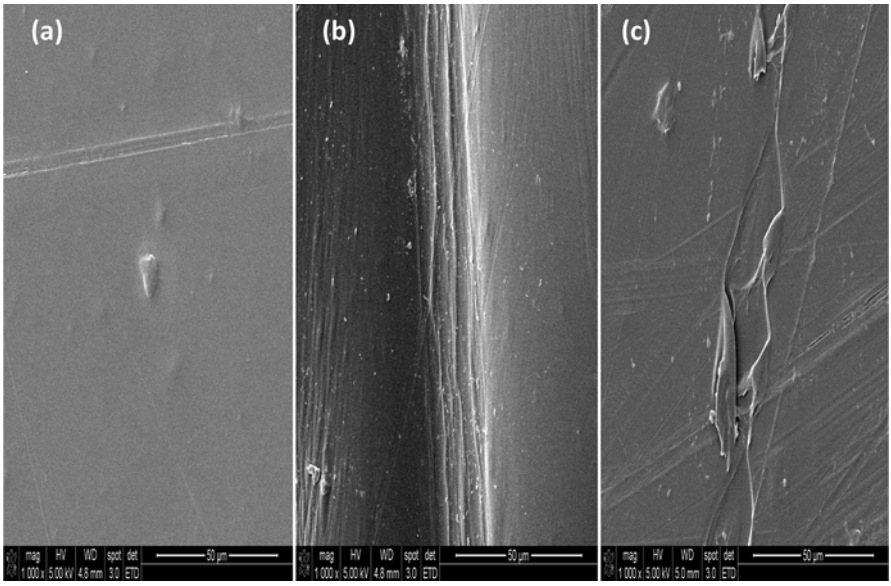

Fig. 5.3 Scanning electron micrographs of Met-PET/PE pouch illustrating the damage caused by PATP treatment: (**a**) control; (**b** and **c**) PATP-treated (magnification ×1000) (reprinted from Ayvaz et al. 2012, with permission from Elsevier Ltd.)

films. They observed the delamination in tested metalized polymers. Figure 5.3 shows how the pressure-assisted thermal processing (600 MPa, 110 °C for 10 min) affected the Met-PET/PE packaging material in research conducted by Ayvaz et al. (2012).

In the same study, pinholes created in pressure-assisted thermally processed Nylon/EVA package and effect of storage for 12 weeks at 25 °C can be seen in Fig. 5.4.

In some other research studies, differential scanning calorimeter (DSC) was utilized to determine the effect of high pressure and pressure-assisted thermal processing on thermal transition characteristics of different packaging materials (Schauwecker et al. 2002; Lopez-Rubio et al. 2005; Galotto et al. 2008; Ayvaz et al. 2012). T_m (melting temperature) and ΔH (heat of fusion) of polymers were calculated from DSC thermograms to support the change in structure of polymers after high pressure applications compared to control samples.

5.4.7 Seal Strength

For high pressure applications of flexible packages, sealing is an important step. Package needs to be sealed properly prior to high pressure applications so that it can survive the process maintaining the packaging integrity with no leakage (Lambert et al. 2000).

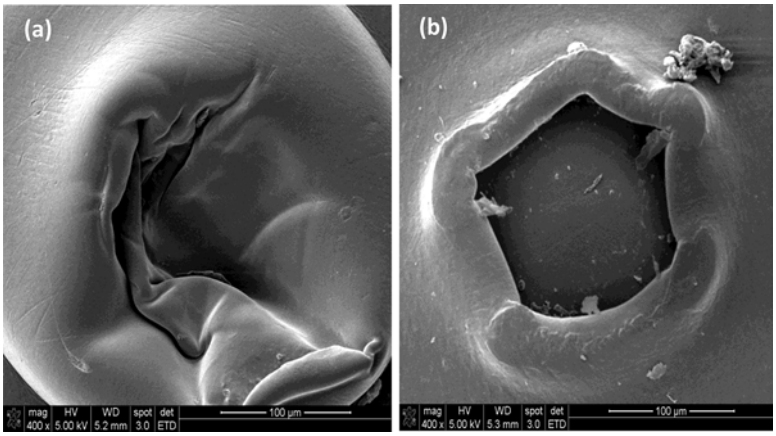

Fig. 5.4 Scanning electron micrographs of Nylon/EVA pouch illustrating the pinholes: (**a**) PATP-treated only with no storage; (**b**) PATP-treated and stored at 25 °C for 12 weeks (magnification ×400) (reprinted from Ayvaz et al. 2012, with permission from Elsevier Ltd.)

Lambert et al. (2000) processed six different types of plastic package. The packages were multilayer (PA/PE) with different thickness, permeability, and stress at yield point and at breakage. They processed at 200–500 MPa and 20 °C for 30 min and found that seal strength of the pouches was not significantly altered by the process except the heat-seal strength of one of the six pouches decreased more than 25 % compared to its initial strength before high pressure processing. Similarly, Dobias et al. (2004) applied 600 MPa pressure at 20 °C on some plastic-laminated films including PA/PE and PE/EVOH/PE and reported that seal strength of the laminates was not affected by high pressure application.

Koutchma et al. (2010) showed that pressure-assisted thermal processing (688 MPa and 121 °C for 3 min) did not affect the seal strength of Nylon/co-extruded ethylene-vinyl alcohol, Nylon/polypropylene, polyethylene terephthalate/aluminum oxide/casted PP, and PET/polyethylene pouches, but increased the seal strength of the two aluminum foil-laminated pouches (PET/aluminum and Nylon/Al/PP). Generally, PATP-treated pouches experienced increased seal strengths compared to traditional thermal treatments. The authors could not find any relationship between the vacuum levels in the pouches and the seal strengths and concluded that pressure-assister thermal processing does not alter the seal strength of plastic-laminated pouches significantly.

Patazca et al. (2013) studied the effect of packaging for inactivation of *Clostridium botulinum* spores by using PATP (750 MPa at 105 °C). Inactivation of spores in plastic pouches, cryovials, and transfer pipettes was compared in laboratory-scale and pilot-scale pressure systems. Approximately 6.6-log reduction of the spores packaged transfer pipettes was obtained after processing for up to 10 min at 118 °C and 700 MPa in both pressure test systems. Reduction of spores packaged in plastic pouches was the lowest (~4.8 log) for both pressure test systems when processed at

the same conditions. It was concluded that the use of high-barrier plastic pouches for packaging of spores and subsequent high pressure processing can result in inaccurate spore inactivation data compared with other packaging systems studied. Since the type of flexible packaging systems might affect inactivation results, additional studies are needed to determine the cause of the packaging effect that was observed in this study.

5.5 Future Research Needs in Pressure Treatment of Packages

Food industry is exploring the concept of nanotechnology applications in food packaging, and it is expected that nanocomposite packages will become more commonly used in the food industry in the near future. Nanotechnology provides the ability to food processors to engineer the structure of the materials at a molecular scale. It may be possible to design packaging material with desired functionalities.

Nanoparticles are defined as particles which are less than 1 nm in at least one dimension; however, other side dimensions can be larger, which can provide high aspect ratios. When used in packaging materials, they provide highly desired exceptional properties (Brody et al. 2008) such as improved barrier, mechanical, and thermal performances as well as extended shelf life of food products (Sorrentino et al. 2007). For instance, montmorillonite clay increases the gas barrier properties of a polymer by simply extending the length of the tortuous path that gases need to pass through (Halim et al. 2009).

According to some modeling approaches, significantly higher barrier properties (up to 50 times higher or even more) can be accomplished by utilizing the high aspect ratio particles. Generally, the difficulty with the nanocomposite packaging is how well the filler is dispersed in the matrix (Lange and Wyser 2003). Another important point involving the usage of nanoparticles in food applications is that nanomaterials need to keep their beneficial properties after different processing conditions, including high pressure levels, elevated temperatures, and so forth (Halim et al. 2009). As for high pressure processing, very limited studies are available on the impact of combined pressure-thermal treatment on nanocomposite packaging materials.

Antimicrobial packaging is another promising tool for controlling the growth of food-borne pathogens or spoilage bacteria in ready-to-eat (RTE) post-processed products. Antimicrobials are impregnated into food packaging to protect the package or to extend the shelf life of the contained food. Known antimicrobials include organic acids and their salts, sulfites, nitrites, antibiotics, alcohols, enzymes, and natural components such as bacteriocins, especially nisin. Nisin is currently the only bacteriocin allowed to be used as a food additive. Enterocins A and B and sakacin K are bacteriocins whose antilisterial activity has been shown in a meat homogenate and have been applied experimentally as ingredients in

several meat products. All uses of antimicrobial substances in or on food-packaging materials are considered food additive uses regardless of their intention. Jofré et al. (2008) studied the effectiveness of the application of interleavers containing enterocins A and B, sakacin K, nisin A, potassium lactate, and nisin plus lactate alone or in combination with a 400 MPa in sliced cooked ham spiked with *Salmonella* spp. It was reported that antimicrobial packaging, HPP, and refrigerated storage appear as an effective combination of hurdles to obtain value-added, ready-to-eat products for 3 months of storage at 6 °C. On the other hand, Marcos et al. (2013) evaluated the combined effect of antimicrobial packaging (polyvinyl alcohol with nisin added) and high pressure processing (at 600 MPa and 12 °C for 5 min) as a post-process treatment on *L. monocytogenes*-inoculated fermented sausages with no added sodium salt. They found that HPP did not contribute any protection against *L. monocytogenes* compared to antimicrobial packaging alone. The authors attributed this to possible protective effect by low water activity and lactate content of the fermented sausages.

5.5.1 Safety of Packaging Materials

Due to the novelty of HPP, food manufacturers may request that their packaging materials providers obtain a letter of no objection regarding the acceptability of their materials for HPP treatment from regulatory agencies such as US FDA or Health Canada. Information on the regulation and pre-market assessment of packaging materials can be found on US FDA and Health Canada's Packaging Materials webpage. In addition, a complete list of all packaging materials accepted by the Canadian Food Inspection Agency (CFIA) including those acceptable for HPP treatment is available on Health Canada and CFIA's website (http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/polymers_tc-polymere_tm-eng.php).

5.6 Conclusions

Pressure treatment has the potential to alter the gas and moisture barrier properties of packaging material. This in turn influence the quality of the packaged product during the extended storage. High pressure pasteurization treatment at ambient or chilled conditions generally do not have adverse effect on properties of the packaging materials commonly used in food industry. However, if the adhesion between the layers of multilayer structure of the packaging film is affected by processing, gaps may appear within the structure resulting in loss of integrity and therefore the safety and quality problems of the packaged food.

More research is necessary to identify suitable polymer packaging material with high barrier properties that can withstand severe treatment conditions during PATP.

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Part III
High Pressure Process Engineering Characterization

Chapter 6
In Situ Thermal, Volumetric and Electrical Properties of Food Matrices Under Elevated Pressure and the Techniques Employed to Measure Them

Sung Hee Park, Loc Thai Nguyen, Stephen Min, V.M. Balasubramaniam, and Sudhir K. Sastry

Abstract This chapter summarizes research efforts in the experimentation and measurement of various in situ thermophysical properties of food and packaging material subjected to combined pressure-thermal treatment. The properties investigated include heat of compression, compressibility, thermal conductivity, specific heat, thermal diffusivity, electrical conductivity, and reaction volume. This information will help to understand the extent of process nonuniformity during pressure treatment, characterize chemical and physical changes, as well as assess the safety and quality of products and processes.

Keywords In-situ properties • Heat of compression • High pressure • Thermal properties • Sensor development

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Nomenclature

A	Area of the intensifier piston
a	Regression model parameter
CH	Heat of compression (°C/100 MPa)
C_p	Specific heat (kJ/kg · K or J/kg · K)
I	Current (A)
k	Thermal conductivity (W/m · °C)
k_e	Cell constant (m^{-1})
P	Pressure (MPa)
Q	Heat generation (W/m)
r	The distance between line heat and second thermocouple probe
T	Temperature (K or °C)
t	Time (s)
V	Voltage (V)
α	Thermal diffusivity (m^2/s)
β	Thermal expansivity (K^{-1})
ρ	Density (kg/m^3)
σ	Electrical conductivity (S/m)
ΔT	Temperature difference (ºC)

Subscript

0-3	Empirical model parameter position of the intensifier piston
1	Start time of heating
2	End time of heating
atm	Atmospheric pressure (0.1 MPa)
bf	Beef
d	Duration of heat pulse
e	End time
H	Heat exchange with the surroundings
i	Initial
m	Time from the start of heating to the temperature reaching maximum
p	Pressure
s	Start time
wt	Water

6.1 Introduction

There has been a recent increase in research evaluating the in situ properties of food matrices under high pressure. In theory, hydrostatic pressure is uniformly and instantaneously applied to food samples, irrespective of the geometry exposed.

Isostatic principles bestow high-pressure processing (HPP) with certain advantages over thermal processing; however, it does not completely eliminate the classical limitations imposed by heat transfer (Denys et al. 2000b). Due to the differences in heat of compression of different food components (e.g., lipids, proteins, etc.) and the differences in the physical heat-transfer boundary conditions, the temperature of food samples processed inside a pressure vessel may vary spatially. Knowledge of the appropriate thermal and physical properties of the processed material during HPP is required in order to effectively analyze, design, and operate the systems (Denys and Hendrickx 1999). Familiarity with in situ properties of various foods under pressure is essential to evaluate the extent of nonuniformity of the treatment and to develop the appropriate kinetics models for microbial and enzymatic inactivation. Developing sensors for in situ measurement of food properties under high pressure is challenging. They should be (1) pressure tolerant (up to 700 MPa), (2) precise and accurate, (3) versatile in terms of measurement of relevant properties in both liquid and solid foods and (4) operation is not interfered by compression media (Min et al. 2009). The current review summarizes various in situ thermal, volumetric, and electrical properties of food matrices during HPP and in situ measurement techniques employed to measure such properties.

6.2 Installation of Thermocouple and Electrical Wires into High-Pressure Vessel

In situ thermal, volumetric or electrical food property measurement under pressure requires custom made instrumented pressure vessels that have provisions for passing wires (or transmitting signals) of thermocouple, power, voltage or current through the walls of the pressure vessel. Diamond anvil type pressure vessels may be useful for the measurement of optical based properties. Whenever estimating the in situ properties of food matrices under pressure using special probes, it important to calibrate the probes. Typically probe specific calibration factors can be obtained by comparing experimental data against those published by standard sources (e.g., NIST data for properties of water). K-type thermocouples have proven to be reliable up to 700 MPa and 100 °C, with less than 2 °C deviations (Bundy 1961). Electrical wire feedthrough is also essential to supply electric power for in situ electrical property studies. One method for such wiring in high-pressure vessels is to drill a hole right through the Bridgman-type metal cone (beryllium copper, stainless steel, mild steel, maraging steel) and silver-solder a continuous thermocouple through the axis of the cone. This silver-soldered cone can subsequently pass through the conical cavity of the high-pressure vessel closure (Fig. 6.1., Sherman and Stadtmuller 1987).

The surface of the metal cone must then be electrically insulated to prevent direct contact between the high-pressure thermocouple cone and vessel enclosure. Kynar heat-shrink tubing has proven to be effective for insulating the conical feedthrough, up to 1000 MPa at 25 °C (Terry and Ruoff 1972). For a high-temperature application, mineral-insulated (MI) cable can be used as the high-pressure feedthrough wires. MI cable is a variety of conductor cables (e.g., thermocouple or power supply

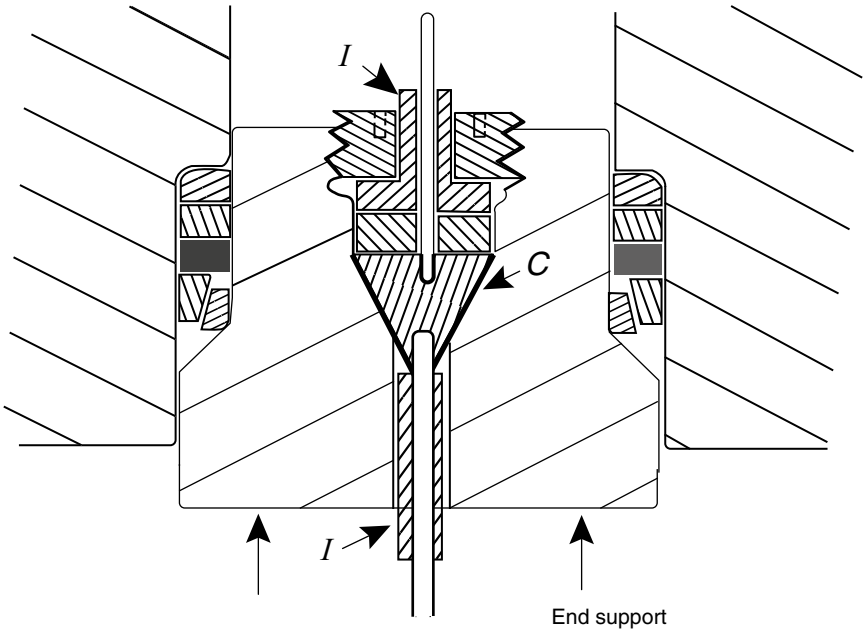

Fig. 6.1 The Bridgman cone-type electrical lead-through. *C* indicates a thin conical shell of pipestone and *I* indicate electrical insulation (from Sherman and Stadtmuller 1987)

copper wire) insulated internally by inorganic magnesium oxide powder (Miller et al. 2009) inside a metal sheath. Therefore, MI cable can be directly silver-soldered onto metal cones without further insulation. Figure 6.2 describes high-pressure feedthrough wires prepared using techniques mentioned above.

6.3 Thermal Properties: Heat of Compression, Thermal Conductivity, Thermal Diffusivity, and Specific Heat

6.3.1 Heat of Compression

All compressible materials adiabatically change temperature during physical compression, depending on their compressibility and specific heat (Balasubramaniam et al. 2004; Houška et al. 2004; Patazca et al. 2007; Ting et al. 2002). During high-pressure processing, the material is compressed by 15–20 % under pressure. This physical compression transiently increases product temperature while the product is under pressure. Upon depressurization, the product gives back the energy gained during compression, resulting in a temperature drop.

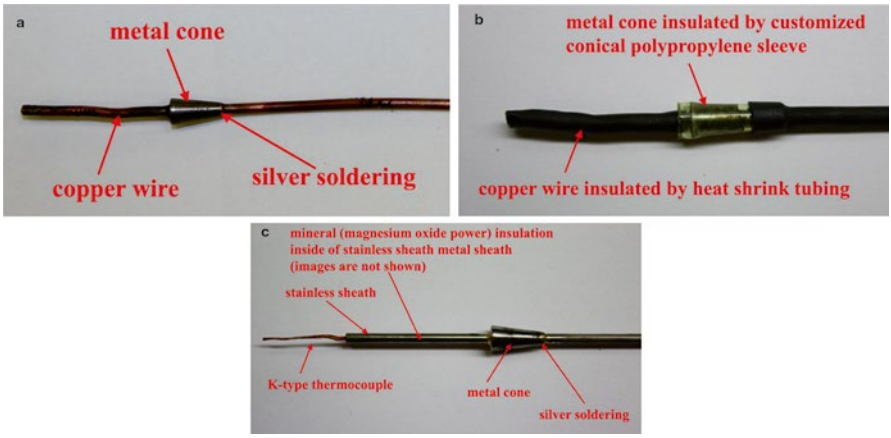

Fig. 6.2 Description of high-pressure feed through wires and their insulation: (**a**) high-pressure electrical feedthrough wire silvered-soldered on the metal cone (non-insulation), (**b**) high-pressure electrical feedthrough wire insulated by heat shrink tubing and customized conical sleeve, (**c**) mineral-insulated (MI) thermocouple

The heat of compression values of the material depends upon the product initial temperature ($T_{i,}$ °K), thermal expansivity (β_p, K^{-1}) at pressure, specific heat (C_p, J/kg · K) at pressure (Pa), and density (ρ_p, kg/m^3) at pressure. The temperature increase due to physical compression can be estimated using the following relationship:

$$\frac{dT}{dP} = \frac{T_i \cdot \beta_p}{Cp_p \cdot \rho_p} \tag{6.1}$$

Under compression, the internal energy of the system increases rapidly as the material is compressed resulting in temperature increase (Rasanayagam et al. 2003; Otero et al. 2000). Due to the challenges in estimating properties of food material in situ under pressure, very limited information is available under such experimental conditions. Earlier researchers estimated the heat of compression values experimentally by monitoring temperature changes in the substance during pressure buildup or decompression (Otero et al. 2000; Rasanayagam et al. 2003; Patazca et al. 2007). Table 6.1 lists heat of compression values of selected food and packaging materials.

Heat of compression values of high-moisture foods (such as fruit juice and milk) are very similar to that of water, 3 °C per 100 MPa at 25 °C (Rasanayagam et al. 2003), whereas vegetable oils and beef fat showed higher heat of compression values due to their higher compressibility with long-chain unsaturated fatty acids and lower specific heat (Rasanayagam et al. 2003). While heat of compression of water and high-moisture-content foods generally increases with increasing initial product temperature, heat of compression of fatty materials does not vary as a function of initial temperature. The differences in the thermal response of water, fats, and oils can be attributed to their molecular structure and phase transition characteristics

Table 6.1 Heat of compression (°C/100 MPa) for selected foods and packaging material at initial temperature of 25 °C

Substances	Temperature increase (°C) per 100 MPa
Water[a]	2.9
Salmon fish[a]	3.0
Cream cheese[b]	4.7
Egg yolk[b]	4.3
Egg white[b]	2.8
Whole Egg[b]	3.3
Hass avocado[b]	3.7
Whole milk[b]	3.2
Skim milk[b]	3.0
Honey[b]	2.9
Extracted beef fat[a]	6.3
Linoleic acid[a]	5.9
Acetic acid[c]	11.4
Propionic acid[c]	6.7
Soybean oil[a]	6.3
Olive oil[a]	7.2
Propylene glycol[a]	5.1
Ethanol[a]	6.8
Polypropylene[d]	4.0

[a]Rasanayagam et al. 2003
[b]Patazca et al. 2007
[c]Ramaswamy and Balasubramaniam 2007
[d]Schauwecker et al. 2002

(Nguyen and Balasubramaniam 2011). Water molecules are compact, polar, and more closely packed than the fat molecules by virtue of hydrogen bonds; thus, water has lower heat of compression than oil and fat (Patazca et al. 2007). In comparison, saturated fatty acids have lower compression heating than unsaturated fatty acids because saturated long-chain fats are more closely packed than unsaturated long-chain fats (Rasanayagam et al. 2003).

Knoerzer et al. (2010) and Schauwecker et al. (2002) investigated the heat of compression characteristics of food packaging materials such as high-density polyethylene (HDPE), polypropylene (PP), and polytetrafluoroethylene (PTFE) during high-pressure processing up to 750 MPa. The authors reported that at temperatures around 20 °C and pressures below 50 MPa, the compression heating coefficients of HDPE are approximately 40 times higher than those of water. This high heat of compression values at low pressure would be attributed to high thermal expansion coefficient values and by the weaker van der Waals forces of the macromolecules, than that of significantly stronger hydrogen bonds in water (Knoerzer et al. 2010).

Knowing the heat of compression of the food material can help to estimate product temperature under pressure using the following empirical relationship (Nguyen et al. 2007):

$$T_i = T_p - [CH \times (P_p - P_{atm}) + \Delta T_H] \tag{6.2}$$

where T_i = initial product temperature (°C), T_p = target process temperature of the product at pressure (°C), CH = heat of compression (°C/100 MPa), P_p = target process pressure (MPa), P_{atm} = atmospheric pressure (0.1 MPa), and ΔTH is the heat gain (or lost) by the test sample from the environment during pressure-holding time.

The difference of heat of compression will influence the magnitude of heat transfer among the pressure-transmitting fluid, food product, and the environment (Nguyen and Balasubramaniam 2011). Subsequently, the thermal gradient in the system could influence microbial inactivation and product quality (Balasubramanian and Balasubramaniam 2003). Product temperature during HPP is influenced by both heat of compression and heat loss. Care must be taken to interpret data obtained from situations involving excessive drop in sample temperature (Ramaswamy et al. 2007). Knoerzer and Chapman (2011) demonstrated the significance of the heat of compression factor in estimating the temperature distribution inside a high-pressure chamber using computational fluid dynamics (CFD) modeling. The stability of product temperature during the holding time at pressure may depend on the insulation characteristics of the pressure vessel (Balasubramaniam et al. 2004).

6.3.2 Thermal Conductivity

The thermal conductivity of water under pressure (over giga-pascal range) has been studied (Bridgman 1923; Kestin et al. 1984; Lawson et al. 1959). Studies of thermal conductivity of water under pressure were summarized by Wagner and Pruss (2002) and reported as an equation of state of water by the International Association of the Properties of Water and Steam (IAPWS). Recently, the National Institute of Standards and Technology (NIST) adopted the IAPWS equation (REFPROP, version 9.0, Lemmon et al. 2010). Very limited information is available about the in situ thermal conductivity of food materials under pressure.

Thermal conductivity values at atmospheric pressure conditions are estimated either by steady-state or unsteady-state (transient) methods. In steady-state methods (unless sample sizes are extremely small), a long time is required to achieve steady conditions (several hours), making these methods incompatible with perishable foods that may change chemically and physically during that time (Nesvadba 2005). Among unsteady-state methods, line heat-source probe technique has been commonly used (Murakami et al. 1996a, b; Reidy and Rippen 1971; Sweat and Haugh 1974). The temperature rise is measured as a function of time, and thermal conductivity is calculated from the following equation (Nesvadba 2005):

$$k = \frac{Q}{4\pi\Delta T}\left[\ln\frac{t_e}{t_s}\right] \tag{6.3}$$

where k = thermal conductivity of the sample (W/m · °C), Q = heat generated by line heat source (W/m), t_s = start time (s) and t_e = end time (s) within the straight-line portion of the curve, and T_s and T_e = temperature (°C) at time t_s and t_e, respectively.

Above transient technique was adopted to measure the in situ thermal conductivity (k_p) of liquid foods under pressure (Denys and Hendrickx 1999; Ramaswamy et al. 2007; Zhu et al. 2008). Figure 6.3 presents the schematic diagram of a line heat-source probe for measuring in situ thermal conductivity (k_p) (Ramaswamy et al. 2007). K-type thermocouple wires along with the insulated constantan heater wire were inserted inside a stainless steel hypodermic needle tube. The wires were insulated to avoid any short circuits and connected to the power source and data acquisition system. Then the probe was aligned approximately in the central axis of the polycarbonate sample holder with a movable piston. The bottom of the outer sample holder housed a removable and free-moving piston, sealed with an O-ring. This allowed pressure to transmit from medium to sample. After pressure equilibration, temperature change was monitored as a function of time to calculate in situ thermal conductivity (k_p) under pressure. k_p of selected foods at elevated pressure and 25 °C has been investigated by previous researchers (Nguyen et al. 2012; Ramaswamy et al. 2007; Zhu et al. 2008). In their studies, experimental data were used to construct regression models to estimate the changes in in situ thermal conductivity (k_p) as a function of pressure (equation 6.4).

$$k_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 + a_3 \cdot P^2 \tag{6.4}$$

Table 6.2 summarizes the coefficients for the regression equation describing the thermal conductivity of water and selected foods as a function of pressure at 25 °C. Since Eq. (6.4) is empirical in nature, it is important that such equations are used to estimate thermal conductivity values only within the range of experimental conditions, as extrapolations may result in erroneous values. All tested samples showed increasing k_p as a function of pressure. Under pressure, the intermolecular distance is decreased hence reducing the mean free path for the molecules, resulting in an increase in thermal conductivity (Nguyen et al. 2012). Apple juice (88 % moisture) showed similar k_p to that of water. Among the tested food samples, clarified butter showed the maximum increase (87 %) in k_p at 700 MPa relative to atmospheric pressure, followed by canola oil (62 % increase) and apple juice (38 % increase). Water and aqueous-based materials have lower compressibility (Bridgman 1923) than fat-based foods. Consequently, their k_p values under pressure increase to a lesser extent (Ramaswamy et al. 2007). Moisture content plays an important role in the thermal conductivity of tested materials (Zhu et al. 2008).

The extent of heat exchange between the product and its surroundings is in part governed by its thermal properties, such as its thermal conductivity and specific heat (Rasanayagam et al. 2003). Whenever foods with high thermal conductivity are pressurized, care is required to monitor any potential heat loss to the surrounding pressure medium or vessel, to ensure the process uniformity and microbial safety. In an unheated/noninsulated pressure vessel, it is recognized that the cold zone (or least treated region) is likely located near the wall or near vessel closures (Balasubramaniam et al. 2004; Knoerzer et al. 2010; Nguyen and Balasubramaniam 2011). Macronutrients in food matrices, such as water, fat, protein, and carbohydrate, have different thermal conductivities under pressure.

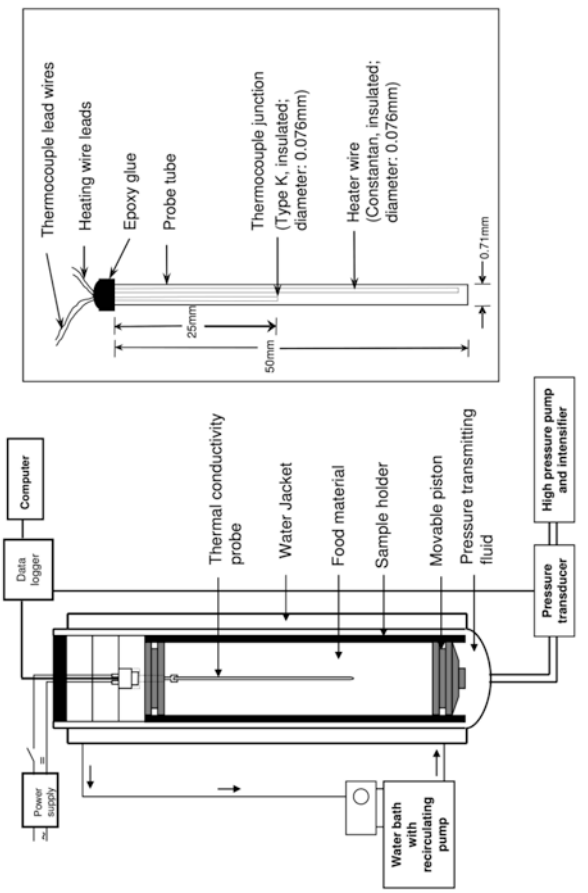

Fig. 6.3 Schematic diagram of the high-pressure experimental setup (**a**) and thermal conductivity probe (**b**) (Ramaswamy et al. 2007)

Table 6.2 Coefficients of regression equation (Eq. 6.4) for in situ thermal conductivity (k_p, W/m · °C) of water and selected food materials as a function of pressure (MPa) at 25 °C

	a_0	a_1	a_2	a_3	R^2
Water[a]	0.607	0.0005	–0.0000007	0.0000000006	1.00
Apple juice[b]	0.6003	0.000329			0.92
Canola oil[b]	0.1987	0.000176			0.99
Clarified butter[b]	0.2356	0.000293			0.91
Cheddar cheese[c]	0.351	0.000266	–0.00000017		0.90
Chicken breast[c]	0.522	0.0003778	–0.000000347		0.88
Potato[c]	0.588	0.000363	–0.000000058		0.99
Tomato puree[d]	0.531	0.0003			0.96
Honey[d]	0.329	0.0001			0.95
Guacamole[d]	0.421	0.0002			0.97
Cream cheese[d]	0.363	0.0002			0.95

[a]Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al. 2010)
[b]Ramaswamy et al. 2007, measured up to 700 MPa at 25 °C
[c]Zhu et al. 2008, measured up to 350 MPa at 25 °C
[d]Nguyen et al. 2012, measured up to 600 MPa at 25 °C

Therefore, the cold zone must be carefully considered for heterogeneous foods (Ardia et al. 2004). Insulating vessels minimize heat loss through the inner wall (Balasubramaniam et al. 2004). Further research is required to evaluate the in situ thermal conductivities of a broad range of food components.

6.3.3 *Thermal Diffusivity*

A dual needle line heat probe has been adopted to estimate in situ thermal diffusivity (α_p) and specific heat (C_p) in food matrices under pressure (Nguyen et al. 2012; Zhu et al. 2007). The basic design is similar to line heat-source probes, except there is a second K-type thermocouple beyond the first probe. The second probe maintains a constant distance from the first probe. Under pressure, a line heater at the first probe heats samples and then the temperature change of the second thermocouple is recorded to calculate in situ thermal diffusivity, using the following relation (Nguyen et al. 2012; Zhu et al. 2007):

$$\alpha_p = \frac{r^2}{4}\left(-\frac{t_m^{-1} - (t_m - t_d)^{-1}}{\ln(t_m) - \ln(t_m - t_d)}\right) \tag{6.5}$$

where r = the distance (m) between line heat and second thermocouple probe, t_d = duration of the heat pulse (s), and t_m = time from the start of heating to maximum temperature (s). Previous researchers developed regression models to estimate the

Table 6.3 Coefficients of regression equation (Eq. 6.6) for in situ thermal diffusivity (α_p, m^2/s) of water and selected food materials as a function of pressure (MPa)

	a_0	a_1	a_2	a_3	R^2
Water[a]	0.146	0.0001	–0.0000003	0.0002	1.00
Soybean oil[b]	0.077	0.00002	0.00000008		0.94
Honey[b]	0.093	0.000001	0.00000008		0.95
Tomato puree[b]	0.130	0.0001	–0.00000006		0.85
Guacamole[b]	0.122	0.00007	–0.00000002		0.83
Cream cheese[b]	0.111	0.00006	0.00000002		0.92
Potato[c]	0.145	0.0000821	–0.0000000255		0.63

[a]Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al. 2010)
[b]Nguyen et al. 2012, measured up to 600 MPa at 25 °C
[c]Zhu et al. 2007, measured up to 350 MPa at 5 °C

changes in in situ thermal diffusivity as a function of pressure using experimental data and described in the form of equation 6.6.

$$\alpha_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 + a_3 \cdot P^2 \tag{6.6}$$

Table 6.3 tabulates the coefficients for the regression equation describing in situ thermal diffusivity (α_p) of water and selected foods as a function of pressure, at 25 °C (Zhu et al. 2007). Water showed a 22 % increase in α_p at 600 MPa in comparison to its initial value at 0.1 MPa. Among tested food samples, soybean oil had the most increase (53 %) at 600 MPa followed by cream cheese (39 %), honey (32 %), tomato puree (30 %), and guacamole (29 %). Thermal diffusivity has a significant impact on the heat flux inside food samples and the surrounding media. When there is a heat flux across the boundary of the system, the transient temperature field, which occurs within the product, must be taken into account (Ardia et al. 2004).

6.3.3.1 Specific Heat

Based on knowledge of in situ thermal conductivity (k_p), thermal diffusivity (α_p), and density data for selected food materials (ρ_p) (Min et al. 2010), in situ specific heat (Cp_p), under pressure, was estimated as follows (Nguyen et al. 2012):

$$Cp_p = \frac{k_p}{\alpha_p \cdot \rho_p} \tag{6.7}$$

where k_p = in situ thermal conductivity (W/m · °C) under pressure, α_p = in situ thermal diffusivity (m^2/s) under pressure, and ρ_p = in situ density under pressure (kg/

Table 6.4 Coefficients of regression equation (Eq. 6.8) for in situ specific heat (Cp_p, kJ/kg·K) of water and selected food materials as a function of pressure (MPa) at 25 °C

	a_0	a_1	a_2	a_3	R^2
Water[a]	4.181	−0.0024	0.000005	−0.000000004	1.00
Soybean oil[b]	2.493	−0.000008	−0.000001		0.83
Honey[b]	2.432	0.00007	−0.000001		0.96
Guacamole[b]	3.190	0.0025	−0.00001	0.00000001	0.75

[a] Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al. 2010)
[b] Nguyen et al. 2012, measured up to 600 MPa at 25 °C

m^3). In situ specific heat (Cp_p) changes under pressure were empirically modeled as follows and are summarized in Table 6.4:

$$Cp_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 + a_3 \cdot P^2 \tag{6.8}$$

Cp_p values of tested materials (water, soybean oil, honey, guacamole) had decreasing trends as a function of pressure, except guacamole. Guacamole did not show a clear Cp_p trend. Initial increase in Cp_p of guacamole may be attributed to entrapped air bubbles in the sample during loading, although an effort was made to deaerate the guacamole by applying vacuum before pressure treatment (Nguyen et al. 2012). Cp_p is the quotient of thermal conductivity to diffusivity, and both conductivity and diffusivity had a similar increasing rate with rising pressures (Zhu et al. 2007).

In the previous studies, significant efforts have been made to measure the in situ thermal properties of various food materials at elevated pressures (350–700 MPa) at room temperature ranges. Previous researchers have tried to simulate the temperature and pressure distribution within the vessel using mathematical modeling and computational fluid dynamics (Abdul Ghani and Farid 2007; Denys et al. 1997; Hartmann et al. 2003) (see chapter 12 and 13 on mathematical modeling and process uniformity). However, in situ thermal properties of real foods were not readily available, so researchers often used the values for that of water. For an example, Abdul and Farid (2007) used computational fluid dynamics to simulate the temperature distributions of solid (beef fat)-water mixtures during conduction in the high-pressure vessel. The in situ thermal conductivity of beef fat ($k_{bf,p}$) under pressure was not available, so the authors estimated $k_{bf,p}$ as adopted by Hartmann et al. (2003):

$$k_{bf,p} = \left(\frac{k_{bf,atm}}{k_{wt,atm}}\right) \cdot k_{wt,p} \tag{6.9}$$

where $k_{bf,p}$ = in situ thermal conductivity of beef fat at pressure; $k_{bf,atm}$ = in situ thermal conductivity of beef fat at atmospheric pressure, 0.1 MPa; $k_{wt,atm}$ = in situ thermal conductivity of water at atmospheric pressure, 0.1 MPa; and $k_{wt,p}$ = in situ thermal conductivity of water at pressure. Validation of the computed temperature distribution was in agreement with those measured experimentally and reported in

the literature. However, a real food consists of multiple components including water, fat, carbohydrates, and protein. Heat-transfer models need accurate thermal and physical property data for the products being studied at the appropriate conditions, and thus, it was necessary to determine the pressure and temperature dependence of the relevant properties, thermal conductivity, density, specific heat, and thermal expansivity (Denys et al. 2000b). Further studies are needed to investigate the in situ thermal properties of foods during combined pressure (350–700 MPa) -thermal (105–121 °C) treatment.

6.4 Volumetric Properties: Density and Compressibility

In high-pressure processing, measurement of a material's volumetric properties under pressure is required to estimate heat of compression values of materials and to model the thermal profiles of pressurized food systems (Barbosa-Cánovas and Rodríguez 2005; Min et al. 2009). The compressibility of molecules and changes in volume influence the pressure-induced changes on proteins, resulting in an equilibrium shift favoring the state with the lowest overall volume (Krešić et al. 2008; Lullien-Pellerin and Balny 2002). In the case of liquid foods under pressure, density differences occur which lead to free convection of fluids (Abdul Ghani and Farid 2007).

Bridgman (1909, 1931) conducted pioneering work on the compressibility of several liquids under elevated pressure. Guignon et al. (2010) investigated the volumetric properties of pressurization fluids (water, ethanol, ethylene glycol, propylene glycol, castor oil, silicon oil) up to 350 MPa. The compressibility of water is available in the NIST reference fluid thermodynamic and transport properties—REFPROP, version 9.0 (Lemmon et al. 2010). Table 6.5 summarizes the isothermal compressibility (MPa^{-1}), volume, and density change of water as functions of pressure. Water reduces its initial volume by 14.8 % at 600 MPa. Water properties are useful to simulate the behavior of high-moisture foods; however, some significant discrepancies may appear in modeling predictions if pure water properties are used instead of corresponding properties for the real food (Guignon et al. 2010).

A number of researchers (Aparicio et al. 2011; Denys et al. 2000a; Guignon et al. 2009; Min et al. 2009; Min et al. 2010) have conducted experiments measuring the compressibility of several food materials under pressure. Various methods, such as the piezometer, piston-displacement method, and hydrometer, were adopted to measure the volumetric properties of food under pressure (Barbosa 2003). Denys et al. (2000b) determined the density of tomato paste and apple pulp by measuring the amount of pressure-transfer medium pumped into the high-pressure equipment. More recently, a variable volume piezometer and linear variable differential transformer were used to measure the compressibility and density of both liquid and solid foods under pressure (Aparicio et al. 2011; Guignon et al. 2009; Min et al. 2009; Min et al. 2010). Min et al. (2009) customized the variable volume piezometer for both liquid and solid foods applicable up to 700 MPa. The modified piezometer utilized the magnet coil and movable copper piston as an eddy current sensor to

Table 6.5 Volumetric properties[a] of water as a function of pressure (MPa) at 25 °C

Pressure (MPa)	Isothermal compressibility (MPa^{-1})	Volume (m^3/kg, 10^{-3})	Density (kg/m^3)
0.1	0.00045246	1.003	997
50	0.00040019	0.982	1018
100	0.00035723	0.964	1038
150	0.00032168	0.947	1056
200	0.00029215	0.933	1072
250	0.00026746	0.920	1087
300	0.00024666	0.908	1101
350	0.00022895	0.898	1114
400	0.00021371	0.888	1127
450	0.00020045	0.879	1138
500	0.00018882	0.870	1149
550	0.00017851	0.862	1160
600	0.00016931	0.855	1170
650	0.00016102	0.848	1180
700	0.00015354	0.841	1189

[a]Data obtained using NIST reference fluid thermodynamic and transport properties—REFPROP software Version 9.0 (Lemmon et al. 2010)

sense piston displacement upon pressurization. A magnet coil, wrapped around a polycarbonate sample tube, produced an electromagnetic field by means of vertical movements of the copper piston. Inductance changes and their empirical relationship to volume changes of water (published NIST database) were determined. Subsequently, this empirical model was used to calculate the volume change of tested food samples depending on inductance changes in the variable volume piezometer under pressure as follows:

$$\rho_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 \tag{6.10}$$

Table 6.6 summarizes the parameters of empirical models to estimate the density changes of selected foods as a function of pressure. The density of all tested foods increased as a function of pressure. The density of water increases from 997 kg/m^3 at 0.1 MPa to 1189 kg/m^3 at 700 MPa. Apple juice has the highest increase in density (17.4 %) with pressure, 0.1 MPa to 700 MPa. Clarified buffer (17.2 %) has the second largest change in density followed by soybean oil (16.9 %), chicken fat (16.2 %), carrot (14.6 %), chicken breast (14.1 %), cheddar cheese (13.7 %), deli ham (13.0 %), honey (8.9 %), and then salmon (8.6 %). Results demonstrate that food densities under pressure, particularly for those relatively high in solids, fat, and porosity, deviate from the behavior of water (Min et al. 2010). Measured differences in food densities under pressure are attributable mostly to differences in compressibility. Published in situ properties of specific heat (Nguyen et al. 2012) and density (Min et al. 2011) can be used to derive theoretical adiabatic temperature increases

Table 6.6 Coefficients of regression equation (Eq. 6.10) for in situ density (ρ_p, kg/m^3) of water and selected food materials as a function of pressure at 25 °C

	a_0	a_1	a_2	R^2
Water[a]	997	0.4025	−0.0002	0.99
Salmon[b]	1013	0.32	−0.00028	0.99
Soybean oil[c]	917	0.4194	−0.0002820	0.99
Apple juice[c]	1044	0.3856	−0.0001804	0.99
Clarified butter[c]	911	0.4042	−0.0002571	0.99
Honey[c]	1464	0.3259	−0.0001995	0.99
Deli ham[c]	1078	0.3970	−0.0002803	0.98
Chicken fat[c]	924	0.4202	−0.0002943	0.99
Chicken breast[c]	1064	0.4174	−0.0002855	0.99
Cheddar cheese[c]	1086	0.4659	−0.0003608	0.98
Carrot[c]	1047	0.4136	−0.0002786	0.97

[a]Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al. 2010)
[b]Min et al. 2009, at 25 °C, measured up to 700 MPa at 25 °C
[c]Min et al. 2010, at 25 °C, measured up to 700 MPa at 25 °C

(dT) using Eq. (6.1). Figure 6.4 shows the theoretical estimates (see equation 6.1) for temperature increases in water, honey, and soybean oil. For the thermal expansivity (β_p, K^{-1}) of soybean oil and honey, there seems to be no published data; so, the β_p of water ($\beta_{p,\ water}$) under pressure was used from NIST reference fluid thermodynamic and transport properties—REFPROP, version 9.0 (Lemmon et al. 2010). Although $\beta_{p,\ water}$ could differ from soybean oil and honey, reasonable values of $\beta_{p,\ water}$ were applied for both soybean oil and honey. In the theoretical estimations of adiabatic temperature increases, soybean oil showed the greatest temperature increase, from 25 °C at 0.1 MPa to 60 °C at 600 MPa. Water and honey increased in temperature up to 43 and 48 °C, respectively, with rising pressures to 600 MPa. Rasanayagam et al. (2003) reported the experimental value of adiabatic temperature increases in soybean oil up to 62.8 °C at 600 MPa when it was compressed from 25 °C at 0.1 MPa. In our study, the theoretical estimation of adiabatic temperature increases had strong agreement with the published experimental values, within 2.8 °C. Water had an experimental adiabatic temperature increase up to 42.4 °C (initial temperature of 25 °C at 0.1 MPa) with elevating pressures up to 600 MPa (Patazca et al. 2007); therefore, water's experimental data was consistent with theoretical estimates based on in situ specific heat and density. It is important to consider adiabatic temperature increase when evaluating microbiological inactivation data under pressure. For example, a difference of 3–4 °C in adiabatic heating has resulted in a difference of up to six log cycles of spore inactivation (Ardia et al. 2004).

In Le Chatelier's principle, phenomena that are accompanied by a decrease in volume are enhanced by pressure and vice versa. Thus, under pressure, reaction equilibriums are shifted toward the most compact state, and the reaction rate constant is increased or decreased (Rastogi et al. 2007). Covalent bonds are highly

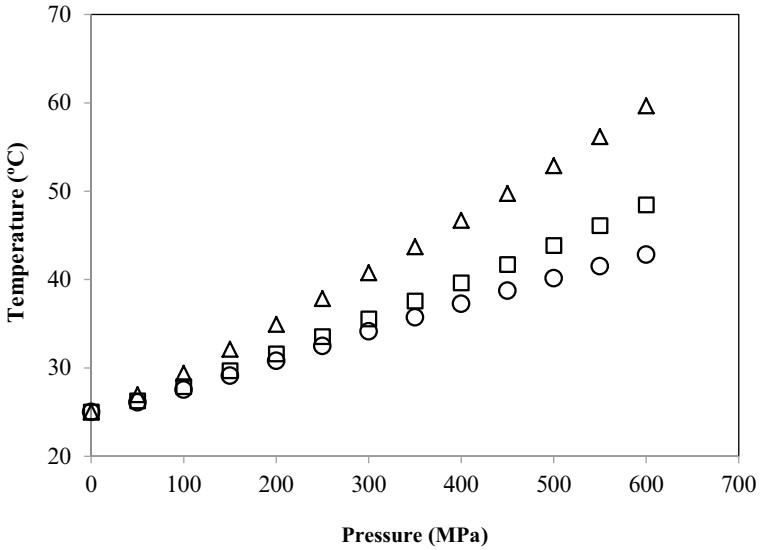

Fig. 6.4 Theoretical estimates of temperature increase based on published in situ property data of specific heat (Nguyen et al. 2012) and density (Min et al. 2010), (○), water; (□), honey; (△), soybean oil

incompressible (Prehoda et al. 1998) and, therefore, not influenced by elevated pressure. Whereas high pressure stimulates some phenomena (e.g., phase transition, chemical reactivity, change in molecular configuration, chemical reaction) that are accompanied by a decrease in volume, it opposes reactions that involve an increase in volume (Linton and Patterson 2000; Norton and Sun 2008).

Volumetric changes of different foods under pressure would influence protein denaturation including collagen denaturation and chemical reactions, beyond microbial inactivation (see Chap. 18). The magnitude of the standard volume change resulting from unfolding globular protein provides unique insight into packing and hydration differences between folded and unfolded proteins (Prehoda et al. 1998). The partial molar volume of the denatured protein system decreases with increasing pressure relative to that of the native protein. In the case of collagen, in contrast to globular proteins, unfolding (collagen denaturation) results in increased partial specific volume at low pressure and decreased partial specific volume at pressures above 324 MPa (Potekhin et al. 2009). It has been shown that the transfer of hydrophobic compounds to water is accompanied by a large decrease in specific volume at low pressures (Masterton 1954; Potekhin et al. 2009). At present, there is limited information relating volumetric properties of foods and components to chemical reactions or structural changes under pressure. Practically applied, high pressure can disrupt the three-dimensional structures of larger molecules or cell structures (i.e., proteins, including enzymes, lipids, cell membranes, etc.) but has no effect on small covalently bonded molecules, such as vitamins, flavor components, and some pigments. However, further work is needed to define this phenomenon further.

6.5 Electrical Properties

The electrical behavior of food materials under pressure has, recently, been investigated to determine the effects of pressure on texture and extent of starch gelatinization. Dielectric properties of biological tissues provide information about tissue structure and composition (Kuang and Nelson 1998). Angersbach et al. (2002) investigated pressure-induced membrane damage in potato tissues due to electrical conductivity changes (non-in situ method), immediately after pressure treatment. The trend of increasing electrical conductivity in potato was observed based on treatment intensity; thus, measuring electrical conductivity can be employed to assess the cellular status of materials under pressure. Bauer and Knorr (2004) described pressure-induced wheat and tapioca starch gelatinization using electrical conductivity measurements (non-in situ method), after pressure treatment. The electrical conductivity of pressure-treated starches increases as a function of pressure and pressure-holding time; and the curves of electrical conductivity are well correlated to curves for degree of gelatinization and pressurization time. Authors proposed that the rise in gelatinized starch conductivity is attributable to an ion release and amylose leaching out of the granule. Consequently, electrical conductivity corresponds with the degree of pressure-induced starch gelatinization and is an effective tool to detect the degree of gelatinization.

6.5.1 In Situ Electrical Conductivity

Earlier studies have measured in situ electrical conductivity of metals and several chemical solutions (Bridgman 1921; Bridgman 1931; Quist et al. 1965; Quist and Marshall 1968; Scaife 1974), but previous cell designs were not suitable for food or biological materials since the electrical properties of biomaterials are field-strength dependent (Cima and Mir 2004; Min et al. 2007). Min et al. (2007) suggested the in situ electrical conductivity cell for food applications should (1) include an insulated electric field (Schiefelbein et al. 1998) and allow for estimation of the cell constant under pressure; (2) provide a uniform electric field, necessary for differentiating between pressure and electromagnetic-induced changes in food and biological samples (Cima and Mir 2004); and (3) include uniform Joule heating of samples under pressure to enable research on the combined effects of pressure, temperature, and electric field strength on conductivity.

Min et al. (2007) and Park et al. (2012) developed an in situ electrical conductivity cell for liquid and solid food applications under pressure (Fig. 6.5). For liquid food testing, samples were simultaneously loaded into both the inner and outer sample holder. Subsequently, liquid foods functioned as a pressure-transmitting fluid inside the conductivity cell. For solid foods, samples were loaded into the inner sample holder and then isoelectrical solution was poured into the outer sample

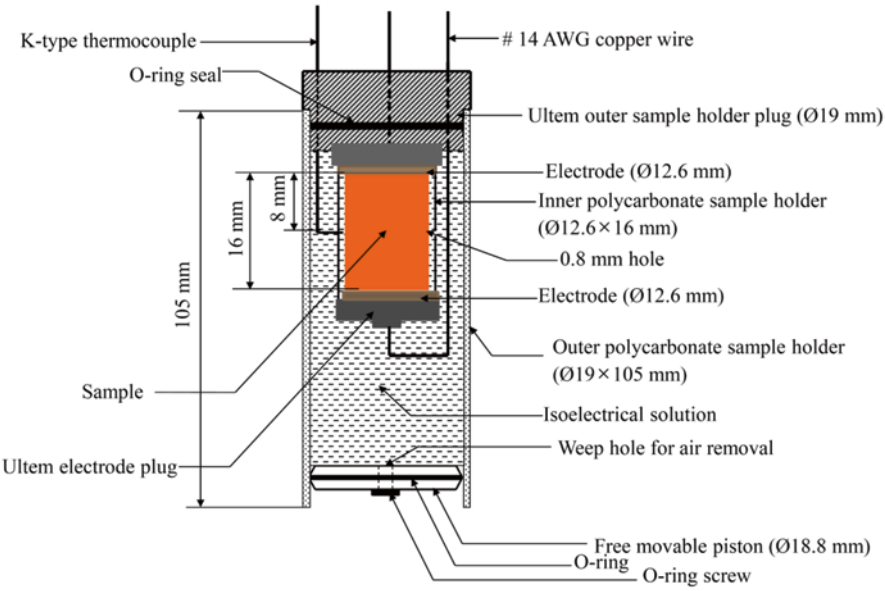

Fig. 6.5 Cross-sectional view of the in situ electrical conductivity cell made for high-pressure application

holder to enable pressure equilibration. The in situ electrical conductivity values (σ_p) for samples were determined using Eq. (6.11) with data for applied voltage (V), current (I), and cell constant (ke_p) under pressure, as follows:

$$\sigma_p = ke_p \times \frac{I}{V} \tag{6.11}$$

In situ electrical conductivity of liquid foods (orange juice, apple juice, and tomato juice) increased as a function of pressure, peaking between 200 and 500 MPa and decreasing between 500 and 800 MPa (25 °C) (Min et al. 2007). For example, the electrical conductivity of tomato juice increased from 0.61 S/m at 0.1 MPa to 0.66 S/m at 400 MPa and then decreased to 0.58 S/m with the pressure increment at 800 MPa. The authors indicated that increasing electrical conductivity between 200 and 500 MPa may have been due to in ionic movement and changes in viscosity, and then the subsequent downward trend of electrical conductivity might be due to a distortion effect on the ions that hinders mobility at the higher pressures. Tomato juice had the highest in situ electrical conductivity among tested samples. Authors proposed that the higher mineral content in tomato juice (278 mg minerals/100 g juice) resulted in the highest electrical conductivity, followed by orange juice (210 mg minerals/100 g juice) and apple juice (139.5 mg minerals/100 g juice) (composition data from USDA 2006). In general, the effect of pressure on electrical

resistance in the solution is very complicated, as might be expected from the numerous factors involved (Bridgman 1931).

In situ electrical conductivity changes of selected vegetables are plotted as a function of pressure and pressure-holding time in (Fig. 6.6). (Park et al. 2012). Pressure treatment increased in situ electrical conductivity values for all processed samples, as a function of target pressure and holding time up to a certain threshold level. Beyond this threshold level, the electrical conductivity values did not change further. For example, the in situ electrical conductivity of raw carrot was 0.027 ± 0.003 S/m, at 0.1 MPa and 25 °C, and then reached up to 0.181 ± 0.032 S/m, at 600 MPa and 3 min holding time. No significant increase in in situ electrical conductivities was observed from 3 to 10 min holding time, at 600 MPa. Pressure treatment induces the transport of solutes from inside to outside the cell (and vice versa) with changes in cell permeability (Préstamo and Arroyo 1998). Cell permeability could lead to increase in situ electrical conductivity under pressure. The compressed structure and increased density seen in vegetable samples under pressure would also likely increase the in situ electrical conductivity in tested samples. Although in situ electrical conductivity changes in relation to tissue damage undergone at pressure are outside the scope of this review, the stabilized electrical conductivity seen in samples suggests that there is a certain threshold of pressure-holding time that could be used to minimize further tissue damage in vegetables. At present, the knowledge of in situ electrical conductivity is not sufficient, and further research needs to focus on liquid and solid foods.

6.6 Reaction Volume and pH

Understanding the pH changes of foods under pressure could be important for controlling microbial inactivation and pH-dependent reactions during high-pressure processing. The initial steps in understanding pH relative to pressure were taken by Min et al. (2011) with weak acid buffers. Weak acid buffer solutions can have pressure-dependent pH changes, due to pressure-dependent ionization equilibrium. Hypotheses state that increasing pressure increases dissociation of weak acids, as ionized products fill smaller volumes due to solvent electrostriction around resulting charged species (Hamann 1980). A consequence of pressure-dependent weak acid dissociation is pressure-dependent pH. As presented by Min et al. (2011), reaction volumes for protonic ionization of weak buffer solutions at different pressures can be used to calculate changes in the molal equilibrium constant for the buffer, due to pressure. Molal equilibrium constants under pressure can then be used to calculate molal pH changes due to pressure. Min et al. (2011) used a variable volume piezometer to measure in situ reaction volumes for protonic ionization of weak acid buffering agents of 2-(*N*-morpholino)ethanesulfonic acid, citric acid, sulfanilic acid, and phosphoric acid under pressure up to 400 MPa (25 °C). The methodology involved initial separation of buffering agents within the piezometer, using gelatin capsules. Under pressure, the volume of the reactants was measured at 25 °C.

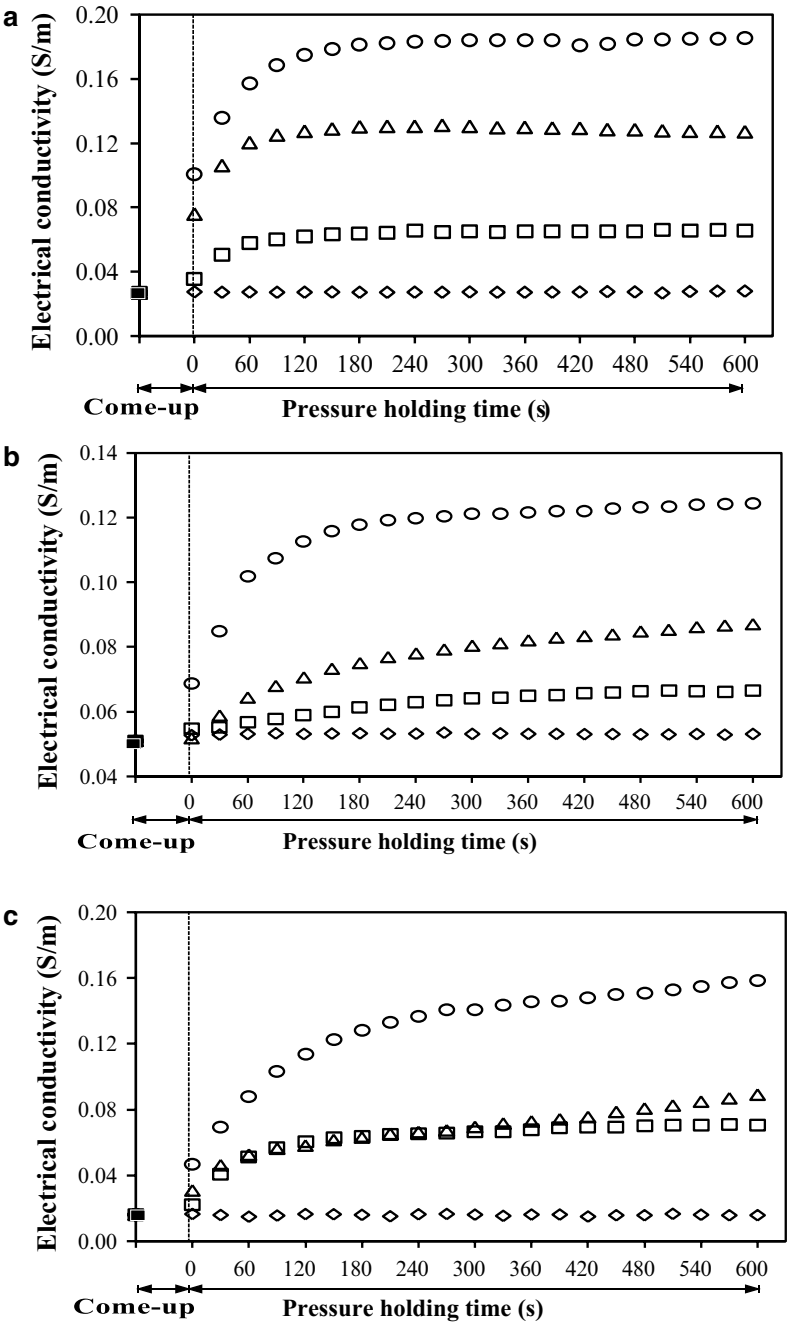

Fig. 6.6 In situ electrical conductivity of the vegetable samples as a function of pressure and holding time: (**a**) carrot (**b**) potato (**c**) red radish ((◇) 0.1 MPa, (□) 200 MPa, (△) 400 MPa, (○) 600 MPa). The *dotted vertical line* indicates the come-up time. Pressurization rate was approximately 20 MPa/s

The contents were then heated to 40 °C to dissolve the gelatin and initiate the reaction. Chamber temperature was cooled to 25 °C and product volume was measured. Reaction volumes were used to calculate the pH of buffer solutions as a function of pressure. The largest pH change was seen in phosphoric acid buffer, which dropped an average of 0.25 pH units per 100 MPa, while the pH of citric acid buffer dropped by 0.13 units per 100 MPa. Sulfanilic acid buffer showed almost no pH sensitivity to pressure, whereas the pH of 2-(*N*-morpholino)ethanesulfonic acid buffer increased by 0.075 units per 100 MPa. These results suggest that increasing ionization of phosphoric acid and citric acid as a function of pressure increases hydrogen ion concentration, promoting pH decrease, whereas 2-(*N*-morpholino)ethanesulfonic and sulfanilic acid have relative pH stability under pressure. pH changes under pressure are clearly complex, and more rigorous study may be needed to fully understand pH changes in complicated food matrices.

6.7 Conclusions

A clear grasp of in situ thermal, volumetric, and electrical properties, reaction volume, and pH changes of food matrices under elevated pressure is critical for validating high-pressure processing uniformity and developing suitable kinetics models of microbial inactivation. Developing appropriate sensors to measure in situ properties under pressure is a challenging work, involving installation of thermocouples, electrical wires, and measurement sensors into pressure vessels. Also, it requires calibrating the process using already published data, such as NIST steam properties under pressure. Previous research has successfully evaluated the in situ thermal conductivity, thermal diffusivity, specific heat, compressibility, density, electrical conductivity, and pH changes of selected foods up to 600–700 MPa at room temperature ranges. This information is useful for optimizing further high-pressure processing with respect to process uniformity and microbial safety. Experimentalin situ property data will enable the development of a mathematical model or computational simulation to validate temperature and pressure distribution for all types of high-pressure processing.

At present, in situ property data for foods is limited to room temperature ranges under elevated pressure. There has been a recent surge in research, which simultaneously studies the effects of elevated pressures (400–600 MPa) and sub-retorting temperatures (105–121 °C) in shelf-stable, low-acid foods in pressure-assisted thermal processing (PATP) and pressure-ohmic-thermal sterilization (POTS). For this purpose, additional knowledge of the in situ properties of foods is needed as a function of both elevated pressure and temperature. Foods are complicated matrices, composed of many macromolecules such as water, fat, carbohydrate, and protein. In situ property measurements under pressure are also needed for each respective nutrient and the effect of mixing, typical to foods.

Acknowledgment References to commercial products or trade names are made with the understanding that no endorsement or discrimination by The Ohio State University is implied. Financial support from the USDA National Institute for Food and Agriculture HATCH project OHO01323, Ohio Agricultural Development Corporation (OARDC), and Center for Advanced Processing and Packaging Studies and the food industry is gratefully acknowledged. References to commercial products or trade names are made with the understanding that no endorsement or discrimination by The Ohio State University is implied.

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Chapter 7
Food Processing by High-Pressure Homogenization

Federico Harte

Abstract High-pressure homogenization is applied to liquid foods by devices that consist of a positive displacement pump (usually a plunger-type pump) and one or more restrictions to flow (stages) created by valves or nozzles. This chapter reviews various valves utilized in high-pressure homogenization of liquid foods. The impact of high-pressure homogenization on various functional properties of protein and polysaccharides and microbial safety of pressure homogenized products is discussed.

Keywords High-pressure homogenization • Valve • Shear • Fluid foods • Microbial safety • Emulsion stability

7.1 Introduction

Homogenization is a term used by food scientists and engineers to describe a wide variety of processes including ultrasonic, rotary, membrane, colloidal mill, and valve homogenization, among others. The ambiguity in the use of the word "homogenization" rises from the fact that any process that reduces the relative heterogeneity of a system can be called homogenization. This chapter will focus on what is typically referred to as "high-pressure valve homogenization" or "dynamic high-pressure homogenization." This process is applied to liquid foods by devices that consist of a positive displacement pump (usually a plunger-type pump) and one or more restrictions to flow (stages) created by valves or nozzles (Fig. 7.1).

We will define "high-pressure homogenization" as homogenization processes where pumps are able to deliver at least 100 MPa hydrostatic pressure to a liquid food before a restriction to flow is imposed, regardless of the flow rate. However, as homogenization technologies keep evolving and higher pressures are achieved, common agreement on how to define "high-pressure homogenization" vs. "low-pressure homogenization" will most certainly evolve too.

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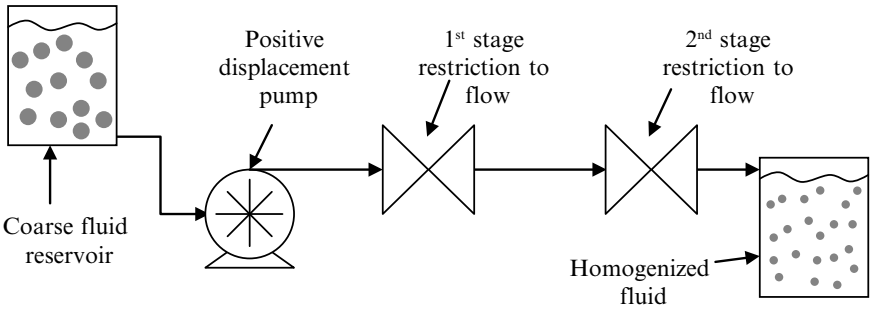

Fig. 7.1 Basic diagram of a valve homogenization system

7.1.1 Low-Pressure Valve Homogenization

In low-pressure valve homogenization, as the pressurized liquid flows through the restriction to flow (valve), there is a large increment in fluid velocity and a corresponding reduction in the fluid pressure. The fluid velocity initiates very intense turbulence in the fluid jet exiting the valve, and this turbulence disrupts the dispersed phase and produces the homogenization effect.

Homogenization using valve homogenizers was first commercially introduced in the early twentieth century for the processing of fluid milk. The main objective at the time was to avoid fat separation so that "every consumer would receive his fair share of milk fat since there could be no stealing of cream" (Trout 1948). Nowadays, virtually all milk is homogenized because consumers reject seeing an upper layer of cream in their milk. Concerns over the potential negative effects of homogenized milk on human health have been dispelled by recent studies or a lack of evidence (Korpela et al. 2005; Michalski and Januel 2006; Paajanen et al. 2005).

In an industrial setup, fat (or cream) is separated from milk by continuous centrifugation, homogenized at pressure below 20 MPa, and then poured back into skim milk to standardize to 1 %, 2 %, or whole (~3.25 %) fat content (Tetra-Pak 2003). Assuming fat globules are spherical particles, the terminal velocity (v_t in m s^{-1}) of a solid sphere moving in laminar flow is described by the Stokes equation as (Bird et al. 2006):

$$v_t = \frac{g \cdot D_P^2 \cdot (\rho_P - \rho_f)}{18 \cdot \mu_f} \tag{7.1}$$

where g is the gravitational force (≈9.81 m s^{-2}), D_P is the particle diameter (m), ρ_P and ρ_f are the density of the particle and fluid, respectively (kg m^{-3}), and μ is the Newtonian viscosity of the fluid (Pa s). The homogenization of milk slows the terminal velocity of fat aggregates by reducing their particle size and increasing their net density through casein micelle aggregation on the surface of fat particles (Fig. 7.2).

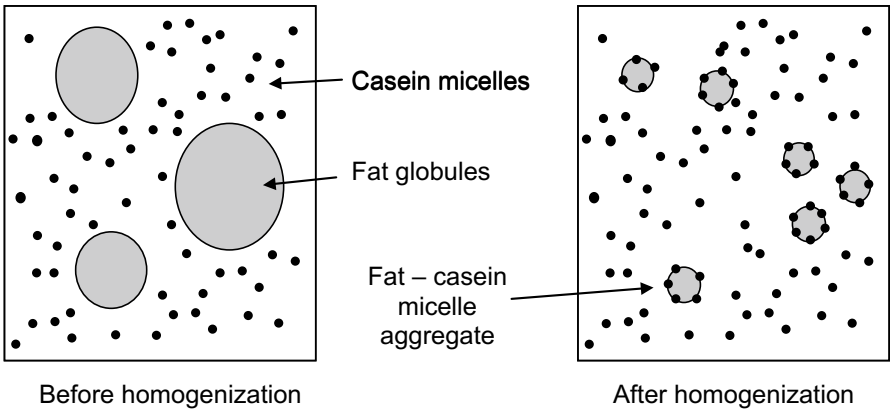

Fig. 7.2 Effect of homogenization on fat and casein fractions in fluid milk

The terminal velocity for a fat globule with 3.5 μm diameter (diameter ranges from 0.2 to 15 μm) and 900 kg m^{-3} density is ≈3.3 mm per hour, whereas a fat aggregate with 0.4 μm diameter and a net density of 960 kg m^{-3} in homogenized milk will move at a terminal velocity of ≈0.6 mm per day.

Most industrial-scale homogenizers are based on two-stage homogenization valves (Fig. 7.3). It is generally agreed that a second-stage valve with back pressure 10–20 % of the primary valve reduces cavitation and improves turbulent flow, promoting particle size reduction (APV 2008).

7.1.2 The High-Pressure Homogenizer

Current industrial-, pilot-, or lab-scale high-pressure homogenizers are equipped with plunger-type pumps and valves or nozzles made from abrasive-resistant ceramics or hard gemstones (Figs. 7.4 and 7.5). Stability in the delivered pressure is achieved through an attenuation volume between the pump and the valve or the use of two or more reciprocating plungers and an overlapping algorithm control. In a typical valve setup (e.g., Stansted Fluid Power), a zirconium or tungsten carbide needle-seat valve or ball-seat valve is used, with homogenization pressure being controlled by the force exerted over the needle blocking the fluid flow (Figs. 7.4b, c and 7.5b). Some homogenizers (e.g., Avestin, BEE International) are equipped with one or two nozzles instead of valves. The technology for nozzle-equipped high-pressure homogenizers was initially developed for water-jet cutting applications. In this case, a high-pressure pump is connected to an attenuator to reduce pressure fluctuations, and homogenization is achieved by nozzle head or "jewel" made from ruby, sapphire, or diamond (Figs. 7.4d and 7.5a). The nozzle orifice is usually <0.35 mm and the specific gemstone depends on the maximum pressure and required nozzle life-span, with diamond being the most resistant and expensive option. In the nozzle setup, homogenization pressure is determined by the pump

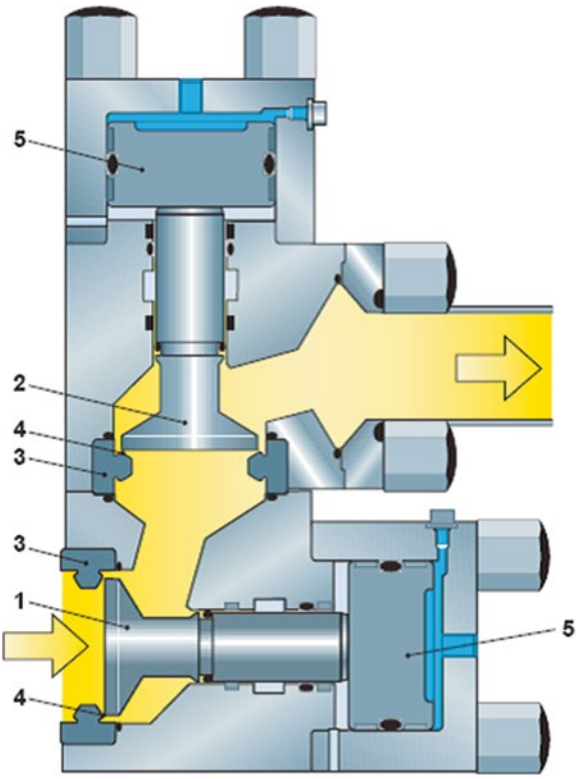

Fig. 7.3 Two-stage low-pressure homogenization valve. First-stage forcer (*1*); second-stage forcer (*2*); seat (*3*); gap (*4*); hydraulic actuator (*5*). Reproduced from Tetra-Pak 2003, with permission

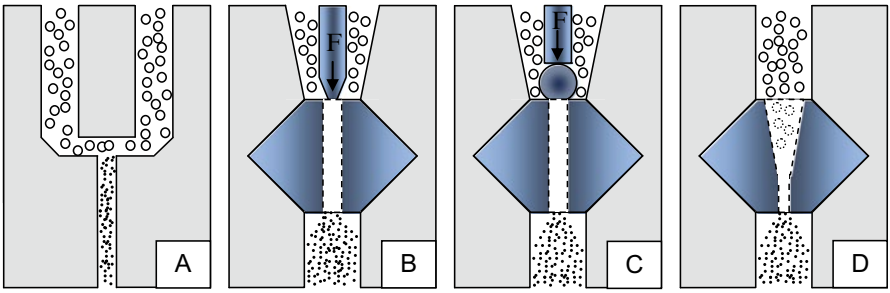

Fig. 7.4 Common high-pressure homogenization valves: (**a**) microfluidics; (**b**) ceramic needle and seat; (**c**) ceramic ball and seat; (**d**) diamond, sapphire, or ruby nozzle (*F* refers to force exerted on the needle)

pressure and/or a diversion to flow. In the microfluidics system (Fig. 7.4a), the flow stream is split in two or more channels that are redirected over the same plane but in right angles and propelled into a single flow stream. The pressure driving pump (up to 300 MPa) promotes a high speed at crossover of the two flows which results in high shear, turbulence, and cavitation over the single outbound flow stream.

Achieving a constant and consistent processing pressure is a major technical challenge in the design of high-pressure homogenizers. This is particularly difficult

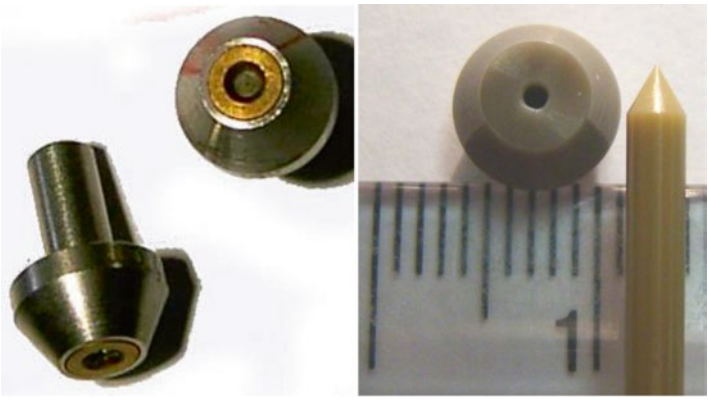

Fig. 7.5 High-pressure homogenization diamond nozzle (**a**) and ceramic needle and seat valves (**b**)

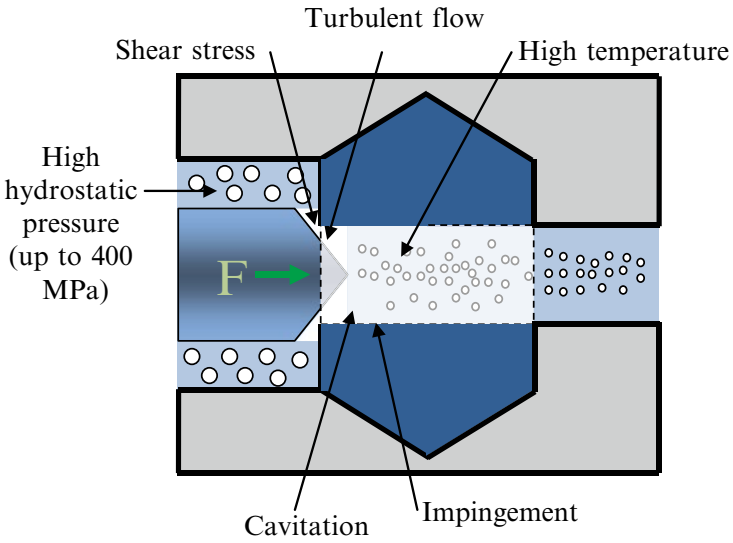

Fig. 7.6 Various physical phenomena simultaneously affecting a fluid during high-pressure homogenization

in homogenizers designed to process small samples where attenuator volumes are not an option. For the latter case, homogenizers equipped with single plunger pumps exhibit low-pressure "valleys" as a result of single pump reciprocating cycles. A partial solution is the use of more than one reciprocating plungers in parallel and overlapping algorithms. Coefficients of variation from 10 to 15 % are still to be expected in currently available devices.

The homogenization effect in high-pressure homogenizers cannot be attributed to a single physical phenomenon. At working pressures >100 MPa, high pressure, shear stress, cavitation, turbulence, impingement, and temperature increase all have a potential effect on microorganisms and food molecules (Fig. 7.6).

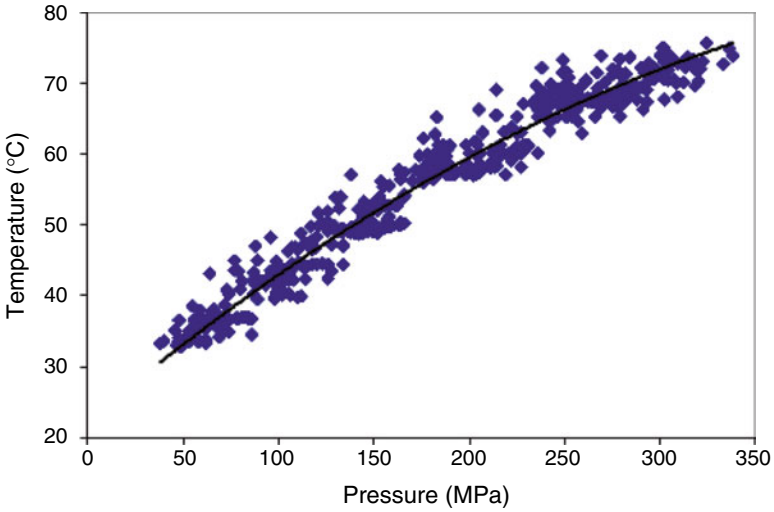

Fig. 7.7 Homogenization pressure-induced increase in temperature for water containing 0.9 % sodium chloride (starting temperature ~20 °C; adapted from Taylor et al. 2007)

Figure 7.7 shows a typical pressure vs. temperature profile for a simple fluid measured immediately after the homogenization valve. Depending on the specific physicochemical properties of the fluid and the refrigeration system used in the homogenization valve enclosure, at least a 15–20 °C shear-induced increase in temperature is typically observed per 100 MPa increment in homogenization pressure (Cortes-Munoz et al. 2009; Desrumaux and Marcand 2002; Heffernan et al. 2009; Taylor et al. 2007).

Current commercially available high-pressure homogenizers are able to reach up to 400 MPa processing pressure at low flow rates (less than 100 L/h). However, it is expected that flow rates and working pressures will increase in the near future since water-jet cutting devices able to reach ~600 MPa are already commercially available (e.g., Flow International).

7.2 Effect on Proteins

Few reports are available concerning the effect of high-pressure homogenization on food proteins. It is known that high hydrostatic pressure itself promotes changes in the secondary, tertiary, and quaternary structures of proteins (Knorr et al. 2006) leading to modifications in protein functionality (López-Fandiño 2006) and enzyme inactivation (Iucci et al. 2007; Valdramidis et al. 2009; Welti-Chanes et al. 2009). However, homogenization subjects proteins to relatively lower hydrostatic pressures and much shorter times (less than 1 s). Some research suggests little change in the secondary or tertiary structure of many proteins with homogenization up to 400 MPa, when a cooling system is connected immediately after the homogenization valve

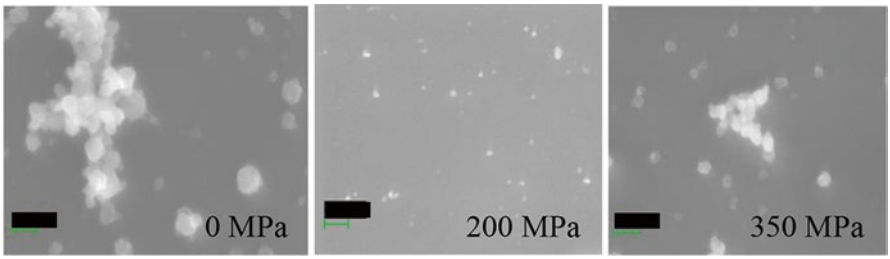

Fig. 7.8 Field-emission scanning electron micrographs (FESEM) of casein micelles from bovine milk processed by high-pressure homogenization (bars are 200 nm)

(Pereda et al. 2008; Pereda et al. 2007). However, there is conflicting data (Datta et al. 2005). For example, Escobar et al. (2011) found that fluid milk processed at 300 MPa and immediately cooled to 10 °C exhibited rapid signs of lipase-induced rancidity suggesting little lipase inactivation (lipase is easily denatured by heat, e.g., 10 s at 80 °C (Walstra et al. 1999)). Similarly, little whey protein disulfide bond formation (typically induced by thermal denaturation) was found in raw milk processed at homogenization pressure up to 300 MPa (Grácia-Juliá et al. 2008; Pereda et al. 2009). Contrary to these findings, some inactivation of pectin methylesterase was observed in orange juice subjected to 250 MPa homogenization pressure (Welti-Chanes et al. 2009).

High-pressure homogenization has been effective in the disruption of protein quaternary structures. Roach and Harte (2008) found that casein micelles from bovine milk exhibited a reduction in micelle diameter (Fig. 7.8) when raw skim milk was homogenized at pressure up to 200 MPa and that micelle reformation occurred at higher pressure. It was suggested that shear-induced increase in temperature would favor hydrophobic interactions among the individual casein proteins, leading to micelle reformation at homogenization pressure >250 MPa.

Changes in the casein micelle size and functionality as a result of processing milk by high-pressure homogenization have an effect on yield and quality of dairy-based foods. Hernandez and Harte (2008) found that model yogurts made from milk processed using high-pressure homogenization and thermal processing (90 °C for 5 min) exhibited better solid-like behavior (increased storage modulus), when compared to controls made from only heated raw or homogenized milks (Fig. 7.9). The combined effect of (1) heat-induced whey protein denaturation and (2) homogenization-induced casein micelle disruption promoted the formation of a thinner casein matrix having an increased surface area, thus improving the strength of acid gels.

The limited denaturing effect of whey proteins makes high-pressure homogenization a promising technology for nonthermal pasteurization of milk used for cheese manufacture (Lopez-Pedemonte et al. 2006). Several studies have shown that high-pressure homogenization improves cheese yield, in most cases by increasing moisture content and to lesser extent protein retention (Table 7.1). The combined effect on casein micelle and fat globule size reduction may explain the increase in protein and water retention in the final cheeses. Zamora et al. (Zamora et al. 2007)

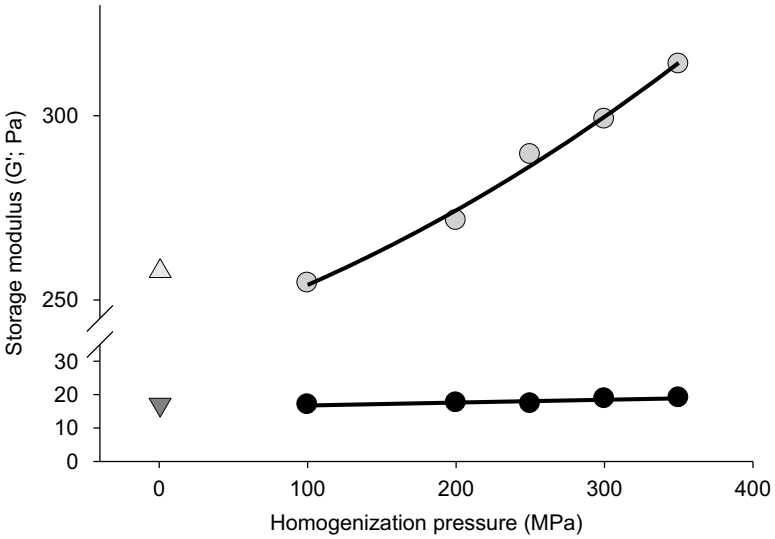

Fig. 7.9 Storage modulus for acid gels made from raw milk (▽), heated milk (90 °C for 5 min,), homogenized milk (△), and heated + homogenized milk (●). (Adapted from Hernandez and Harte 2008)

Table 7.1 Summary of reports on the effect of high-pressure homogenization in cheese yield (mass of cheese/mass of milk)

Type of cheese	Source	Processing conditions					References
		Raw	Thermal		Homogenized		
		Yield (%)	Yield (%)	Process	Yield (%)	Process	
Pecorino	Ewe	12.7	14.3	65 °C–15 s	17.6 %	100 MPa	(Vannini et al. 2008)
Crescenza	Cow	–	–	72.5 °C–15 s	>1 % vs. thermal	100 MPa	(Burns et al. 2008)
Caciotta	Cow	10.2	9.5	72 °C–30 s	12.5	100 MPa	(Lanciotti et al. 2006)
Not specified	Goat	16.0[a]	20.3[a]	72 °C–15 s	32.0[a]	100 MPa	(Guerzoni et al. 1999)

[a]In this case cheese yield was measured as mass of curd/mass of milk

suggested that changes in cheese-making properties of milk processed by high-pressure homogenization could also be explained by modifications to the protein-fat structures. Significant changes in lipolysis, proteolysis, and formation of biogenic amines have been also observed in cheeses that are stored for ripening as a result of high-pressure homogenization of milk (Burns et al. 2008; Lanciotti et al. 2007; Lanciotti et al. 2006; Vannini et al. 2008).

Escobar et al. (2011) found a linear relationship between homogenization pressure and yield when "queso fresco" cheeses were made using a combination of thermal treatment (65 °C for 30 min) and homogenization (Fig. 7.10). Most of the

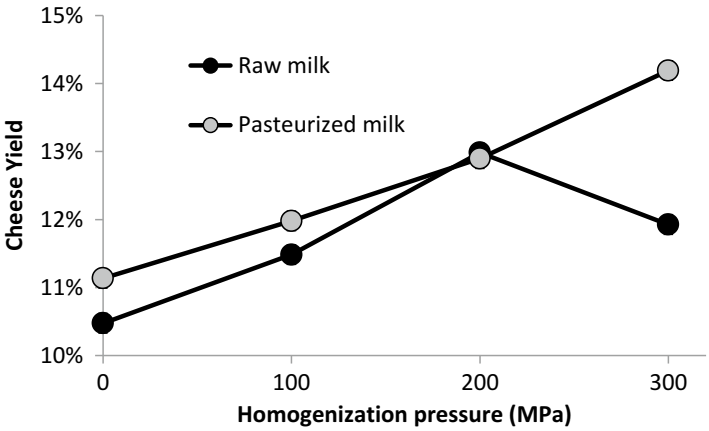

Fig. 7.10 High-pressure homogenization effect on "queso fresco" cheese yield (kg cheese/kg milk). Pasteurization at 65 °C for 30 min. Bars are 95 % confidence intervals (Adapted from Escobar et al. 2009)

yield increase was attributed to larger moisture content of the cheeses as a result of a thinner protein matrix. It was hypothesized that the thermally induced interaction of whey proteins and casein micelles prior to homogenization would protect the micelles from re-coalescence when milk was further subjected to high-pressure homogenization at 300 MPa. On the other hand, cheeses made from milk only homogenized at 300 MPa (i.e., no thermal processing) exhibited a relative reduction in yield, due to the reformation of casein aggregates through heat-induced hydrophobic interactions immediately after homogenization (Fig. 7.8).

7.3 Effect on Polysaccharides

High-pressure homogenization has shown good potential for improvement of polysaccharide functionality. Polysaccharides are known to modify hydration, solubility, rheological, and interfacial properties when used as food ingredients. For a specific polysaccharide, functionality is strongly correlated to molecular weight and one of the key unit operations in the processing of natural polysaccharide to make food ingredients is the reduction of molecular weight (depolymerization) to a target value. However, most methods used for depolymerization yield macromolecules with a wide range in molecular weight (polydispersity) and even multimodal distributions (see Heinze et al. 2006 for an example on dextran polydispersity; see Allan and Peyron 1995; Cravotto et al. 2005; Jedrzejas 2000; Van Den Einde et al. 2003 for depolymerization methods). Furthermore, currently available methods (chromatography based) for the recovery and isolation of specific molecular weight fractions are expensive and have limited application for the development of food ingredients on an industrial scale.

To avoid the uncertainty in the physical properties of polysaccharide ingredients having similar average molecular weight but dissimilar polydispersity, most commercial polysaccharides are sold based on average viscosity at a given concentration and set conditions rather than molecular weight. This is a partial solution to the problem of polydispersity, since similar rheological behavior at a given concentration and temperature does not assure that same properties will be maintained under other conditions.

One of the first attempts to determine the effect of high-pressure homogenization on molecular weight of food polysaccharides was carried out by Corredig and Wicker in pectin (Corredig and Wicker 2001). They demonstrated that homogenization at 124 MPa affected both average molecular weight and molecular weight polydispersity. By measuring Newtonian viscosity in k-carrageenan dispersion, it was shown that high-pressure homogenization up to 300 MPa is able to reduce molecular weight in an asymptotic trend indicating that pressure-dependent critical molecular weight is potentially achievable (Fig. 7.11). Similar behavior has been found in alginates, xanthan gum, and chitosan (Harte and Venegas 2010; Li et al. 2010).

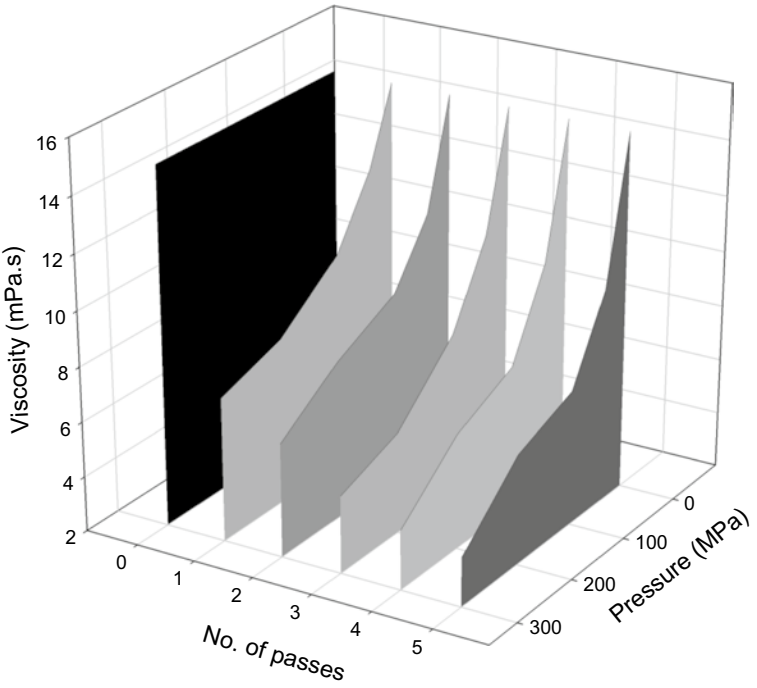

Fig. 7.11 Viscosity reduction in k-carrageenan dispersions by high-pressure homogenization (0–300 MPa and 0–5 homogenization steps) (Adapted from Harte and Venegas 2010)

7.4 Microbial Inactivation by High-Pressure Homogenization

Several studies have detailed the utility of high-pressure homogenization for the inactivation of microorganisms (see Chaps. 14–17). Early studies were based on processing pressures ≤50 MPa and were focused on the inactivation of various yeasts (Engler and Robinson 1981; Keshavarz Moore et al. 1990). Early studies relied on the use of single pump homogenizers with no attenuators, where strong fluctuations in processing pressure were inevitable and variable results due to "low-pressure valleys" were observed.

Pioneering work on the inactivation of microorganisms by high-pressure homogenization was started by Toledo and Moorman (Toledo and Moorman 2000). Since then several research groups have published results showing the potential of this technology for the nonthermal inactivation of yeasts, bacteria, molds, and viruses (Table 7.2). It is generally agreed that gram-negative bacteria exhibit higher

Table 7.2 Recent reports on the inactivation of molds, yeasts, bacteria, and viruses by high-pressure homogenization

Microorganism	Substrate or juice	Max pressure (MPa)	Steps	Decimal reduction	References
Gram-negative bacteria					
Escherichia coli	Apple	350	1	>5	(Kumar et al. 2009)
	Apple, carrot	350	1	>6	(Pathanibul et al. 2009)
	Buffer	300	1	4.5	(Wuytack et al. 2002)
	Buffer	350	1	>7	(Taylor et al. 2007)
	Buffer	300	1	6	(Wuytack et al. 2002)
	Water	250	1	6	(Donsì et al. 2009)
Yersinia enterocolitica	Buffer	250	1	2–3	(Wuytack et al. 2002)
	Buffer	300	1	>6	(Diels et al. 2003)
Shigella flexneri	Buffer	300	1	5	(Wuytack et al. 2002)
Pseudomonas fluorescens	Buffer	300	1	6	(Wuytack et al. 2002)
Salmonella enterica serovar Typhimurium	Buffer	275	1	2–3	(Wuytack et al. 2002)
Gram-positive bacteria					
Enterococcus faecalis	Buffer	300	1	<1	(Wuytack et al. 2002)
Staphylococcus aureus	Buffer	300	1	3	(Diels et al. 2003)
	Buffer	300	1	<1	(Wuytack et al. 2002)
	Milk, orange	300	1	2.8–4.0	(Briñez et al. 2007)
Staphylococcus carnosus	Milk, orange	300	1	0.0–3.6	(Briñez et al. 2007)
Lactobacillus plantarum	Buffer	300	1	<1	(Wuytack et al. 2002)
	Orange	300	1	>7.1	(Campos and Cristianini 2007)
Listeria monocytogenes	Milk	400	1	>3	(Roig-Sagués et al. 2009)

(continued)

Table 7.2 (continued)

Microorganism	Substrate or juice	Max pressure (MPa)	Steps	Decimal reduction	References
Listeria innocua	Buffer	250	1	<1	(Wuytack et al. 2002)
	Apple, carrot	350	1	5–6	(Pathanibul et al. 2009)
Leuconostoc dextranicum	Buffer	300	1	1–2	(Wuytack et al. 2002)
Lactobacillus delbrueckii	Water	250	1	0.5	(Donsì et al. 2009)
Bacillus cereus	Water	150	3	~5	(Chaves-López et al. 2009)
Bacillus subtilis	Water	150	3	~5	(Chaves-López et al. 2009)
Yeast and molds					
Saccharomyces cerevisiae	Orange	300	1	>5.6	(Campos and Cristianini 2007)
	Carrot,	100	1	1	(Patrignani et al. 2009)
	Apricot		8	5	
	Water	250	1	5	(Donsì et al. 2009)
Zygosaccharomyces bailii	Carrot	100	1	0.8	(Patrignani et al. 2010)
	Apricot		8	2.6	
Aspergillus niger	Mango	300	1	>6.2	(Tribst et al. 2009)
Virus					
MNV-1 murine *Norovirus*	Buffer	300	1	0.8	(D'Souza et al. 2009)
MS2 coliphage	Buffer	300	1	0.3	(D'Souza et al. 2009)
Various phages	Skim milk	100	1	0.2–3	(Capra et al. 2009)
			8	3–6	
Heterotrophic count					
Heterotrophic count	Apple	300	1	3.6	(Suarez-Jacobo et al. 2009)

susceptibility to high-pressure homogenization inactivation than gram-positive bacteria, due to reduced peptidoglycan content in the cell membrane of the former (Wuytack et al. 2002). Diels and Michiels (Diels and Michiels 2006) presented an extended review on potential mechanisms for the inactivation of microorganisms by high-pressure homogenization. It was initially suggested that the inactivation effect by homogenizers could be explained by impingement of cells in the outer ring of homogenizers or by cavitation-induced membrane damage (Shirgaonkar et al. 1998). At processing pressures >100 MPa, it is probable that shear stress, pressure, cavitation, temperature, turbulence, and impingement may all contribute to microbial inactivation and the relative importance of each physical phenomenon may vary depending on the specific processing pressure.

Pathanibul et al. (2009) studied the inactivation of *Escherichia coli* K12 and *Listeria innocua* in apple and carrot juices. Using previously reported decimal reduction time (D-value) and the change in temperature for a decimal change in

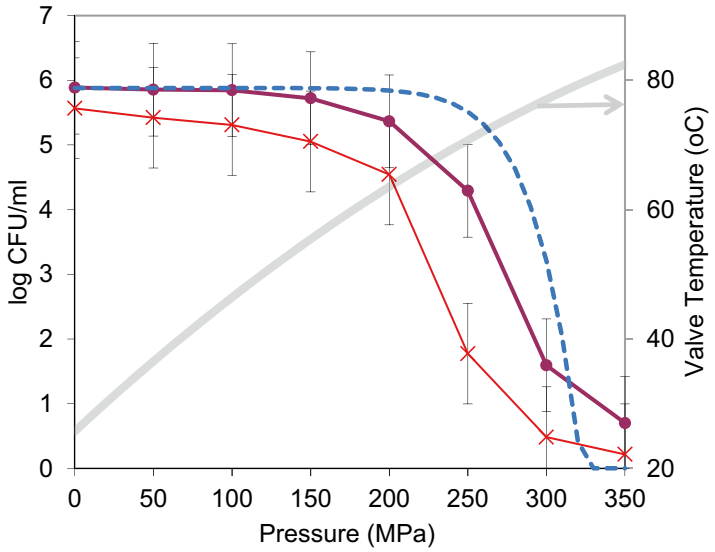

Fig. 7.12 Inactivation of *Listeria innocua* in apple juice processed by high-pressure homogenization inactivation. *Purple line* indicates no nisin added; *red line* indicates nisin added (10 IU/ml); *dotted blue line* indicates equivalent thermal inactivation; *gray line* is the temperature immediately after the homogenization valve. Bars are 95 % confidence intervals for the mean (Adapted from Pathanibul et al. 2009, with permission)

D (z-value) for both bacteria, it was concluded that the shear-induced increment in temperature in the homogenization valve could completely explain the observed inactivation at pressure ≥350 MPa (see Fig. 7.12). It was also observed that the addition of nisin to the juices increased the effectiveness of higher homogenization pressure indicating potential for synergism between the antimicrobial and the homogenization process.

7.5 Emulsion Stability and Delivery Systems

High-pressure homogenization has received strong attention as a physical means for achieving stable suspensions of solids in liquids (sols) or liquids in liquids (emulsions) in the neo-nanoscale (80–300 nm). For example, the pharmaceutical industry has aggressively investigated methods for stabilizing highly hydrophobic drugs in aqueous phases. Generally a transient (yet unstable) uniform suspension is made by using rotary homogenizers or colloidal mills before further particle size reduction using high-pressure homogenization (Date and Patravale 2004; Wang et al. 2008). Key advantages of the process include enabling (1) a narrow size distribution, (2) aseptic processing, and (3) highly concentrated as well as diluted formulations. Mechanisms for droplet reduction and empirical droplet vs. processing pressure

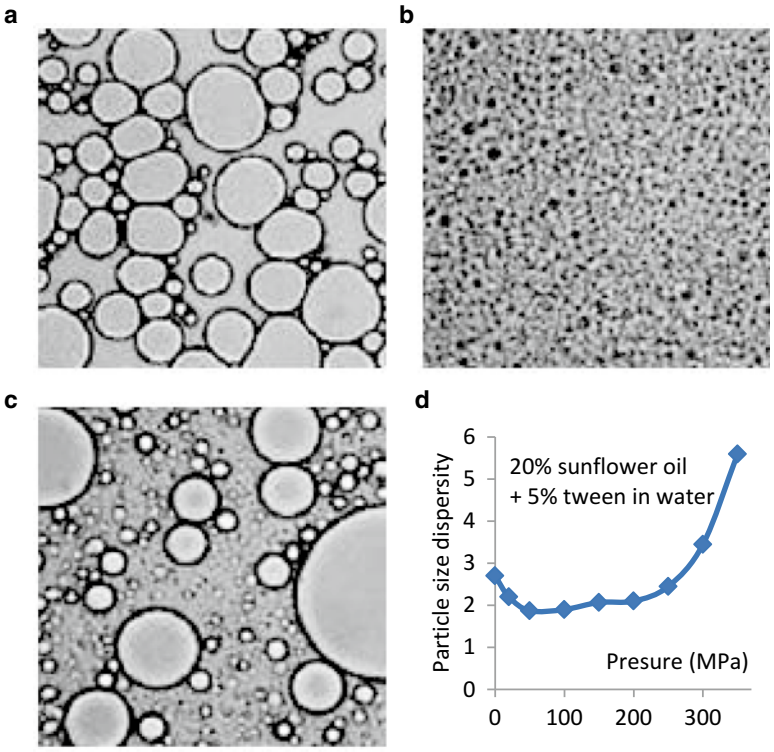

Fig. 7.13 (**a**–**c**) Light microscopy images (×100) of 20 % sunflower oil + 0.75 % methylcellulose in water emulsions after (**a**) rotary homogenization, (**b**) 100 MPa, (**c**) 350 MPa homogenization (adapted from Floury et al. 2003), (**d**) relative width distribution [(upper 90 % diameter—lower 10 % diameter)/midpoint diameter] of sunflower oil in water emulsion surfactant. Diameter is the Sauter diameter (d_{32}) (Adapted from Floury et al. 2004)

have been reviewed elsewhere (Floury et al. 2004; Håkansson et al. 2009; Raikar et al. 2010). Higher homogenization pressures lead to smaller monodispersed droplets up to a point (>200 MPa) where shear-induced increase in temperature becomes an important factor promoting droplet re-coalescence (probably through hydrophobic interactions), resulting in polydispersed systems (Fig. 7.13; (Floury et al. 2004; Marie et al. 2002)).

High-pressure homogenization alone, or in combination with polysaccharides, proteins, or surfactant molecules, has been used to stabilize oils in aqueous phases. Reports have shown improvements in the stabilization of peanut oil (up to 45 % (Cortes-Munoz et al. 2009)), sunflower oil (up to 20 % (Floury et al. 2002)), corn oil (up to 30 % (San Martin et al. 2009)), β-carotene (Yuan et al. 2008), and triglycerides (Henry et al. 2008) in water using homogenization alone or in combination with proteins (whey proteins (Cortes-Munoz et al. 2009; Floury et al. 2000; Lee et al. 2009), soy globulins (Floury et al. 2002), caseins (San Martin et al. 2009)) and polysaccharides (methylcellulose (Floury et al. 2003), acacia gum (Tipvarakarnkoon et al.)).

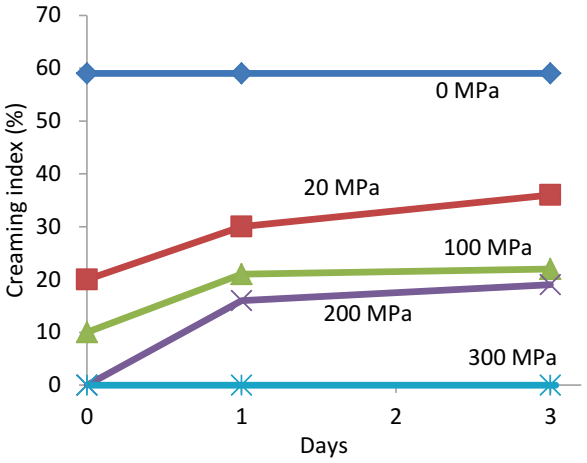

Fig. 7.14 Creaming index [100×(volume of aqueous phase/total volume)] of 30 % corn oil in water emulsions containing 0.5 % (w/w) micellar casein (Adapted from San Martin et al. 2009)

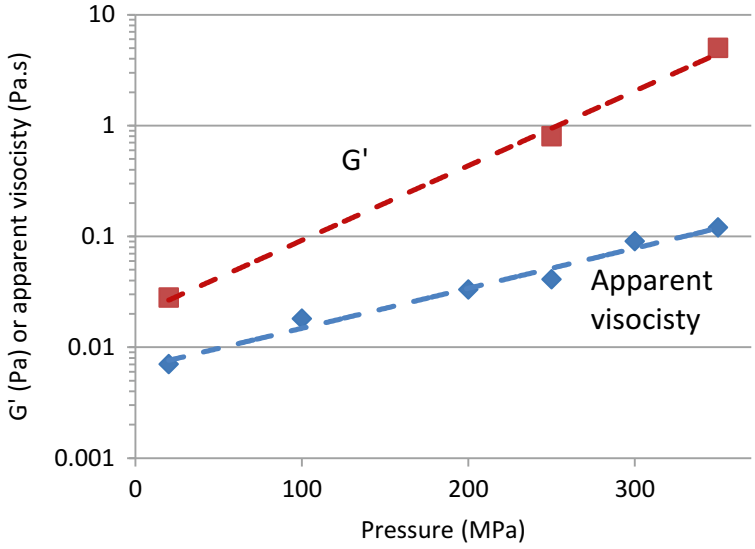

Fig. 7.15 Apparent viscosity (Pa s, $\dot{\gamma} = 100\,s^{-1}$) and storage modulus (G', Pa, ω = 0.1 Hz, strain = 5 %) of 20 % sunflower oil in water emulsions containing 2 % w/w 11S subunit soy protein (Adapted from Floury et al. 2002)

An interesting finding has been the improvement in emulsion stability and change in rheological properties (pseudoplasticity and gel formation) in highly concentrated oil in water emulsions where proteins and high-pressure homogenization have been used together (Figs. 7.14 and 7.15) (Cortes-Munoz et al. 2009; Floury et al. 2002; San Martin et al. 2009). San Martin et al. reported the stabilization of 30 % corn oil in water emulsions for several days when 0.5 % micellar casein was

used in combination with high-pressure homogenization up to 350 MPa (Fig. 7.14). Similarly, Floury et al. (Floury et al. 2002) observed changes in flow behavior including gel formation in 20 % sunflower oil in water emulsions where soy protein (11S subunit, 2 % w/w; Fig. 7.15) was used. Improvements in emulsion stability are probably due to both pressure- and interface-induced tertiary and quaternary conformational changes in protein structure that block temperature-induced droplet coalescence.

7.6 Concluding Remarks

High-pressure homogenization has proven to be an effective technology for improving safety and functionality of fluid foods and ingredients. As new materials and novel engineering designs continue to evolve, homogenizers exhibiting higher operational pressures, stable delivery pressures, better temperature control, and increased flow rate will be shortly available. Areas where high-pressure homogenization is expected to have an impact include (1) sterilization of fluid foods with limited thermal damage to flavor and nutritional food components (e.g., fruit juices), (2) stabilization of highly hydrophobic flavors and nutraceutical components (e.g., ω-3 fatty acids, sitosterols), (3) formulation of novel ingredients through homogenization-induced molecular interactions (e.g., protein-polysaccharide coacervation), and (4) improved polysaccharide functionality by molecular weight targeting, among others. In summary high-pressure homogenization remains a field where research is needed and novel food applications are foreseen.

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Chapter 8
Pressure Shift Freezing and Thawing

Jia You, Maryam Habibi, Navneet Rattan, and Hosahalli S. Ramaswamy

Abstract This chapter focuses on the basic principles that are involved in using high pressure for freezing and thawing purposes. Some emphasis will be given to the equipment used and on the effect of high-pressure freezing and thawing on the growth of microorganisms. The impact of pressure treatment on the quality of foods is also discussed.

Keywords High pressure • Freezing and thawing • Microbial safety • Quality

8.1 Introduction

Over the years, scientists have been attempting to improve food processing methods by employing innovative concepts to extend the advantages of conventional processing techniques. Pasteurization, sterilization, drying, chilling, and freezing are some of the methods which have been traditionally applied to keep foods safe and to extend their shelf life. Among these methods, freezing has been known to impart least effect on texture and nutritional value of food products, so freezing technology has been widely used in food processing because of its advantages.

In conventional freezing processes, heat transfer within food is generally limited, which eventually results in slow freezing rates. These conditions result in the formation of large extracellular ice crystals and thereby are responsible for damaging the product texture. Novel rapid freezing techniques offer potential for producing very small and uniform ice crystals within the cellular matters, and they have been found successful with various food products. If heat is removed at a slow rate, the ice crystals will have few nucleation sites, resulting in large ice crystals filling the extracellular areas. Large ice crystals, particularly needle-shaped crystals, cause maximum damage to the food structure and result in dehydration of the cells. Instead, if the product is cooled at a faster rate, smaller ice crystals are produced and they will

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be distributed evenly all over the tissue, and thus, less mechanical damage will happen to cell walls and texture and there will also be a lesser loss of nutrients through dripping upon thawing. This is because, during rapid freezing, heat gets removed faster so that before the growth of ice crystals is initiated to take place, newer nucleation locations are created that will result in the formation of smaller ice crystals in larger numbers (Fennema et al. 1973). Rapid freezing conditions prevail in those high-capacity and high-efficiency modern techniques such as air-blast, fluidized bed, cryogenic, plate, and spiral freezing of food particles of smaller size yielding individual quick freezing (IQF) conditions. However, even in air-blast and other rapid freezing methods, large size products have a relatively lower heat transfer rate with smaller surface-to-mass ratios and therefore large ice crystals are formed within food cells (North and Lovatt 2005). Cryogenic freezing is a relatively more rapid freezing method than liquid immersion or air-blast freezing due to high rate of heat transfer (low temperature and boiling of cryogens), but it can negatively influence the cell walls and form intra- and extracellular ice crystals due to thermal gradients (Otero et al. 2008). Pressure shift freezing (PSF) is one of the newer freezing techniques. PSF takes advantage of depression of freezing point of water under pressure and can be useful for creating uniform and tiny ice crystals in order to reduce the tissue damage and improve the product texture.

Except some products including frozen desserts and ice creams, most frozen foods are thawed before consumption. Thawing is generally a slow process as compared with freezing. During thawing process, the food surface is normally exposed to air or water at a relatively higher temperature and humidity, which provides free water on the product surface and potentially presents conditions suitable for microbial growth. Longer time for thawing will also lead to more destruction in quality and reduced stability of the final product. So for these reasons, thawing is much more critical than freezing in terms of controlling the changes in the product (Mascheroni 2012).

There are many different methods employed for thawing of frozen foods. Providing heat to achieve the phase change in the frozen food is common to all these methods but the way it is applied varies a lot. It can be categorized into three major groups: external heating methods, internal heating methods, and miscellaneous methods (Mascheroni 2012). In the last couple of decades, several novel techniques have been explored for the purpose of thawing frozen food, such as microwave, radio-frequency, and ohmic heating, which are based on internal heat generation (Mascheroni 2012). These methods have shown good promise of thawing for various food products, but there have also been few concerns associated during thawing by these methods. For example, with ohmic heating, uneven thawing is observed which is found to result due to channeling of electric current through already thawed regions of food product. The channeling effect is observed because the conductivity of thawed food is higher than the food which is in frozen state and hence it conducts more heat. In RF and microwave thawing, “runaway heating” can be observed which also results in distinctly thawed and frozen zones within a food product, because the thawed food absorbs more energy than the frozen parts; hence, the formation of separate zones takes place (Liu et al. 2005). A new method belonging to

miscellaneous group is being explored in order to achieve appropriate thawing in lesser time so as to have better quality thawed product. This method is based on using water characteristics at different pressures and it is called high-pressure thawing. The early research on high-pressure thawing was carried out by Taylor (1960) who applied high pressure on frozen biological materials. He found out that slow freezing when accompanied by pressure-assisted thawing (225 MPa) helped to better the retention of surviving conjunctiva cells which was the objective of his study. The application of high-pressure thawing as a novel technique for frozen foods started about 20 years ago. Food texturization, preservation, and phase change are the major fields of high-pressure application in food industry (Cheftel 1995; Knorr et al. 1998; Kalichevsky et al. 1995) and they can further be combined with sanitation effects (LeBail et al. 2002b).

8.2 Pressure Shift Freezing

8.2.1 Principles

Volume increases when water is frozen at atmospheric pressure. During the transformation at atmospheric pressure from water to ice (called ice I), pure water volume expands up to nearly 9 % at 0 °C and about up to 13 % at −20 °C, since ice I uniquely has a lower density than that of liquid water, and therefore it can cause significant structural and textural damage (Kalichevsky et al. 1995). During freezing, most foods expand with a lesser extent as compared to pure water, so the volume of food does not change uniformly because many other components shrink to different extents when the temperature gets lower and thus mechanical damage of tissue structures takes place (Zaritzky 2006). Typically, ice I is formed by the stress-inducing freezing front moving across food in conventional freezing processes (LeBail et al. 2003), which leads to the formation of large extracellular ice crystals, and hence cell damage occurs. However, the ice II to ice IX, which are formed under high pressure, have larger densities than that of liquid water, and hence they can contribute to less damage to cells without volume increases (Li and Sun 2002). As shown in Fig. 8.1, water still remains in nonfreezing state below 0 °C under high pressure. Pressure-supported freezing promotes instantaneous and homogeneous formation of ice throughout the whole product due to a high degree of supercooling, since the ice nucleation rate increases nearly tenfold for each degree of Kelvin of supercooling (Burke et al. 1975; Kalichevsky et al. 1995).

Pressure shift freezing (PSF) has been regarded as a promising freezing method in the food industry. PSF takes advantage of the principle of water-ice phase transition under pressure: pressure application depresses the freezing point of water from 0 °C to −21 °C at 210 MPa (Bridgman 1912). The general purpose of PSF is to reduce the temperature below 0 °C under pressure and then release the pressure to induce rapid ice nucleation throughout the cooled sample (Sun and Zheng 2006).

This novel freezing technique allows materials to produce rapid and uniform ice nucleation and growth of ice crystals and therefore leads to less tissue damage and good quality retention in the product.

8.2.2 *Pressure Shift Freezing Curve*

Figure 8.1 schematically shows the PSF process. After the product is installed into high-pressure vessel, pressure is elevated to desired level (~200 MPa). Then the product is cooled under pressure to around −20 °C or until the desired temperature is reached (steps A–B–C), and water still remains in the liquid state at this stage as indicated in Fig. 8.1. Pressure is then released rapidly, thereby causing uniform and high supercooling conditions throughout the product, and consequently the phase transition takes place (steps C–D). The initial formation of nuclei is instantaneous and homogeneous throughout the whole product due to this high supercooling of the samples (Alizadeh et al. 2007; Thiebaud et al. 2002). Numerous fine ice nuclei grow into large numbers of small ice crystals, and latent heat is released accompanied with the formation of ice which eventually increases the product temperature to the corresponding freezing point (point D). For a given cooling temperature, higher pressure and lower temperature before expansion promote more ice formation and thus the phase transition time is also reduced (Otero and Sanz 2000). Since only partial freezing is obtained in PSF, freezing is then completed at atmospheric pressure (steps D–E) (Sun and Zheng 2006).

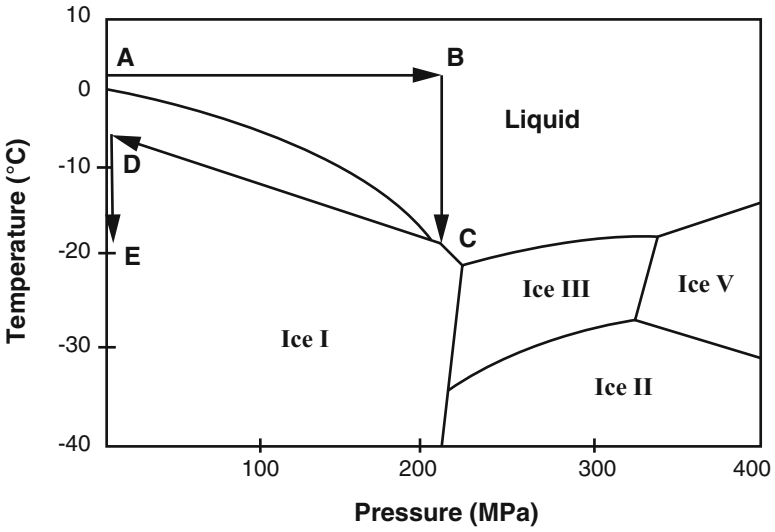

Fig. 8.1 A typical pressure shift freezing curve

In conventional freezing methods, slow freezing rates result in the formation of extracellular ice and moisture movement from original location to form large ice crystals (Zaritzky 2006). For most foods (meat, fish, and vegetables), classical methods would possibly cause freeze-cracking damage due to thermal gradients at very low freezing medium temperature and lead to structural damage and texture degradation and quality losses (Fernandez et al. 2006; Zhu et al. 2005a). Compared with efficient classical freezing techniques including cryogenic freezing, PSF is also useful to freeze large food products in which uniform ice distribution is required (Otero et al. 2008). Therefore, PSF is an effective technique to freeze foods with a large number of small ice crystals that are distributed evenly within the whole food system and good structural and quality retention.

Instantaneous ice nuclei formed by rapid depressurization are very important since the amount of initial ice nuclei determines the quality of PSF-frozen products. Sanz et al. (1997) reported that 36 % of total water was formed into ice after rapid depressurization from 210 MPa to atmospheric pressure. In case of meat, 16 % instantaneous ice was formed during expansion from 200 MPa to 0.1 MPa (Massaux et al. 1999b). For β-lactoglobulin gels, 28 % of water was reported to be converted to ice instantaneously after expansion from 207 MPa to 0.1 MPa (Barry et al. 1998). Based on thermodynamic considerations, a crude estimate of the amount of ice crystals formed can be computed. Assuming a freezing point depression to −20 °C, the supercooling of water by 20 °C will be achieved by removing sensible heat of water of approximately 80 kJ/kg (4.2 kJ/kgC × 20). Upon the release of pressure, this heat must be converted to latent heat. Based on the latent heat of water of 330 kJ/kg, this could result in 80/330 or ~25 % of water to be instantaneously frozen. The number of ice crystals produced by PSF is so large that even after the continued slow freezing conditions at atmospheric pressure, the crystal remains predominantly small.

8.2.3 Pressure Shift Freezing Equipment

High-pressure equipment is schematically shown in Fig. 8.2. The system includes a jacketed high-pressure vessel, a pressure generation system, and control panel for pressure and temperature. The high-pressure system is connected to a cooling bath, which provides cooled liquid to circulate continuously around the pressure vessel in order to achieve the purpose of freezing. The pressure vessel is a hollow cylinder and is constructed with stainless steel, which allows high-pressure vessel to be resistant to corrosion and to maintain its mechanical properties at low temperatures (Otero and Sanz 2011). At first, the cooling bath temperature is set for a given sub-zero temperature. Packaged sample is installed into pressure vessel and is directly immersed into pressure medium when the temperature decreases to desired temperature. Several pressurizing media have been reported in many previous studies, such as glycol/water, 62/38, v/v (Kalichevsky-Dong et al. 2000); ethylene glycol/water, 75/25, v/v (Otero et al. 2008); and ethanol/water, 50/50, v/v (Chevalier et al. 2000b). After installation, the pressure vessel is closed

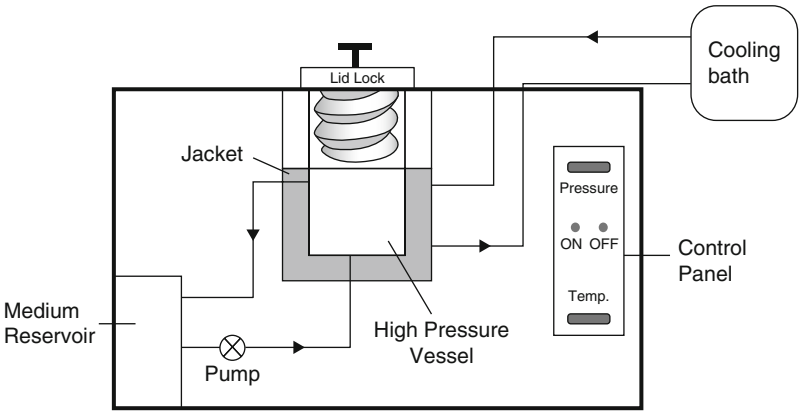

Fig. 8.2 Flow diagram of a pressure shift freezing/thawing equipment

properly and then the pressure generation is initiated. The air in the pressure vessel is removed out by pumping pressure medium from the container into the pressure vessel. After the pressure reaches a desired pressure level, it is held under this condition until the desired temperature is achieved in the product after which the pressure is released rapidly by using drain valve. Finally, the sample is allowed to complete the freezing process in the pressure vessel at atmospheric pressure. During pressure shift freezing process, the temperature of the sample and pressure medium is generally monitored by using thermocouples.

8.2.4 Pressure Shift Freezing and Microbial Growth

Many studies have been performed on high-pressure processes to evaluate their performance on the inactivation of microorganisms (Hiremath and Ramaswamy 2012; Black et al. 2011; Ramaswamy and Shao 2010; Garcia-Graells et al. 1999). However, for these works, high-pressure treatments were above 0 °C. It is also worth investigating the combination of high pressure and sub-zero temperatures' effect on the microbial growth.

Hashizume et al. (1995) reported the effects of pressure shift freezing on *Saccharomyces cerevisiae* in 0.85 % NaCl. The inactivation ratios were improved by PSF treatment at −10 °C and −20 °C in comparison with 0 °C, 5 °C, and 25 °C. A three log cycle of inactivation was reached by 180 MPa in 7 min at −20 °C and in 20 min at −10 °C. Ponce et al. (1998) observed that *E. coli* 405 CECT added to liquid whole egg and distributed evenly, when treated at 450 MPa and −15 °C for 15 min, gave an inactivation ratio of three log cycles. The author pointed out that the reason might be that food constituents provide microorganisms with baroprotection and can have an effect on the pressure resistance of microorganisms. Takahashi (1992) observed that *E. coli* processed at 200 MPa/−20 °C/20 min in

buffer had the reduction of eight log cycles as compared to that at room temperature, which only had four log cycle reductions.

Picart et al. (2004) studied the inactivation of smoked salmon mince inoculated with *Pseudomonas fluorescens*, *Micrococcus luteus*, and *Listeria innocua* at −21 °C under 207 MPa. The temperature of samples was first decreased to −21 °C at 207 MPa for 23 min. Then the pressure was slowly released to 0.1 MPa, which took nearly 18 min, and the freezing process was completed to −25 °C. The results indicated that inactivation ratios of *L. innocua* and *M. luteus* were 2–2.5 log cycles and that of *P. fluorescens* were 4.6 log cycles.

On the other hand, it was found that high costs are required for industrial applications in order to freeze lactic acid bacteria due to the high technical demands of pressure shift freezing equipment (Volkert et al. 2008). Hence, there is always a need to review the cost of installing the PSF equipment before using it for industrial applications. But it has been proposed that in long-term usage, the equipment costs can be repaid back (LeBail et al. 1997).

8.2.5 Pressure Shift Freezing and Quality Changes during Storage

During freezing storage, foods generally have quality losses over the storage period. The storage temperature of frozen foods is usually kept higher than their glass transition temperature in order to decrease quality losses. However, quality degradation still occurs during storage. The quality losses not only depend on freezing processes but also are related to storage conditions (Otero and Sanz 2011). Moreover, the storage and distribution temperature is recommended to decrease to −18 °C to −20 °C to better preserve frozen food quality with slow growth of crystals. In case of long storage conditions, it is suggested to lower the storage temperature to −25 °C to −30 °C. However, the temperature fluctuations remind an important problem. The control of storage temperature is very important since temperature fluctuations could cause a recrystallization process, which can have a marked influence on the ice crystals' distribution and can even destroy tissues (Martine Le and Genevieve 2004). In a study, after high-pressure treatment, potato cubes were stored at −30 °C, which resulted in no changes in quality as compared to the original ones, while samples frozen at −18 °C showed remarkable quality losses which were similar to the samples treated with conventional techniques (Koch et al. 1996).

For PSF-frozen fish and meat products, the storage time has no effect on their texture, color, and drip losses (Otero and Sanz 2011). In a study conducted by Sequeira-Munoz et al. (2005), the carp fillets were cooled under high pressure at 140 MPa at −14 °C for 12 min and under air-blast freezing at −20 °C with 4 m/s air velocity. It was observed that the texture of frozen carp fillets was similar to unfrozen control samples and PSF was more effective in reducing drip losses of cooked samples than air-blast freezing. Moreover, they also stated that PSF can reduce lipid degradation effectively during the frozen storage compared to air-blast freezing.

Table 8.1 Studies on high-pressure freezing of foods

Material	Process	Conditions	Research focus	Reference
Peach, mango	PSF	200 MPa, −20 °C	Microstructure	Otero et al. (2008)
Carrot	PSF	200 MPa, −19 °C	Microstructure	Fuchigami et al. (2006)
Gelatin gels	PSF	100/150/200 MPa	Microstructure	Zhu et al. (2005b)
Norway lobsters	PSF	200 MPa, −20 °C	Microstructure	Chevalier et al. (2000a)
Eggplant	PSF	200 MPa, −20 °C	Microstructure, texture	Otero et al. (1998)
Pork	PSF	200 MPa, −20 °C	Microstructure	Martino et al. (1998)
Potato	PSF	400 MPa	Microstructure, kinetics, quality	Koch et al. (1996)
Tofu	PSF	200 MPa, −18 °C	Microstructure	Kanda et al. (1992)

8.2.6 Pressure Shift Freezing of Selected Food Products

Owing to the advantages of pressure shift freezing, several studies have been carried on various foods. This technique has been developed both in research and commercial areas in Japan, as well as in the USA and Europe (Swientek 1992). Table 8.1 lists some products that have been treated by PSF.

8.2.6.1 Food Models

Lévy et al. (1999) reported that oil-in-water emulsions stabilized by sodium caseinate were frozen separately, by pressure shift freezing with rapid depressurization at −18 °C with 207 MPa and also under a constant pressure of 100 MPa at −9 °C. The ice crystals formed by PSF with rapid depressurization were a large number of small ice crystals and irregularly shaped without specific orientation. In comparison, emulsions frozen under constant pressure of 100 MPa had large ice crystals with a needle shape and were oriented radially. In addition, in terms of the duration of freezing plateau and ice crystal arrangement, the results of releasing pressure from 300 MPa at −16.5 °C are similar to the release from 207 MPa at −18 °C. However, larger ice crystals and a longer freezing plateau were caused when pressure was released from 100 MPa at −8 °C. So the author suggested that pressure release from 207 MPa was the optimal way to produce small ice crystals.

In case of gels of β-lactoglobulin, pressure shift freezing under 207 MPa at −19 °C could better preserve the structure of the gel network, as compared with gels frozen by still air at −30 °C and in gaseous nitrogen at −80 °C, and also the pressure shift frozen gels were quite similar to nonfrozen ones. The changes in firmness of the gels frozen by different techniques were not significantly different (Barry et al. 1998). The results were similar to the observation of gelatin gels. Zhu et al. (2005b) found that conventional air freezing (CAF) and liquid immersion freezing (LIF) produced larger ice crystals than those formed by PSF. They showed

that, for PSF at three pressures, the higher pressure and lower temperature resulted in the formation of smaller ice crystals.

Kanda et al. (1992) pressurized kinu tofu at 200 MPa at −18 °C and analyzed the microstructure of samples by using scanning electron microscopy. They found that smaller ice crystals were formed in tofu in the case of pressure shift freezing. Fuchigami and Teramoto (1997) also pointed out that kinu tofu frozen at 200–400 MPa was similar to control untreated samples; in fact, the texture of frozen tofu was found to improve, since stress, strain, and rupture energy changed insignificantly. However, the amount of drip loss and ice pores and rupture stress increased when pressure rose from 400 to 700 MPa.

8.2.6.2 Meat Products

Muscle cells are flexible and elongated fibers that have minimal air spaces (Haard 1997). It is accepted that freezing has an impact on the muscle structure, leading to the shrinking of muscle fibers due to moisture movement into extracellular spaces (Hurling and McArthur 1996). The effect of freezing temperature and freezing rate on muscle structure has been studied to some extent. Muscle structural damage during rapid freezing is less than that during slow freezing (Alizadeh et al. 2007).

Alizadeh et al. (2007) reported that, according to the microstructure of ice crystals, most cells in Atlantic salmon associated with air-blast freezing (ABF) and direct-contact freezing were occupied with cross section of the ice crystals, which were larger than the muscle fibers. Ice crystals formed during ABF (−30 °C, 1.4 m/s) process and direct-contact freezing had larger and irregular shapes. The ice crystals caused deformation of tissue due to poor muscle fibers for beef and pork (Grujic et al. 1993; Martino et al. 1998). However, PSF samples can keep muscle fibers intact and reduce tissue deformation and shrinkage, since PSF process creates smaller and more uniform ice crystals and also maintains isotropic spread of ice crystals in the fish tissues, particularly for the 200 MPa treatments. The size of ice crystals formed by PSF at 100 MPa had a higher stability for long frozen storage duration. Zhu et al. (2003) also reported that the PSF-treated salmon tissues were well maintained and PSF produced a large number of fine and regular intracellular ice crystals which contributed to a better preserved microstructure as compared with air-frozen and bath-frozen samples. These researches were in agreement with the findings on pressure shift-frozen pork and lobster (Chevalier et al. 2000a; Martino et al. 1998). Chevalier et al. (2000a) also suggested that it is better to use low pressure in order to minimize protein denaturation caused by high pressure.

There is a debate on the effect of small ice crystals and pressure-induced protein denaturation to the quality of HP-treated frozen meat (Norton and Sun 2008). By using DSC tests, the results showed that the toughness of PSF-frozen meat increased, especially at the 150 and 200 MPa, which might be caused by denaturation of myofibrillar protein of pork. It is recommended that raw meat be frozen by applying high pressure under 150 MPa in order to preserve microstructure and quality (Zhu et al. 2004b). Fernandez-Martin et al. (2000) also found similar results and they also

reported reduction in water holding capacity of pork sample which further results in higher drip loss when PSF was carried under 200 MPa and −20 °C.

PSF leads to significant color changes in meat and red meat might become pale due to small ice crystals scattering more light than the larger ones. Thus, meat is more opaque and has a lighter color (Pérez-Chabela et al. 2004). The chromatic changes increase along with increasing pressure level employed (Zhu et al. 2004b).

8.2.6.3 Fruits and Vegetables

In plant tissues, structure damage is associated with the semirigid nature of cells and the less orderly packing of the cells in the tissue (Haard 1997). Mechanical damage to local structure on fast freezing is lesser than that at slow freezing. Fast freezing protects the structure of the muscle better due to the production of a large number of smaller ice crystals and more uniform extracellular spacing resulting in less water movement and less breakage of muscle fiber and hence less separation of cell walls and consequently better retention of the quality of frozen food (Otero et al. 1998; Van Buggenhout et al. 2006a). Otero et al. (2008) reported that PSF (200 MPa, −20 °C) produced smaller ice crystals and resulted in reducing structural damage in peach and mango as compared with conventional methods, such as air-blast freezing (−40 °C) and cryogenic freezing by liquid N_2 evaporation. Air-blast freezing had the lowest freezing rate of the three methods studied, and therefore it caused the formation of larger ice crystals which led to major damage in the fruit tissue and broke the cells. Freezing rate of liquid nitrogen is faster than air blast, but it also negatively influences the cell walls and forms intra- and extracellular ice crystals due to thermal gradients. PSF samples formed a large number of small intra- and extracellular crystals without affecting the microstructure.

In case of frozen carrots, structure damage was clearly visible in the samples frozen by conventional freezing which is still-air freezing (−18 °C), while the samples frozen by cryostat bath (−18 °C) and cryogenic freezing (−80 °C) were similar to the fresh sample. However, carrots frozen by PSF (200 MPa, −18 °C) also had freezing damage. This result seemed to be in contrast with results of several other authors (Van Buggenhout et al. 2006a). Otero et al. (1998) reported that PSF can reduce structural damage of eggplant as compared with conventional still-air and air-blast freezing methods.

The textural attributes of vegetables are mainly about firmness, tenderness, and crispness (Vickers 1987). Rapid freezing leads to optimal texture of most foods due to less time taken for osmotic dehydration of cells. In the case of eggplant, Otero et al. (1998) reported that PSF caused less textural damage and lower drip losses in comparison to conventional still-air and air-blast freezing. The quality of PSF-frozen potato cube showed smaller difference between that of untreated controls than conventional freezing, in terms of texture and color quality (Koch et al. 1996).

Van Buggenhout et al. (2006b) found that the texture and drip loss in strawberries treated by PSF were not improved. The drip loss of PSF-frozen samples was slightly higher than that of conventionally treated samples. Moreover, the firmness

of frozen strawberries treated by both methods was greatly decreased. The author concluded that the influences of PSF in inducing quality loss exceeded the contributions of the small ice crystal information.

Studies on mangoes and peaches, demonstrated by using scanning electron microscopy, showed that pressure shift freezing leads to less textural changes and has an acceptable microstructure and it also reduces freeze-cracking problems (Otero et al. 2008). In the case of frozen carrots, Van Buggenhout et al. (2005) investigated the potential of pressure shift freezing combined with low-temperature blanching for preserving texture. The hardness of PSF-frozen carrots was improved markedly by pretreating the samples with calcium soaking and then followed by thermal treatment (60 °C, 30 min) or high-pressure treatment (300 MPa, 60 °C, 15 min) before PSF processes. However, the improved hardness of carrot could not be maintained under −18 °C storage. Blanched broccoli by PSF did not change in color and flavor. Hence, it can be safely concluded that the color of fruits and vegetables is slightly affected by PSF (Otero and Sanz 2011).

8.3 High-Pressure Thawing

8.3.1 Definition

The term high-pressure thawing includes two types of processes such as high-pressure-assisted thawing (HPAT) and high-pressure-induced thawing (HPIT). Some authors do not differentiate between them and use them interchangeably (Otero and Sanz 2003).

The difference between high-pressure-assisted thawing and high-pressure-induced thawing as described by Knorr et al. (1998) is that in HPAT, pressure increases up to 100 MPa and temperature increases throughout the holding time under the constant pressure, which leads to phase change during holding time. When pressure increases above 200 MPa, the phase change starts during pressure loading and it continues during the holding time under constant pressure and this process is termed as HPIT.

Otero and Sanz (2003) also tried to distinguish between HPAT and HPIT, so they applied different pressure level treatments to agar gel samples at different initial temperatures (−5 °C to −20 °C). They found HPAT to be prevalent at lower pressure levels of 50 and 60 MPa (−10 °C, −15 °C, and −20 °C) and at 90 MPa (−15 °C and −20 °C), wherein the phase change and melting did not start during the pressure loading phase, but during the holding phase, the temperature of the sample increased and completion of phase change occurred, whereas, in HPIT, partial melting of ice occurred during the pressure loading phase and it was observed at pressure levels of 110 and 210 MPa at all the temperatures and also at 50 and 60 MPa (−5 °C) and at 90 MPa (−5 °C and −10 °C) as well. During the holding phase, the melting reaches completion just like HPAT. So they stated that the sample thawing throughout the whole pressurization cycle (loading and holding) during HPIT is the main difference between these two processes.

8.3.2 Principles

The principles of high-pressure thawing are based on the studies of Bridgman (1912). He found out that water subjected to the pressure shows different behavior and different physical properties as compared to those at the atmospheric pressure. At atmospheric pressure, the melting point of ice is 0 °C, while by increasing the pressure to 210 MPa, it reduces to −21 °C. Under pressurized conditions, the phase changing time from ice to water will be shorter than the conventional thawing, because the temperature gradient is higher and the thawing is rapid (Okamoto and Suzuki 2002). Due to microbial growth restriction, the ambient temperature cannot be more than 4 °C during atmospheric thawing, which makes thawing process long (Boillereaux et al. 1999). In high-pressure thawing, the temperature difference between the frozen sample and ambient air provides significant driving force and allows large heat flow which results in increasing the thawing rate (Zhu et al. 2004a). The rapid thawing rate will further help to keep the better quality of the food product (LeBail et al. 2002a). Makita (1992) reported that the necessary time for high-pressure thawing is almost one third of the conventional method; moreover, the pressure did not affect the quality of the food product.

Zhao et al. (1998) reported that product size and initial temperature do not have any effect on thawing rate, while the level of pressure and the time of thawing process do affect the quality of product and the thawing rate as well. So they suggested that performing thawing under higher levels of pressure on even large amount of product would be beneficial. The size and dimensions of food are not a big deal in high-pressure processing because the pressure is transferred throughout the food uniformly and rapidly which makes this method a unique system (Galazka and Ledward 1995).

Karino et al. (1994) reported that necessary melting enthalpy reduces from 333 kJ/kg at atmospheric pressure to 241 kJ/kg at 193 MPa pressure. During high-pressure processing, this phenomenon decreases the energy involved in the phase change; hence, it requires lesser time for processing, so it results in better retention of food quality as compared to conventional thawing processes (Chevalier et al. 1999).

8.3.3 High-Pressure Thawing Curve

Figure 8.3 denotes a typical high-pressure thawing curve which is plotted between phase change temperatures of water versus pressure. The steps D–C–B–A show the pressure-assisted thawing, which takes place under constant pressure. In this process, reduction of the melting point of ice to −21 °C at 210 MPa (point C) leads to increase in the temperature difference between the product and surrounding, so this increased gap results in acceleration of heat flux. Higher heat transfer rate decreases the thawing time which is one of the most important attributes of high-pressure thawing.

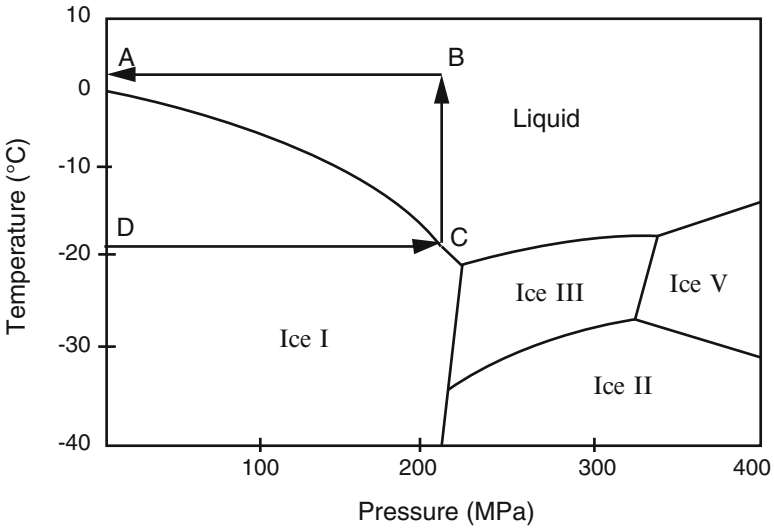

Fig. 8.3 A typical high-pressure thawing curve

It can be observed that by increasing the pressure from 0.1 to 210 MPa (steps D to C), the temperature of water for phase change decreases from 0 °C to −21 °C and melting does not start during the pressure loading phase (LeBail et al. 2002a, b). Temperature increases throughout the holding time under the constant pressure (steps C to B), which leads to phase change during holding time (Knorr et al. 1998), and completion of phase change occurs.

LeBail et al. (2002a) also described the high-pressure-assisted thawing of pure water sample. In their study, the temperature of pressurizing fluid medium was set above 0 °C. It was observed that during pressure loading cycle, the temperature of the sample decreased and then thawing took place under constant pressure cycle. When the temperature of the sample reached above the initial freezing point, the pressure was released. Lastly, the temperature of the sample reduced during depressurization and cooling occurred. They also stated that a similar phenomenon occurs in foods with high water content.

8.3.4 Advantages of High-Pressure Thawing

Conventional thawing takes a long time to complete, so many chemical and physical changes occur in foods during this process. High-pressure thawing is a good method to significantly decrease the side effects of conventional thawing and to keep the high retention of quality of food.

From an economic point of view, even due to the reduction of only the drip loss during high-pressure thawing, the investment for designing a high-pressure thawing

machine can be paid back very quickly. LeBail et al. (1997) stated that if even there is only 5 % saving in drip loss, by assumption, an equipment of 150–300 tonnes capacity per year with a product cost of 7.7–15 EU per kilogram will only take 2–3 years on an average to pay back the expenditure of installing the unit. In addition, reduction in microbial load can reduce the time required for subsequent processes such as pasteurization of ready-to-eat meals which eventually can help to decrease energy consumption and to increase productivity in industry.

From a productivity point of view, one high-pressure thawing equipment is worth 2–5 times more productive than a conventional atmospheric thawing system, because it is twice or in special situations five times faster than the latter. Furthermore, reduction in costs of labor and maintenance plays an important role in competition among companies in the market.

8.3.5 High-Pressure Thawing Equipment

High-pressure thawing is also carried out in similar equipment as pressure shift freezing (Fig. 8.2). However, there are certain differences in the components that have been used during thawing. Firstly, the type of pressure-transmitting medium in HPT has been different, although the same medium as PSF can also be used. In HPT few different pressurizing fluids have been applied in various previous studies, such as mineral oil/distilled water, 2/98, v/v (Zhu et al. 2004a), pure water (Otero and Sanz 2003), and hydraulic oil/water, 5/95, v/v (Zhao et al. 1998). Secondly, the temperature of the medium used for thawing under pressure is also higher as compared to freezing because they are meant for totally different purposes. From literature studies, it was found that many researchers have used different temperatures of pressure thawing media such as 20 °C (Zhu et al. 2004a, Otero and Sanz 2003) and 25 °C (Zhao et al. 1998).

8.3.6 High-Pressure Thawing and Microbial Growth

Favorable conditions for the growth of microorganisms are provided during atmospheric thawing because there is high water activity due to the melting of ice and/or condensing of water on the surface and pH is also around 7 which favors microbial growth. Hence, the temperature of surrounding air should be kept low throughout process to inhibit microbial growth (Mascheroni 2012) but this will lead to increased thawing time. However, high temperature difference between ambient air and the product during high-pressure thawing accelerates the heat transfer, which further leads to reduction in the thawing time, and it will also inhibit the growth of microorganisms.

While the surface and the corners of a product are at microbial growth risk in atmospheric thawing (Mascheroni 2012), they can be ignored in pressure thawing,

due to uniformity in passing of the pressure throughout the frozen product. Temperature and pressure have a synergetic effect on the lethality of microorganisms during high-pressure thawing (Chevalier et al. 1999).

Due to the importance of microorganisms' growth during thawing especially in fish and meat products, many studies have been done in this field. Destruction of microorganisms and limitations of their growth throughout high-pressure thawing have been reported for different types of foods.

While Hite (1899) reported that pressures more than 100 MPa can inhibit microbial growth, Murakami et al. (1992) showed a constant load of microbes during high-pressure thawing. Eshtiaghi and Knorr (1996) found that two log cycle reductions in vegetative microorganisms occurred during application of high-pressure thawing of frozen strawberries in which sugar solution was used as pressurization fluid.

In other studies, it has been described that high-pressure thawing at 100 and 200 MPa, respectively, resulted in 1.5 and 3 log cycle reductions in the number of *Listeria innocua* inoculated in salmon (Mussa and LeBail 2000) and also reductions up to four log cycles in indigenous flora and three log cycles in inoculated *Listeria innocua* in salmon were found when thawing was carried out at 200 MPa at 5 °C (LeBail et al. 2002b). Overall, the combination of low temperature and high pressure levels accompanied by rapid heat transfer restricts microbial growth during high-pressure thawing.

8.3.7 High-Pressure Thawing Effects on Proteins

High pressure disrupts the structure of secondary and tertiary bonded molecules but has no effect on covalently bonded molecules, so it results in denaturation of large protein molecules. Hence, protein denaturation and meat discoloration have been seen as some of the side effects of high-pressure application in many studies (Kalichevsky et al. 1995; Mertens and Deplace 1993). Similar results have been seen in studies conducted on tuna fish and surimi (Takai et al. 1991) and also in beef at pressure levels higher than 50 MPa during high-pressure-assisted thawing (Deuchi and Hayashi 1992). A pressure level of 150 MPa is known as the starting pressure for denaturation of proteins (Chourot 1997). Cheftel and Culioli (1997) also reported that protein modifications may occur at pressures from 100 to 210 MPa.

In Fig. 8.4, the structures of the high-pressure-thawed pork muscle strips are shown. A pressure of 100 MPa shrinks the sarcomere and there is no significant difference observed between A band and I band in comparison with the control. Samples thawed at 200 MPa show significant break of the filamentous structure of the I band and a loss of the M band. Higher pressures (300 MPa) make the sarcomere's structure unstable and break A and I filaments. Broken I filament results in loss of M line and thickening of Z line. At pressures over 400 and 500 MPa, many changes including the splitting of A band can be observed (Okamoto and Suzuki 2002).

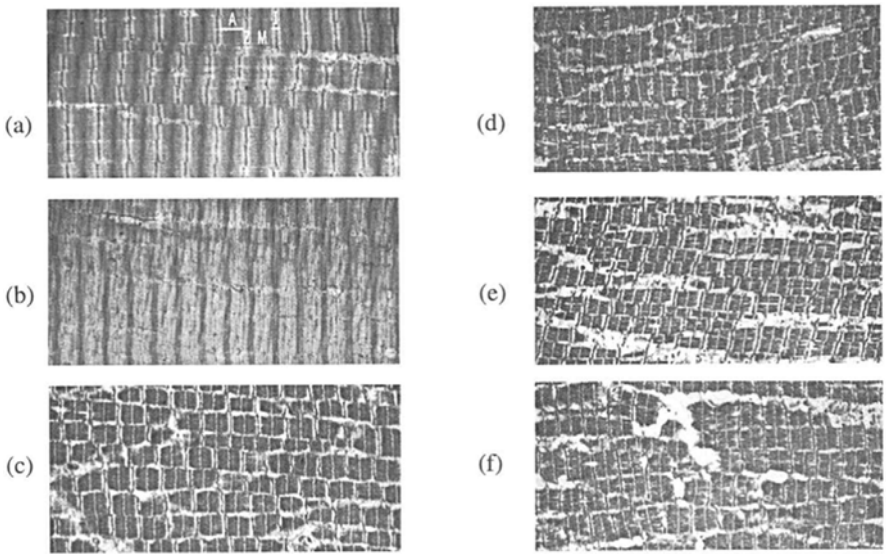

Fig. 8.4 Electron micrographs of high-pressure-thawed pork's muscle strips. (**a**) Control (thawed by running water); (**b**) thawed at 100 MPa; (**c**) thawed at 200 MPa; (**d**) thawed at 300 MPa; (**e**) thawed at 400 MPa; (**f**) thawed at 500 MPa (Okamoto and Suzuki 2002)

8.3.8 High-Pressure Thawing of Different Frozen Foods

8.3.8.1 Fish

The muscle structure of frozen fish is more sensitive to thawing than the meat muscle; hence, scientists have done many researches to improve and to maintain the quality of thawed frozen fish. From the economic point of view, both producers and consumers are concerned about the preservation and the loss of nutritional and sensory value of frozen or thawed fish by conventional methods.

Takai et al. (1991) reported that pressure can cause protein denaturation, which eventually causes color changes in tuna and surimi during pressure thawing and also results in hardening the texture of both fishes as compared with raw samples. But the positive side is that pressure can shorten the time of thawing and the temperature can also remain under 5 °C which is useful in restricting the growth of microorganisms. Another study that was done on tuna showed that high-pressure-assisted thawing can lead to color changes (increased L value) and result in reduced in drip loss, and further, no effect on microbial flora was observed due to HPAT (Murakami et al. 1992).

The results of comparison between high-pressure- and running-water-thawed carps showed that the carps exposed to 100–300 MPa pressure for 10 min had

similar quality attributes as the fresh ones, whereas running-water-thawed carps (15–17 °C) were characterized by lower breaking stress and lower elasticity, and hence they had more loss of textural quality. High-pressure application during thawing made the carp muscle lose its transparency and resulted in increase in L values. The color changes in muscle carps thawed at 100 MPa pressure and under running-water conditions were found to be similar. Protein denaturation was also recognized in high-pressure-treated carp muscles (Yoshioka et al. 1996).

High-pressure thawing has some effects on organoleptic characteristics of fish fillets, and in comparison with conventionally thawed ones (i.e., in water), the former is better in taste and texture (Schubring et al. 2003). Zhu et al. (2004a) reported that pressure levels of more than 150 MPa during thawing can produce color change in Atlantic salmon (*Salmo salar*) and can also modify texture at around 200 MPa. They also reported that different freezing rates before thawing have an effect on drip loss but have no effect on color and texture. Liquid nitrogen-frozen samples showed significant decrease in drip loss, whereas water immersion samples had a lot of drip volume.

Chourot (1997) found that pressure application of 150 MPa on pollock whiting fillets can minimize thawing and cooking drips. It was also reported that reduction of drip loss occurs during high-pressure-assisted thawing when the pressurization takes longer than the required time for appropriate thawing. The reason of this phenomenon was related to the effect of high pressure on improvement of heat transfer between fish fillets and drip loss after completion of thawing. In another study also, it was found that high-pressure thawing of aiguillat and salmon fishes resulted in significantly higher reduction of drip volume and also resulted in more reduction in both indigenous and inoculated *Listeria innocua* microbial loads as compared to those thawed in water at atmospheric pressure (LeBail et al. 2002b).

Rouillé et al. (2002) indicated that at 150 MPa pressure level, thawing drip loss was reduced to 70 % in spiny dogfish and 31 % for scallops, both on dry basis. At the same pressure level, total drip loss after thawing and cooking was found to decrease by 20 % for spiny dogfish, whereas it increased by up to 15 % for scallops.

Takai et al. (1991) concluded from his study that by performing high-pressure thawing at 150 MPa for aiguillat (spiny dogfish or *Squalus acanthias*) and scallops (*Pecten irradians*), an optimal decrease in drip loss can be observed. In comparison with atmospheric thawing, high-pressure thawing leads to reduced total drip (thawing and cooking), higher in magnitude for scallops and smaller for aiguillat, and for more reduction in drip loss, more time for pressurizing is required.

In addition, the main advantage of high-pressure thawing is reduction in thawing time. Increasing the pressure from 0.1 to 200 MPa in frozen fish fillets will lead to decrease in the thawing time from 1 h to 15 min (Chevalier et al. 1999).

It seems that restricted microbial growth, decreased drip loss, and reduced thawing time are some of the positive effects of thawing under high pressure which are common among most studies, while some other aspects such as color changes and protein denaturation need more consideration.

8.3.8.2 Pork

Massaux et al. (1999a) found that there is increase of pressure-induced discoloration and toughening in pork, and he further (Massaux et al. 1999b) proposed that freezing and thawing under 100 MPa is suitable for pork, due to the lack of exudation and slight discoloration and toughening.

Frozen pork meat has shown similar behavior in thawing losses as fish when exposed to high pressure levels. The maximum drip loss reduction was observed at 400 MPa. On the contrary, the cooking drip loss increased by increasing pressure levels, but as a whole, total loss in pressurized thawing was less than running-water thawing. By using high pressure, the water holding capacity also increased and meat tenderization occurred as well. The best results were obtained at 200 MPa pressure because, above this level, there was a significant effect on the structure of myofibrils (Okamoto and Suzuki 2002).

Park et al. (2006) studied the physical characteristics of frozen pork when exposed to high-pressure-assisted thawing at different pressures (50, 100, 150, and 200 MPa) at 15 °C. From his study, water holding capacity was found to increase by high pressure, whereas thawing and cooking drip losses at 50 and 100 MPa pressure were found to decrease. Pressures more than 150 MPa resulted in increased L and b values but a value got decreased.

8.3.8.3 Beef

There have been few studies on the application of high-pressure thawing on beef as compared with fish and pork. As large volumes of frozen meat are utilized in meat industry and a long time is being spent for thawing, substituting the regular method with an appropriate method for thawing time reduction will be advantageous for the industry. It was found that the quality of high-pressure-thawed beef at 120 MPa/−10 °C is the same as atmospherically thawed ones and, moreover, the pressure application shortens the thawing time to one third in comparison with atmospheric thawing. Higher pressures and lower temperatures make the beef harder and change the surface color to whitish due to protein denaturation (Makita 1992). Another research stated that optimal pressure-assisted thawing for beef with a significant effect on drip loss and color changes can be performed at 50 MPa, while higher pressures may result in denaturation of proteins and subsequently have an effect on meat whitening (Deuchi and Hayashi 1992).

Zhao et al. (1998) studied the effect of atmospheric thawing and high-pressure thawing (140, 210, 280, and 350 MPa) on ground beef. Some sample characteristics such as size and initial temperature had no effect on the rate of thawing, so it is beneficial to use this method for a larger amount of meat to decrease thawing time. High pressures of 210 or 280 MPa decreased the thawing time of a 250 g ground sample with an initial temperature −25 °C to 30 and 25 min, respectively, whereas in a 3 °C cooler, it took almost 12 h for the thawing process. The drip loss of high-pressure-thawed samples was negligible and no negative effect of high-pressure thawing was observed in cooking loss and color and texture changes.

8.3.8.4 Fruits and Vegetables

There have not been any considerable studies done on the high-pressure-assisted/high-pressure-induced thawing for fruits and vegetables. In one study, Eshtiaghi and Knorr (1996) demonstrated that by applying 600 MPa pressure (for 15 min at 25 and 50 °C) on slices and whole frozen strawberries in vacuum-packaged, heat-sealed polyethylene bags, there was a decrease in the total microbial count by 21 % and 140 % and an increase of sucrose uptake by 45.6 ± 2.4°Brix and 34.7 ± 0.9°Brix in slices and whole strawberries, respectively, as compared to atmospherically thawed strawberries at 92 °C for 20 min. Applied pressure makes cell walls permeable, so it improves the sugar uptake, and also due to degassing of the sample tissues, heat transfer is increased which results in more destruction of microbes.

8.3.8.5 Tofu

The texture of tofu is an important quality factor and it influences the final product quality. Fuchigami et al. (1998) studied the effects of high-pressure thawing on high-pressure-frozen kinu tofu (soybean curd) and compared the results with high-pressure-frozen tofu thawed at atmospheric pressure. Tofu samples were frozen at 100, 200, 340, 400, 500, and 600 MPa pressures and were then thawed at the same pressures. Based on the results, it was found that, in pressure-thawed tofu, pore size was the same as in untreated tofu, and also it was noticed that the ice crystals did not grow during freezing at 200–500 MPa. When the pressure was reduced after high-pressure freezing, the growth of ice crystals was noticed and it continued during the thawing at atmospheric pressure as well. This was attributed to the fact that the phase transition took place only when the pressure was reduced.

8.3.8.6 Cheese

Some dairy products are temporarily stored frozen, but conventional freezing and thawing can produce some unfavorable texture changes and exudation. In a study conducted on cheddar and mozzarella cheeses, high-pressure freezing of cheddar cheese at −20 °C in 200 MPa followed by pressure thawing has been shown to impart less negative side effects on the rheology of the samples, as compared with the freezing in a bath at −20 °C in 0.1 MPa pressure, but there were differences observed between them and nonfrozen cheeses in terms of deformation and compression. Deformation of frozen mozzarella cheese was more in rapid pressure-released samples (from 200 MPa, −20 °C) and thawed either at 0.1 or 200 MPa (in the fluid at 20 °C in both cases), whereas the effect of freezing at −20 °C in 0.1 MPa on the rheology of thawed mozzarella was negligible. Pressure-frozen and pressure-thawed mozzarella had lower elasticity and higher coherence and showed two cycles of 25 % compression without any break (Johnston 2000).

8.4 Conclusions

Freezing is a common and quick method for food preservation. In order to improve the quality of frozen foods, novel freezing methods have been developed recently. Pressure shift freezing as a novel freezing technique can provide benefits in accelerating freezing process, forming small ice crystals, and thus causing less tissue damage and more quality retention. Pressure shift freezing has been applied in various food products in Japan, the USA, and Europe. Many studies have been done on application of PSF on raw meat, fruits and vegetables, and gels and also storage and microorganism inactivation. The degree of influence of PSF on foods depends on properties of specific foods due to their complex constituents, so further investigations on pressure shift freezing application on various foods and their storage stability, quality improvement, microbial inactivation, and equipment development are necessary.

During thawing of frozen products, in addition to many different chemical and physical changes in foods, heat transfer makes the process more complex. High pressure shortens the thawing process by increasing the temperature difference between products and the surrounding and decreases drip volume and keeps quality better. Some factors including temperature, pressure level, pressure holding time, and characteristics of the product can affect the behavior of frozen food products during high-pressure-assisted thawing. In comparison with pressure shift freezing, less attention has been paid to high-pressure shift thawing. More studies about pressure-induced protein denaturation, color changes, and correlation between the drip loss and over-thawing time should be done to optimize this process for commercial application.

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Chapter 9
Pulsed High Pressure

Richard Meyer

Abstract This chapter briefly discusses the microbial efficacy of high-pressure pulsing on inactivation of various microorganisms. The importance of controlling key experimental variables for obtaining consistent results is discussed.

Keywords High pressure • Pulsing • Microbial efficacy • Lethality

9.1 Introduction

The impact of pulsed high pressure to achieve improved results versus a single pulse has been tested on prions, bacterial spores, various nonspore-forming bacteria, molds, and yeasts. The results have been mixed partly because the early studies did not account for all the variables impacting lethality, such as pH shift under pressure, fluid used in the vessel, and adiabatic heating (Balasubramaniam et al. 2004). No studies utilizing high-pressure pulsing on viruses appear in the literature (Hirnesien et al. 2010).

9.2 Microbial Efficacy of Pulsing

Table 9.1 compares the microbial efficacy of pressure pulsing. Prions are the most heat and pressure resistant to inactivation compared to bacterial spores and microbes in general. Pulsed pressure-assisted thermal processing was utilized in the initial research involving prions (Brown et al. 2003). Subsequent studies utilizing a single pulse and multiple pulses demonstrated that it was the time at pressure and temperature which inactivated the infectious prions and not pulsing (Cardone et al. 2006a, b). Once a threshold pressure (1000–1200 MPa) and temperature (135–142 ° C) were achieved with a hold time of at least 5 min (with or without pulses), there was greater than 6 log reduction in infectious prions (Cardone et al. 2006c).

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Table 9.1 Microbial efficacy of pressure pulsing

Authors	Peak Pressure (MPa)	Time at Peak Pressure (minutes)	Temperature at Peak Pressure (°C)	Pulses	Pressurization Time (seconds)	De-pressurization time (seconds)	Time at base pressure (seconds)	Media Treated				Microbiology			Vessel		
								Composition	pH	Water activity	Initial Temperature (°C)	Strain	Initial load (logs/gram)	Post HP load (logs/gram)	Pressure Fluid	Initial temperature (°C)	Sample Insulated
PRIONS																	
Brown, 2003; Cardone, 2006	1200	1	142	10	30	20	30	hot dog	6.8	0.98	85	1	6	0	water	85	polyethylene cartridge filled with castor oil
		5	135	1	30	20	0	hot dog	6.8	0.98	85	1	6	0	water	85	
BACTERIA	NR																
Hayakawa, 1994	800	60	60	1	NR	NR	0	0.1 M phosphate buffer	6.5	NR	NR	2	6	2	water	NR	NR
	400	5	70	6	NR	NR	NR		6.5	NR	NR	2	6	2	water	NR	NR
	600	5	70	6	NR	NR	NR		6.5	NR	NR	2	6	0	water	NR	NR
Sojka, 1997	500	60 MPa/1' & 500 MPa/1'	70	11	NR	NR	NR		NR	NR	NR	3	3	0	NR	NR	NR
Wilson, 1995	621	5	117.2	1	300	15	NR	raw meat	NR	NR	98	2, 3, 4	6	0	water	98	NR
Meyer, 2000	690	1	119	2	90	10	30	macaroni & cheese	5.87	0.98	90	3, 4	6	0	distilled water	90	yes
Ramaswamy, 2003	400	0	25	1	180	<15	0	apple juice	3.5	NR	13	5	8	0	distilled water + 2% mineral oil	13	
	300	5	25	4	135		0		3.5	NR	13	5		0		25	
	350 & 400	1 second						orange juice				6	3 to 4				
Yuste, 2001	450	15	NR	1	90	30	0	poultry meat	6.51	NR	20	9	7.93*	4.2 for both treatments	water	20	NR
	450	60 MPa/30' + 450 MPa/10' + 60MPa/30' + 450MPa/5'	NR	4			NR			NR	20		7.86**	2.9 for both treatments	water	20	NR
Lopez-Caballero, 2000	400	10	7	1	NR	NR	NR	oysters	NR	NR	2	10	5	0	NR	2	NR
	400	5	7	2	NR	NR	NR		NR	NR	2		5	0	NR	2	NR
YEASTS																	
Aleman, 1996	270	6.67	NR	1	0	NR	0	pineapple juice	NR	NR	23	7 & 8	5.1	0	distilled	23	NR
	270	0.6	NR	167	0.1	NR	0.2		NR	NR	23	7 & 8	4	0	water	23	NR
Donsi, 2007	250	1	25	10	10	3	60	pineapple juice	3.82	0.986	18	7	6	0	NR	18	NR
	250	1	25	10	10	3	60	orange juice	3.81	0.99	18	7	6	0	NR	18	NR
Donsi, 2010	300	60	50	10	30	2 to 5	0	orange juice	3.46	NR	NR	10	2	0	NR	50	temperature controlled vessel
	300	60	50	10	30	2 to 5	0	apple juice	3.42	NR	NR	10	2	0	NR	50	

Strain Number	Description
1	BSE & CWD Prions
2	Bacillus stearothermophilus
3	Bacillus subtilis
4	Clostridium sporogenes PA 3679
5	Escherichia coli ATCC 29055
6	Leuconostoc mesenteroides
7	Saccharomyces cerevisiae
8	Saccharomycopsis fibulugera
9	Unspecified mesophiles* & psychrophiles**
10	natural flora

9.3 Influence of Pressure Pulsing on Bacterial Spores

Research on the use of pulsed high pressure to inactivate bacterial spores started with *Bacillus stearothermophilus* IFO 12550 (Hayakawa et al. 1994) which is the process indicator organism for measuring sterility as specified by the US military specification MIL-S-36586 and by the FDA (CFR 21, 800–898). This research reported that a pressure of 600 MPa for 5 min with a peak temperature under pressure of 70 °C, which was repeated in six pulses, was sufficient to inactivate a spore load of 10^6/g in a spore suspension. This research was followed by Sojka and Ludwig (1997) which reported that using 11 one-minute pulses at 500 MPa with a starting temperature in the vessel at 70 °C inactivated a spore load of 10^8/g of *Bacillus subtilis* ATCC 9372. Wilson and Baker (1997) claimed to achieve complete inactivation of a spore load of 10^6/g from *Bacillus subtilis*, *Bacillus stearothermophilus*, and *Clostridium sporogenes* with a single pulse at 621 MPa and peak temperature under pressure at 98 ° C for 5 min. However, subsequent research found that if microbiological testing was conducted within 7 days, no growth would be indicated. However, microbiological testing after 30 days would show growth because injured spores would recover and begin growing (Meyer et al. 2000). Total inactivation was not achieved in these studies.

Bacterial spore inactivation was eventually achieved with both a single pulse and two pulses. For a single pulse, the lethality followed conventional thermal processing with a 6-min hold at 690 MPa at 121 °C under peak pressure but inactivated only 2–3 logs of the most heat- and pressure-resistant strain of *Clostridium botulinum* (strain 69A). However, pulsed high pressure (two 1-min pulses) at 690 MPa and 119 °C peak temperature under pressure inactivated 10^6/g of *Clostridium sporogenes* PA 3679 (Meyer et al. 2000) and 10^6/g of the same heat- and pressure-resistant strain of *Clostridium botulinum* 69A (unpublished data). This research was conducted at the Institute for Food Safety and Health, Illinois Institute of Technology, 6502 South Archer Road, Bedford Park, IL 60501-1957, USA, on the institute's 35 L Avure Technologies high-pressure vessel which was equipped with an insulated cartridge (Teflon). The test samples were preheated to 95 °C in the cartridge and then the cartridge was loaded into the vessel. The temperature was recorded in the samples at all times.

9.4 Influence of Pressure Pulsing on Vegetative Bacteria

The impact of pulsed high pressure on vegetative cells was reported by Ramaswamy et al. (2003) where multiple pulses at 350 MPa and 400 MPa with no hold time between pulses enhanced the lethality in eliminating *Escherichia coli* (29055) in apple juice compared to pressure and hold. Pulsed high pressure achieved the same log reduction but in less time at pressure compared to pressure and hold; four pulses at 1-s holds gave the same reduction as a single pulse with a hold time of 5 min. This is consistent with results found by Basak (2001) where a no-hold-time

pressure pulse of 1 s at 350 MPa and 400 MPa achieved 3 and 4 log reductions in the concentration of *Leuconostoc mesenteroides* in orange juice. Ramaswamy et al. (2003) concluded that a hold time of 1.7 min at 350 MPa will result in the same effect as a single pressure pulse. However, Yuste et al. (2001) found no benefit from pulsing at room temperature when applied to mechanically recovered poultry meat at 450 MPa with naturally occurring mesophiles and psychrotrophs. Lopez-Caballero et al. (2000) also found no benefit between pulsing and a static pressurization on the inactivation of the microflora in oysters.

9.5 Influence of Pulsing on Yeast

Pulsing appears to enhance the lethality against yeasts. Aleman et al. (1996) compared sinusoidal pulses, step pulses, and static-pressure pulses on pineapple juice inoculated with *Saccharomyces cerevisiae* 2407-1a. They found that sinusoidal pulses have no beneficial effects compared to a static pulse, but a step pulse was sharply more effective. Compared to a static pulse, the step pulse (0.66 s on pressure and 0.22 s off pressure) achieved a 4 log reduction on yeast counts in 100 s versus a single pulse between 300 and 900 s at 270 MPa at room temperature. Pulse times of 10, 100, and 200 per second gave the same results. Donsi et al. (2007) found that pulsing was effective in inactivating *Saccharomyces cerevisiae* in a model medium and commercial fruit juices. They noted that the number of pulses was most critical with a minimum hold at each pulse and that the pressurization rate was not a factor. By pulsing, the pressure could be reduced from 400 MPa to 250 MPa for effective stabilization of fruit juices. A follow-up study (Donsi et al. 2010) indicated that the effectiveness of pulsing depends on a combination of pulse number and pressure and temperature levels.

9.6 Conclusion

In conclusion, most of the research on pulsing did not control nor measure all the critical variables identified in the research protocols outlined by Balasubramaniam et al. (2004). This would explain why there are so many contradicting results reported in the literature. Without following the same protocols in executing the experiments and also following the same set of protocols in the follow-up microbiological testing, it is difficult to compare results. The differences between the results can be attributed to the lack of control of the key variables. Given that pulsing does not consistently provide an improvement over non-pulsing, there are no strong arguments for utilizing pulsing for commercial production except for sterilizing. In this application, pulsing was consistently effective in eliminating spores from all sources.

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Chapter 10
Applications and Opportunities for Pressure-Assisted Extraction

Stephanie Jung

Abstract Besides being an attractive alternative processing tool to enhance the shelf life and microbial safety of food while preserving their nutritional and quality attributes, high-pressure processing has the potential to modify the extractability of food compounds. This effect could improve pressurized food bioavailability and produce enriched extract for food and/or pharmaceutical applications obtained with improved extraction procedures and/or at a higher yield. This review presents some of the advancements of the use of high-pressure processing as an extraction processing aid.

Keywords High pressure • Extraction • Bioactive compound • Fruits and vegetables

10.1 Introduction

High-pressure processing (HPP) is an alternative technology, mostly used to inactivate pathogenic and spoilage microorganisms along with undesired enzymes affecting quality attributes, thereby increasing microbial safety and extending the shelf life of food products, including those free of chemical preservatives. Pressurized chemical preservative-free avocado puree (guacamole), beverages, and cooked meat products have been among the first ones commercialized, more than a decade ago. Since then, the variety of pressurized products commercially available has continuously increased, and new applications of this technology are constantly being identified. Because of the effects of high-pressure treatment on food components and on their inter- and intramolecular interactions and on micro- and macrostructure, there is indeed an array of other potential applications for this technology. Some of the potential applications having a recent particular interest include improving the

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texture of "healthy" products such as fat- and salt-reduced meat-based products or increased infusion of food products (Sikes et al. 2009, Villacis et al. 2008), while others, such as tenderization of *pre-rigor* meat, have regained interest (Smith et al. 2010). Others, such as the treatment of raw ground beef patties, recently reached commercialization stage under the brand Fressure™, with a process patented by Cargill. Another application having great potential is to use high pressure to modify (bio)accessibility and/or extraction of valuable compounds that are difficult to release from their matrix (Rastogi et al. 2007). Examples of these components include essential oils, bioactive compounds (including those having antioxidant and/or coloring properties), and aromas. One of the advantages of HPP is that, in most cases, for low molecular weight and covalent-bond-stabilized compounds, the biological function of the molecules of interest is not degraded during treatment. Also studies have shown that HPP has the potential to affect mass transfer, solvent penetration, and molecule interactions, thereby modifying parameters such as rates, duration, and extraction temperature and availability of bioactive molecules, along with positively affecting other parameters such as energy requirement and opening opportunities for alternative use of hydrocarbon solvents such as methanol or hexane. These assets are important considering the steadily growing interest of the food industry and consumers in sustainable aspects of food production and processing. HPP already has the advantage of being considered as a green "environmentally friendly" technology (Jung et al. 2011a, b) and is well perceived by the consumers compared to other alternative food processing technologies (Olsen et al. 2010). The consumers are also increasingly conscientious about the healthiness of the foods they are consuming, as illustrated by the impressive growth of the organic food market (Van Camp et al. 2010). Thereby the treatment that would improve the bioavailability of nutrients and increase nutrient intake could address consumer requests for healthier food. There is also increasing interest in the recovery of "natural" extracts by using alternative technologies to solvent-based methods that could replace the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) for food and/or pharmaceutical use (Zhang et al. 2004; Prasad et al. 2010) (Fig. 10.1).

Bioactive compounds are available at a higher content in plant materials; therefore, most of the studies involving the potential of pressure extractability use fruits, vegetables, and herbs as starting materials. It is important to note however that high-pressure processing is successfully and widely used by the seafood industry as an extraction/separation tool. Besides its potential of promoting the safety of seafood products, high-pressure processing has the unique property to promote an easy separation of the meat from the seafood shell, thereby greatly improving the separation/extraction yield of crustacean and mollusks (http://www.youtube.com/watch?v=GZL6jQjWu-M, Murchie et al. 2005). The potential of high-pressure processing for the seafood industry is reviewed in Chap. 27 and will not be further discussed in this chapter.

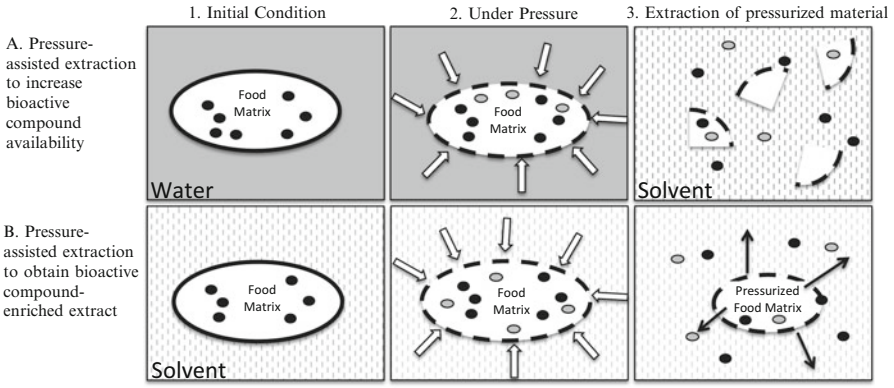

Fig. 10.1 Schematic representation of the steps commonly applied in studies reporting effect of high-pressure processing (HPP) on food compound extractability. (*A*) HPP is applied to study its effect on extractability/bioavailability of food. After treatment in water, the pressurized matrix is transferred into a solvent and often submitted to a mechanical treatment (steps 2 to 3). (*B*) HPP is applied to obtain an enriched extract for food and pharmaceutical applications. The matrix (usually dried) is dispersed into a solvent in which the food is pressurized, and extractability into this solvent is determined after treatment. Prior to extractability determination, a centrifugation step is often applied (i.e., reduction of the volume). Mechanical treatment can also be applied, but this option was not represented here. In step 2, change of membrane permeability can be one of the mechanisms behind change of extractability. It is more likely that some of the solvent in which the food matrix is dispersed will enter into the product during treatment. *Black circles*: compound of interest. *Gray and dashed circles*: water or other solvents

10.2 Difficulties in Understanding Mechanisms behind Increased Extraction

There is a broad range of explanation and collection of evidence in the literature when a change (increase or decrease) of the concentration of a compound in an extract recovered from pressurized food is observed. This variation has been related to (1) a change of its extractability due to a change in cell permeability and/or properties of the compound itself and/or surrounding compounds in the matrix; (2) a better barostability vs. thermostability of the component; (3) changes in chemical/enzymatic reactions under pressure; and/or (4) residual enzyme activities which impact the compound degradability. Depending on the food matrix, some of these effects may have greater consequences on extractability, and potentially all these parameters, but at different extent, impact the effect of the process on the final concentration of a compound in an extract. The complexity of the phenomena occurring during treatment, along with the challenges to not only characterize mechanisms occurring under pressure but also to translate results that are obtained in a purified environment to the ones obtained in a food matrix, contributes to this difficulty. This fact can be illustrated by the following example: When lysosomal enzymes, which are involved

in acquisition of meat tenderness, are either extracted from meat in a crude extract or purified and dissolved in a buffer solution prior to pressure treatment, a significant decrease of their activities above a pressure of ~400 MPa is reported (Jung et al. 2000, Buckow et al. 2010a). However, when meat is directly submitted to the treatment and lysosomal activities determined from pressurized meat, an increase in activities is reported with increasing pressure, which has been correlated to rupture of the lysosomes, the structure in which lysosomal enzymes are trapped (Jung et al., 2000). Similarly, the activity of commercial pectin methylesterase gradually decreased with increased pressure (200, 400, 600 MPa), while the enzyme activity of a crude pectin methylesterase in extracts from pressure-treated whole tomatoes was maintained regardless of pressure level (Tangwongchai et al. 2000). Another example to illustrate the difficulty of identifying the mechanisms responsible for variation of bioactive compound content in an extract recovered from a pressurized food is the case of the anthocyanins. Enzymes such as polyphenol oxidase and peroxidase are able to degrade anthocyanins, and therefore residual activities of these enzymes after treatment could have an important effect of final anthocyanin content (Zabetakis et al. 2000; Terefe et al. 2009; Buckow et al. 2010b).

The majority of the studies reported in this chapter related a partial or total change in the concentration of a compound in an extract obtained from a pressurized food to an effect of HPP on its extractability. The case of increased enzyme activities observed after treatment of a food product, which can involve, among other reactions, an increase in the enzyme extractability, is not the focus of this chapter.

10.3 Pressure-Assisted Extractability of Vitamins

The effect of HPP on vitamin content of pressurized food products varied widely depending on the processing conditions and the product being treated. An increase of 24 % of vitamin A was reported after treatment of tomato puree at 400 MPa for 15 min at 25 °C (Sanchez-Moreno et al. 2004). Similarly, when a moderate treatment (100 and 200 MPa, 10 and 20 min, initial temperature of 18–20 °C) was applied to yellow bell peppers as an alternative to thermal blanching, the ascorbic acid (AA) content of the extracts from the pressurized yellow bell peppers was significantly increased in all conditions, with an increase from 166 (control) to ~250 mg/100 g fresh weight after treatment at 200 MPa for 20 min (Castro et al. 2011). This increase was related to a higher extractability along with the barostability of the AA and its oxidation inhibitors. The protein content of the soluble enzyme fraction was also maximized in these conditions, increasing from ~15 to 21 g protein/100 kg fresh weight. Such an increase of AA content was not observed after treatment of cashew juice (250 and 400 MPa, 25 °C, 3, 5, or 7 min) (Queiroz et al. 2010). When compared to a thermal treatment of 98 °C for 21 s, a treatment in the 100–400 MPa range for a duration of 420 s or above, of a vegetable beverage made of tomato, green pepper, green celery, onion, carrot, lemon, and olive oil, decreased the ascorbic acid content by 9 % vs. 11 % with the thermal treatment (Barba et al. 2010).

In many studies reporting the effects of HPP on vitamins, no increase in the vitamin (B, C) content of extracts obtained from a wide variety of pressurized foods was observed (Sanchez-Moreno et al. 2009; Butz et al. 2003). Loss of vitamins was reported for strawberry puree after treatment at 400, 500, or 600 MPa for 15 min at initial temperature of ~20 °C (Patras et al. 2009). Similarly, for Granny Smith apple puree (pH 3.4), 57 and 14 % residual AA content was observed after 400 and 600 MPa, respectively (5 min, 20 °C), vs. 61 % for the pasteurization treatment (Landl et al. 2010).

From these few examples, it can therefore be observed that caution must be used regarding the benefits of applying HPP treatment to maintain vitamin content similar to the "fresh" products. Starting material, HPP processing conditions, storage conditions, and also the treatment conditions chosen for the control (often thermal treatment) need to be considered. However, high-pressure processing remains in many cases an attractive alternative to thermal treatment as the vitamin loss is usually lower than what is obtained with a thermal treatment, and the quality attributes are also maintained compared to thermal treatment.

10.4 Pressure-Assisted Extractability to Improve Bioactive Compound Availability

Due to their potential in reducing the risk of major chronic disease, effects of HPP on health-related compounds including phytochemicals have been reported (Kris-Etherton et al. 2002; Sanchez-Moreno et al. 2009). Phytochemicals are classified into four main groups: (1) the terpenoids, which include the carotenoids, which are tetraterpenes; (2) the phenolics and polyphenolics including the flavonols, having a polycyclic structure; (3) the nitrogen-containing alkaloids; and (4) the sulfur-containing compounds (Jaganath 2008). Among them, the effect of HPP on the carotenoids and the phenolics has been of major interest. These compounds, because of their structure, are not likely to be barosensitive. High concentrations of phytochemicals are found in fruits and vegetables, which have been the matrix of choice for studying the effect of the treatment on nutritional quality, including bioactive compound extractability/bioavailability (Oey et al. 2008).

10.4.1 Vegetables

Because of the potential importance of the impact of processing conditions (pressure level, temperature, and dwell time) on the food matrix structure and the corresponding compound extractability, characterization of plant cell properties, including structure, has been investigated (Gonzalez and Barrett 2010). It was observed that onions submitted to pressures of 300 MPa and above experienced a total loss of membrane integrity. Evidence of these structural changes was obtained with neutral

red staining (Gonzalez et al. 2010a) and ion leakage measurements (Gonzalez et al. 2010b). Conductivity (expressed as % of total) increased from ~30 to 100 % after treatment at 200 and 300 MPa, respectively. When submitted to pressures from 20 to 200 MPa (5 min), changes in membrane permeability determined by ^{1}H-NMR quantification occurred at 200 MPa, when initial temperature of the treatment was 20 °C, while no significant changes were observed for a initial temperature of treatment of 5 °C (Gonzalez et al. 2010b). The treatment at 400 MPa applied at 20 °C was less important than a thermal treatment of 60 or 90 °C, as illustrated by the T_2 values of 0.95 and 0.70 s vs. 1.18 s for the raw onion. The treatment also decreased water mobility in pressurized onions, which was attributed to the effect of the treatment on other macromolecules present, which may impact binding properties. Characterizing the micro- and macrostructure of the food being treated is an important step in understanding the effects of the treatment and how to select best processing conditions. There is, however, no straight correlation between structural changes and extractability, as well as many other parameters, such as changes in physicochemical properties of various components and chemical and enzymatic conversion reactions. The structural changes observed in the onion structure most likely contributed to the increased total phenol and flavonol content reported in the study of Roldan-Marin et al. (2009), which covered pressure and temperature range of 100–400 MPa and 5–50 °C, respectively. Interestingly, better extractabilities were obtained at a temperature of 5 °C. For example, after treatment at 100 MPa and 5 °C, total quercetin content increased (when compared to the untreated onion) by 26 % vs. 18 % at 50 °C, quercetin-4′-glucoside increased by 33 % vs. 28 %, and quercetin-3,4′-diglucoside increased by 17 % vs. 10 %. An optimal low temperature for the recovery of these bioactive compounds was explained by the fact that this temperature would not be responsible for bioactive content loss.

Treatment at pressures of 300 and 400 MPa significantly impacted the structure of carrots, with a treatment of 2 min increasing the cell wall thickness by 10 % (Trejo Araya et al. 2007), with lower pressures (100 and 200 MPa) having a minimum impact. The authors reported cell wall buckling, folding, and reduction of cell contact for the pressurized carrots. The treatment favored the elongation of the cells, with elongation indices increasing from <5 to up to 10 after HPP (100, 300, or 550 MPa; 2, 10, or 30 min; initial temperature of 20 °C), an index of 1 and 20 characterizing a more spherical particle compared to a highly elongated particle, respectively. The cell shape factor also illustrated deformation due to pressure treatment, which was related to the compression and decompression effects. In a recent study, the structure of a carrot subjected to mild and severe pasteurization and sterilization treatments obtained with thermal vs. pressure treatment was compared (Knockaert et al. 2011).

A treatment at 400 and 600 MPa for 2 min at room temperature, however, did not modify the α- and β-carotene content of extracts recovered from pressurized carrots in the study of McInerney et al. (2007). In the same study, the treatments also failed to improve lutein content in green bean extract and lutein and β-carotene content in broccoli extract. The authors, however, reported, in the case of beans treated at 600 MPa, an increase of lutein availability following simulated gastrointestinal digestion.

Tomato is among the top consumed vegetables in the world. Therefore, the effect of high-pressure treatment on tomato products has been of particular interest. While tomato is a fruit, most of the studies investigating its behavior under pressure refer to it as a vegetable and will therefore be reported as such in this section. Among the carotenoids present in tomatoes, lycopene is of particular interest because of the red color it confers to ripe tomatoes and its protective effect against epithelial cancers (Shi and Le Maguer 2000). The visual appearance of cherry tomatoes pressurized at 20 °C for 20 min was pressure dependent. As pressure increased from 200 to 400 MPa, a degradation of their appearance was observed, while above 400 MPa, textural damage was reduced (Tangwongchai et al. 2000). Similarly, the percentage of cell rupture reached a maximum at a 400 MPa pressure level, and pressure-treated tomato tissue structure revealed modifications that were pressure-level dependent.

Various effects of HPP have been observed on the extractability of bioactive compounds from pressurized tomato-based products. With tomato puree, no effect of HPP was observed after treatment at 300 and 600 MPa for 10 and 60 min and an initial temperature of 20 °C on total carotenoids (extracted with tetrahydrofuran) (Fernandez Garcia et al. 2001). The extraction of readily extractable carotenoids (extracted with petroleum ether) was however significantly reduced with a recovery of ~40 % at 300 MPa (increasing treatment time from 10 to 60 min having no impact) compared to ~60 % recovery after a 95 °C thermal treatment for 10 or 60 min. Pressure level had a significant impact on readily extractable carotenoids, decreasing to ~20 % at 600 MPa. These differences were attributed to the alterations in the microstructure of the tomato pulp with a formation of a barrier around the structure containing the carotenoids and involving exposition of hydrophilic structures and cell decompartmentation. These changes also affected the water and glucose binding, with water release from the pressurized tomato pulp at 600 MPa for 60 min decreasing from 80 % for the control to 49 %. Interestingly, retention of total lycopene content in an extract from pressurized tomato puree was reported to be ~160 % with a 500 MPa treatment (20 °C for 2 min), while pressures of 300 and 700 MPa, at the same conditions, or 700 MPa, 80 °C, two pulses of 30 s; 90 °C, 700 MPa, 30 s; and 90 °C, 700 MPa, two pulses of 30 s, did not have a significant effect (Krebbers et al. 2003). This study also observed variations in viscosities of pressurized tomato purees, which were suggested to be due to changes in extractability of cell wall components. HPP can indeed affect pectin extractability as illustrated with variations of uronic acid extractability of berries treated at 400 MPa for 15 min, at an initial temperature of 35 °C (Hilz et al. 2006). It was also demonstrated that further degradation of pectic polysaccharides can be achieved by addition of pectic enzymes during treatment.

Interestingly, when tomato juice was treated at an initial temperature of 4 and 25 °C for 10 min at a pressure above 200 MPa (300–500 MPa), the extractability of lycopene and carotenoids gradually increased up to 62 and 56 %, respectively, at 500 MPa (Hsu 2008). Increased pressurized tomato juice redness was correlated to this improved lycopene extractability. However, when the initial processing temperature was increased to 50 °C, the extractability was not improved regardless of the pressure level (100–500 MPa), which was attributed to the effect of treatment on

cell membrane and other macromolecules in the juice matrix including proteins. During the refrigerated storage up to 28 days at 4 °C of the pressurized tomato juices treated at an initial temperature of 25 °C for 10 min, the total carotenoid and lycopene contents were maintained, contrary to the heat-treated juices for which the contents decreased during storage (Hsu et al. 2008). Similarly, Gupta et al. (2010) observed an improvement in lycopene extractability from pressurized tomato juice. After treatment at 700 MPa, at an initial temperature of 45 °C for 10 min, extractability of total lycopene from tomato juice was increased by up to 12 %, and after increasing the initial temperature to 100 °C, a 7 % increase was still reported at a pressure level of 600 MPa applied for 10 min. Differences in the extent of lycopene extractability between studies were attributed to variations in different parameters including processing conditions and tomato cultivar, which was shown in the study of Gupta et al. (2010) to have a significant impact on lycopene extractability. This observation concurred with the study of Pudney et al. (2011), which reports, using confocal Raman microspectroscopy, changes in the carotenoid location of tomatoes from different cultivars, which more likely can affect their extractability. Carotenoids are in a microcrystalline structure in untreated tomatoes, which could be a major drawback in their bioavailability when ingested (Pudney et al. 2011), and the effect of high-pressure treatment on their physical state should also be determined. After a thermal treatment, carotenoids are indeed solvated in a lipid globule, which could improve their bioavailability. Another possible reason for the variability in the extent of extraction due to pressure treatment between studies is the difficulty to differentiate between the degradation rate of the compound of interest and the simultaneous increase or decrease of its extractability. A degradation of total lycopene of 20.8 and 56.3 % was reported when treated in hexane solution at 500 and 600 MPa, respectively (initial temperature of 20 °C, 12 min), while the lycopene content of an extract from tomato puree treated at 500 MPa increased from 5.2 to 6.2 mg/100 g (Qiu et al. 2006). The use of hexane probably had an important effect on the temperature increase due to the difference in adiabatic compression heating of hexane compared to water. However, this study pointed out that the treatment can have, for some conditions, an effect on lycopene structure.

10.4.2 Fruits

Consumption of berries such as raspberries and strawberries has significantly increased over the last couple of years, and these highly perishable fruits could benefit from a shelf-life extension using HPP. However, when treated as a whole fruit, a significant change in the appearance and texture of these delicate fruits occurred. Using nuclear magnetic resonance, Otero and Prestamo (2009) observed a significant water migration in strawberries treated at a pressure of 100 and 200 MPa (10 min, 7.5 °C) and occurrences of structural modifications due to the presence of air bubbles in the fruits (air being more compressible than liquid and solid present in the matrix). Changes in the whole fruit appearance could be avoided by the treatment of

the berries as a puree and/or juice. When treated at 600 MPa for 15 min at 20 °C, the total phenol content of an extract from pressurized strawberry puree increased from 855.02 mg GAE/100 g DW to 939.01 mg GAE/100 g DW, which was attributed to an increase of their extractability, while it decreased to 817.01 mg GAE/100 g DW after thermal treatment at 70 °C for 2 min (Patras et al. 2009). The anthocyanin content was not altered through the pressure treatment, while the thermal treatment significantly decreased its content. Similar trends regarding the anthocyanin and total phenol content of pressurized vs. thermal-treated samples were observed for blackberry puree. When combined with a thermal treatment varying from 20 to 60 °C, applying pressures ranging from 300 to 600 MPa for about 10 min did not significantly affect the total phenol and total anthocyanin content of pressurized strawberry extracts (Terefe et al. 2009). However, 22 and 27 % degradation of total phenol content and total anthocyanin content, respectively, was observed after 3 months of refrigerated storage of the pressurized strawberries, which could be due to residual activities of anthocyanin-degrading enzymes. Determination of the kinetics of anthocyanin degradation of pasteurized blueberry juices treated at 100–700 MPa at (initial or process temperature ranging from) 60–121 °C showed that pressure enhanced the anthocyanin degradation, which was attributed to pressure promoting condensation reactions involving anthocyanins (Buckow et al. 2010b). Effect of treatment conditions on activities of enzymes responsible for anthocyanin degradation was also indicated as being responsible for the variation of its content in pomegranate juice treated at 400–600 MPa at 20, 45, or 50 °C for 5 or 10 min (Ferrari et al. 2010). A slight increase of its content was observed when treatment was applied at 50 °C, for 500 MPa, 5 or 10 min, or 600 MPa, 10 min.

The benefits of pressure on Brazilian native cashew fruits have been investigated. Soluble polyphenolic content of cashew apple juice was increased by up to 28 % after treatment of 3 and 5 min at 250 MPa or 400 MPa (25 °C), but this effect was no longer observed after a 7-min treatment time (Queiroz et al. 2010). Benefits of applying HPP to promote extractability of polyphenolic compounds were also dependent on processing conditions (450 MPa, initial temperature of 20 °C, for 1, 3, and 5 min) for a fruit smoothie made with whole apple, apple juice from concentrate, strawberry, banana, and orange (Keenan et al. 2011). A treatment applied for 3 min increased the procyanidin B1 and hesperidin contents, initially at ~185 μmol/100 g DW and ~200 μmol/100 g DW, respectively, to ~200 and 230 μmol/100 g DW, respectively, while increasing the processing time to 5 min decreased significantly the hesperidin content to ~135 μmol/100 g DW. In the same study, a treatment of 5 min did not affect the level of chlorogenic acid compared to the unprocessed smoothie. The same authors previously reported that the thermal-treated smoothie had a higher level of total antioxidants than the pressure-treated smoothies and the control smoothies, while the pressurized samples had higher phenol contents than the thermally processed samples (Keenan et al. 2010). HPP treatment failed to increase the total polyphenol content of Granny Smith apple puree (pH 3.4); the total polyphenol content remained similar to the one of raw puree (1,180 mg/kg) after a treatment at 400 MPa (5 min, 20 °C), while it decreased to 888 mg/kg with a 600-MPa treatment (Landl et al. 2010).

10.4.3 Additives to Improve Pressure Extractability

In order to prevent reactions enabling degradation of bioactive compounds, the potential of adding additives before pressure treatment of the product of interest has been investigated. The results showed mixed benefits of such an addition. When muscadine grape juice was treated at 600 MPa for 15 min, with no additives added, the total soluble phenolic contents increased significantly from 1360 for the unprocessed juice to 1420 mg/L, while the monomeric anthocyanin and total anthocyanin contents (responsible for the juice color) remained unaffected by the treatment (Talcott et al. 2003). When the juice was fortified with ascorbic acid (~800 mg/L), some decreases in the total soluble phenolic and monomeric anthocyanin contents were observed, compared to the control (fortified juices not submitted to HPP). In the presence of ascorbic acid, pressurized juices with added rosemary extract (0.1–0.4 %), added because it contains polyphenolic cofactors, had higher total soluble phenolics compared to the ascorbic-free pressurized juices containing the same concentration of rosemary extract.

Addition of copigments extracted from thyme and rosemary to grape juice treated at 400 MPa and 550 MPa prevented degradation of total anthocyanin compared to the controls (juices pressurized without additives) (Del Pozo-Insfran et al. 2007). The same trend was observed for ascorbic acid-fortified juice. Addition of sodium chloride (0–0.8 %) and citric acid (0–2 %) on tomato puree did not improve carotenoid extractability which was found to reach maximum at 400 MPa, the highest pressure level applied (Sanchez-Moreno et al. 2004).

10.4.4 Change in Extracted Bioactive Profile

High pressure has another unique potential, which is to produce products with distinct bioactive compound profiles, being the result of partial or total change in their extractability. This property was illustrated in the studies of Plaza et al. (2011) and Sanchez-Moreno et al. (2005). When a treatment of 400 MPa, initial temperature of 36 °C, was applied for 1 min to orange juice, the extractability of individual carotenoids, lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene, increased by 33.7, 74.0, 89.4, 27.6, 46.9, and 57.0, respectively, while vitamin A and flavanone hesperetin increased by 34.7 and 16.1 %, respectively. Similarly, the content of total carotenoids extracted from a vegetable soup (*gazpacho*), containing 50 % tomatoes, 15 % cucumber, 10 % green pepper, and 3 % onions, treated at 150 and 350 MPa (60 °C, 15 min), was not statistically different from the control on the day of treatment (Plaza et al. 2006). However, the treatment at 350 MPa changed the carotenoid profile, increasing the lutein content by 31 % and the β-carotenoid content by 17 %, which was attributed to the denaturation of the proteins under pressure, releasing more of the carotenoids from the protein/carotenoid complexes. This explanation differed from other studies, which usually correlate a pressure-induced protein denaturation to a decrease of matrix compound extractability.

Selectivity in the treatment was also observed during pressurization of black table olives (250 MPa, 35 °C, 5 min), with an increase of total phenolic content by a factor of up to 2.5; the individual phenolic hydroxytyrosol increased by a factor of up to 2, while the oleuropein content decreased. This selectivity in the effect of the content of these two phenolic components is of particular interest because of the bitterness characteristic of oleuropein (Tokusoglu et al. 2010).

10.5 Application of High-Pressure Treatment Extraction in Food and Pharmaceutical Industry

Microwave, ultrasound, supercritical fluid, and accelerated solvent are among the conventional and nonconventional extraction techniques studied most extensively for the recovery of enriched bioactive extract from plant sources, with each of these methods having its own advantages and drawbacks (Chemat et al. 2011; Virot et al. 2010; Masom et al. 1996). High-pressure processing, as an extraction tool, compared to these technologies is still in its infancy but could be an environmentally friendly alternative process, which has the advantage of being well perceived by the consumers.

For this application, in most cases, the starting material is dried before HPP treatment and parameters such as pressure level, dwell time, solvent-to-sample ratio, and concentration of solvent optimized (Fig. 10.1, Table 10.1). In some of the studies, a significant increase in extraction yields was reported. When a significant increase was not observed, HPP significantly reduced the required time, when compared to treatment applied at atmospheric pressure (Table 10.1). HPP can also be used as a means to add value to coproducts, often under valorized waste generated during production of a certain food. For example, when grape by-products were treated at 600 MPa for 1 h at 70 °C, a significant change in the profile of the anthocyanins extracted was noticed (Corrales et al. 2008). The extraction of acylated anthocyanin monoglucosides was favored, while the content of anthocyanin monoglucosides remained the same with a control sample. According to the authors, this selectivity could be attributed to a pH decrease of the solvent due to higher release of phenolics but also to deprotonation of the molecules.

Extracting gelatin from fish skins is a time-consuming treatment, and high-pressure application at 10 °C was investigated during the acid swelling and the water extraction step (250 MPa for 10 or 20 min, 400 MPa for 10 min or for two pulses of 5 min) (Gomez-Guillen et al. 2003) as a tool to accelerate this process.

10.6 Other Applications Using HPP as an Extraction Tool

HPP has been investigated as a processing aid to improve extractability of soybean oil using environmentally friendly technologies (Campbell et al. 2011; Kapchie et al. 2008; Jung and Mahfuz 2009). These green alternative technologies have the

Table 10.1 Examples of use of HPP as a tool to recover an enriched extract for food and/or pharmaceutical application

Starting material	Molecule(s) of interest	Parameters tested	Major results	Authors
Bitter melon	Momordicosides (saponins)	Pressure (300–500 MPa)	Best extraction yield (pressurized vs. control): 3.258 vs. 2.478 g/100 g	Ji et al. (2010)
		Dwell time (3–9 min)		
		Solvent-to-sample ratio 30–50 mL/g		
		Ethanol concentration (60–80 %)		
Deodeok (perennial flowering herb)	Phenolic acids	Roots were treated at 500 MPa, 50 °C, for 30 min in 70 % ethanol, compared with conventional extraction (70 % ethanol, 80 °C for 24 h), and followed or not with fermentation	Extraction yield increased from 21.76 % to 32.14 %. Extraction was not enhanced by fermentation	He et al. (2010)
Grape by-products	Anthocyanins	Solid-to-liquid ratio 1:4.5, 50 % ethanol, 600 MPa, 1 h, initial temperature 70 °C	Total phenolic content was increased 1.5-fold and antioxidant capacity threefold. Treatment allows extraction selectivity in compound of interest	Corrales et al. (2008)
Green tea leaves	Caffeine	Pressure (100–600 MPa)	Treatment at 500 MPa for 1 min at room temperature in 50 % ethanol at a 20:1 ratio gives same extraction yield than 20 h at room temperature	Xi (2009)
		Dwell time (1–10 min)		
		Ethanol vs. acetone, methanol, and water		
		Ethanol concentration (0–100 mL/mL)		
		Liquid-to-solid ratio (10:1–25:1 ml/g)		
Jerusalem artichoke	Total phenols	100 MPa, 50 °C, 24 h, combined or not with enzymatic treatment and fermentation	Pressure alone increased extractability from 35.5 to 48.37 mg TAE/g extract. Combination of HPP, enzyme, and fermentation increased value to 73.8 TAE/g extract	Kim et al. (2010)
Korean barberry (medicinal plant)	Total phenols	100 g of dried stem in 900 mL water, 500 MPa, 30 °C, 30 min vs. 80 °C for 24 h (conventional extraction)	Content increased from 118 mg GAE/g to 228 mg GAE/g. Probiotic fermentation did not enhance extractability	Lee et al. (2010)

Litchi	Total phenolic content, flavonoids	Pressure (200, 400 MPa) 30 min, 25 °C 5 g in 200 mL ethanol:HCl (85:15)	Compared to conventional extraction (30-min stirring at 25 °C), extraction yield increased from 1.83 to 30 % for pressurized samples. Total phenolic content remained similar to control but flavonoid concentration increased by a factor 10	Prasad et al. (2008)
Longan fruit pericarp	Total phenolic, antioxidant, and antityrosinase activities	Pressure (200–500 MPa) Dwell time (2.5–30 min) Initial temperature (30–70 °C) Ethanol concentration (25–100 %) Solid-to-liquid ratio (1:25–1:100, w/v)	At 500 MPa, 30 °C, and 10 g in 500 mL of 50 % ethanol for 30 min, total phenolic extraction was 20.8 vs. 11.9 mg/g DW for the control. Dwell time has no effect on total phenolic content and extraction yield	Prasad et al. (2009a, b, c, 2010)
Panax ginseng	Ginsenosides	Pressure (100–600 MPa) Dwell time (1–5 min) Ethanol concentration (10, 25, 50, 70, 90 %) 50 % ethanol vs. water, methanol, *n*-butanol Solid-to-liquid ratio (1:10–1:100)	Dwell time has no effect while at 600 MPa yield was 7.5 vs. 7.25 % at 100 MPa. Yield varied with ethanol concentration from 5.13 to 7.51 and from 6.17 to 7.51 with solid-to-liquid ratio	Zhang et al. (2007)
Propolis[a]	Total polyphenol and flavonoid content	Pressure (100–600 MPa) Dwell time (1–10 min) 95 % ethanol vs. 95 % methanol, 5 % sodium bicarbonate, water Ethanol concentration (35–95 %) Solid-to-liquid ratio (1:5–1:45 g.cm^3)	At 1:3.5 (g/mL) solid-to-liquid ratio, 75 % ethanol, 500 MPa, 1 min, and room temperature, the total polyphenol and flavonoid contents were similar to an extraction of 7 days in 70 % ethanol	Zhang et al. 2005; Xi and Zhang 2007
Rhodiola sachalinensis (herb)	Salidroside, flavonoids	Pressure (100–500 MPa) Dwell time (1, 3, 5, 7 min) Ethanol concentration (0, 30, 50, 70, 90 %) Solvent-to-material ratio (10:1–70:1)	At 500 MPa, 25 °C, 3 min, 60 % ethanol, and 50:1 ratio, 82.7 % salidroside extraction yield was obtained vs. 100 % after 240 min at 80 °C with Soxhlet extraction, and flavonoid extraction was improved from 2.1 % (Soxhlet) to 5.2 %	Zhang et al. (2007); Bi et al. (2009)

*These data represent extraction due to leaching as only filtration or centrifugation was applied after treatment to recover the extract

[a]Natural substance collected by honeybees from buds and exudates of certain trees and plants. This product was not dried before HPP treatment

potential to replace the use of the hydrocarbon solvent hexane during extraction of oil-bearing materials such as soybeans. Two aqueous-based processes have been recently developed, both using enzymes, which are key in the release of oil into water. One process is using proteases on extruded flake material and the other one uses cellulase/pectinase on soybean flour (Kapchie et al. 2008; Jung and Mahfuz 2009; Jung et al. 2009). A pretreatment at 200 MPa of flour or flakes before aqueous extraction (without enzyme) slightly improved soybean oil recoveries from 49 % to 56 % for flour (5 min, initial temperature 25 °C) and 60–64 % for flakes (15 min, initial temperature 25 °C) (Kapchie et al. 2008; Jung and Mahfuz 2009). An increase of the pressure to 500 MPa significantly decreased oil recovery. At this pressure, denaturation of soy proteins occurred and oil was trapped into insoluble aggregates, and the treatment favored noncovalent interactions and disulfide bond formation (Jung and Mahfuz 2009; Jung et al. 2009). Adding proteases to the flakes after the 500 MPa treatment in the aqueous extract restored the oil extraction yield (similar to the control), but when cellulases/pectinases were added, significant lower extraction yields were still obtained. It is likely that the proteases were able to release the oil trapped in the pressure-induced aggregates. These results illustrate the primary importance of the effects of the treatment on the matrix component and aggregation of the proteins decreasing extraction of oil. Pressure level (100–700 MPa, initial temperature, 10 min, 25 °C) applied to soaked soybeans (12 h at 20 °C, 25 min) (Omi et al. 1996) used for soymilk production had a significant impact on both isoflavone concentration and isoflavone profile, which were attributed to a combination of variation in selectivity in extraction, interaction with proteins, and activity of β-glucosidase (Jung et al. 2008). Pressure at 400 MPa and above decreased the protein extractability, with a protein content of the soymilk of 47.7 % vs. 51.6 % for the control (Jung et al. 2008). Changes in the water distribution and disruption of protein bodies in seeds soaked under 400 MPa for 8 h was previously reported (Zhang et al. 2004). Up to 2.5 % of proteins were recovered into the water surrounding presoaked soybeans (17 h at 5 °C) and submitted to 100–700 MPa treatment 20 °C, 25 min) (Omi et al. 1996). Similarly, leaching of proteins was observed when rice was treated by HPP at 100–400 MPa (Kato et al. 2000). Increasing pressurization time at 300 MPa gradually increased protein content in the water surrounding the rice during treatment, with the protein content increasing from 0.1 for the control to 0.5 mg/g rice after 120 min. Because the proteins released during treatment are major rice allergens, it was claimed that HPP could reduce allergenicity of rice (Kato et al. 2000). There was, however, no benefit of applying high-pressure processing (100–300 MPa, 10–60 min at 20 °C) to reduce allergenicity of silver carp allergens (Liu and Xue 2010).

While not a change in extractability per se, HPP was also investigated as a pretreatment prior to drying foods in the studies of Vega-Galvez et al. (2011), Yucel et al. (2010), and Kingsly et al. (2009). When comparing the effect of HPP treatment (100–300 MPa, 5–45 min, 20 and 35 °C) prior to hot-air tunnel drying (27–85 °C, 0.4 and 0.8 m/s) on characteristic drying time (t_c) of carrot, apple, and green bean, the benefit of applying HPP was dependent on the matrix and the drying conditions (Yucel et al. 2010). For example, for drying at 45 °C, 0.4 m/s after treatment at 200 MPa, 15 min, and 20 °C, the t_c values for the control and pressurized samples were

533.9 vs. 706.4, 148.1 vs. 113.9, 145.11 vs. 139.0, and 124.5 vs. 98.9 for green beans, carrots, Amasya apples, and red delicious apples, respectively. For carrots, if drying conditions were changed to 27 °C and air velocity maintained, there was no significant difference in the t_c values between the control and pressurized samples. When improvement was observed, it was attributed to increased water diffusivity due to permeabilization of cell structure or enlargement of micropores. A moisture loss due to pressure treatment was also observed, which ranged from 2 to 5 %, and was attributed to cell disintegration. Loss of water due to the treatment was also observed for pineapple, which increased with pressure level (50–700 MPa, 10 min, 25 °C treatment temperature) (Kingsly et al. 2009). Higher moisture diffusivity of the pineapple slices after HPP and blanching was also observed due to treatment-induced enhancement of the internal mass transfer during drying, and a treatment at 500 MPa for 10 min was proposed as an alternative to blanching before drying. A comparison of enzymatic, blanching, and microwave vs. HPP (350 MPa, 30 s, room temperature) as pretreatments prior to convective drying (70 °C) of aloe vera gel showed the benefit of using pressure. The treatment increased the water diffusion coefficient, increased the firmness of the final product, and significantly improved the antioxidant activity (by a factor of ~3.25), which was attributed to better extractability of bioactive components due to changes in the tissue matrix (Vega-Galvez et al. 2011).

10.7 Conclusion

Research on HPP as an extraction tool is still in its infancy when compared to the investigations done on microbial inactivation under pressure to improve food product safety. Several parameters will contribute to the sustainable identification and implementation of food applications using high-pressure hydrostatic processing.

Bioactive components depend on their bioavailability to provide beneficial effects, and an increase of their extractability in vitro is an encouraging indicator that their bioavailability can be improved. However, bioaccessibility and human studies need to complete these data sets to claim potential benefits of a technology to increase the bioavailability of a product. There is an urgent need to use additional indicators such as in vivo bioaccessibility in the small intestine. Interactions between macromolecules which the process may promote and effects of the treatment on other macromolecules may indeed have a significant impact on the overall bioavailability of a processed product.

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Chapter 11
High Pressure Processing in Combination with High Temperature and Other Preservation Factors

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Abstract High pressure processing (HPP) has shown its efficacy in treating a number of food products of very diverse origin. For this reason, HPP has been adopted by a number of food processing companies to replace or partially replace existing preservation techniques. At the same time, HPP offers the possibility to develop new products with very attractive characteristics. Nevertheless, this novel technology has some limitations, and one way to enhance its efficacy is to combine it with others where synergistic or additive effects are attained. The most noticeable combination is what is known as pressure assisted thermal sterilization (PATS), an approach which is developing interest at the academic and industrial levels because it offers the opportunity to sterilize foods where the quality of the finished product is superior to those processed by conventional thermal treatments. HPP could also be combined with other so-called hurdles such as carbon dioxide, bacteriocins, vegetable extracts, organic acids, osmotic dehydration, pulsed electric fields, and ohmic heating, to name a few. These combinations and a few others are covered in this chapter where PATS receives the most attention.

Keywords Hurdles • Pressure assisted thermal sterilization • Synergistic • Bacteriocins • Organic acids • Vegetable extracts • Osmotic dehydration • Pulsed electric fields • Ohmic heating

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11.1 Introduction

High pressure processing (HPP) or high hydrostatic pressure (HHP) is an industrial reality; many years of research is supporting the development of this technology with thousands of experiments, various food products currently being commercialized and tested, and a large number of microbial and enzymatic species also being examined. Advances in this technology have been well documented in the literature regarding equipment, processing, food products, and tested characteristics. Recently, a new era of high pressure processing began with the development and validation of a sterilization process. In 2009, the FDA approved the use of high pressure in combination with high temperature (pressure assisted thermal sterilization or PATS) as a sterilization process to be used in the industry. In this chapter, a detailed description of the advances on PATS is included regarding microbial and enzymatic inactivation, the effect of this sterilization process in vitamins and other nutritional components in some products, the use of chemical compounds to validate the process, and the effect on texture for some foods. Also a brief discussion related to reduction of the generation of undesirable chemical compounds in food after PATS is presented.

Scientists have been investigating the use of high pressure in combination with other preservation factors. In the past, the combination of pH, water activity, and temperature, for example, was used together to extend the shelf life of a product giving the opportunity to develop the so-called hurdle technology approach. Today, the combination of pressure plus carbon dioxide, natural compounds such as bacteriocins, and osmotic dehydration and even combination with other emerging technology such as ohmic heating or pulsed electric fields, just to mention a few, have been tested. Some of these combinations, with specific examples, are also included in this chapter.

Finally, modeling is an area that requires special attention when several factors are used together to inactivate microorganisms and enzymes or to study the degradation of certain compounds. It is well known that emerging technologies do not follow a first-order kinetics, either for microbial inactivation or enzymatic activity, and several years have been devoted to finding new mathematical models to fit the survivor curve and predict inactivation trends using these novel technologies. Some drawbacks were found, such as nonuniform processing conditions or mixed populations of microorganisms. Today, the big challenge is to find mathematical models to fit inactivation curves and predict other trends using two lethal agents at the same time: pressure plus heat or electromagnetic fields and addition of natural or chemical compounds. The combination of these factors sometimes presents a synergistic, additive, null, or antagonistic effect, and the mathematical models should be able to describe those responses. At the end of this chapter, a brief section about the use of mathematical approaches to describe inactivation trends using at least two preservation factors is presented.

11.2 Recent Uses of High Pressure Processing

A nonthermal technology that has shown advances in the last few years is high hydrostatic pressure (HHP) or high pressure processing (HPP). During the 1990s, this technology was fully explored as an alternative technology for pasteurization of high acid products, with successful results. Hundreds of research papers, book chapters, and books related to this technology were published around the world. Within a few years after results of this research were presented, the industry began to adopt the technology to process classic items such as guacamole and smoothies, to pasteurize deli meats after being cut and packed, to process jams and marmalades, and also to open and remove the meat from oysters. This technology became very popular around 2000; every conference about food engineering at that time mentioned high pressure. Researchers explored hundreds of items related to this technology including ethnic foods, microbial and enzymatic inactivation, nutrient degradation, sensory evaluation, development of new commercial products, and military rations. High pressure showed its potential to pasteurize food products by inactivating most pathogenic species such as *Escherichia coli* O157:H7 or *Salmonella* spp., to mention two typical examples, without changing the fresh-like characteristics of the product. After showing the efficacy of the technology, researchers faced a new challenge, i.e., the inactivation of very resistant spores. Since that time, new combinations with pressure were tested. First, researchers used very high pressure (1 GPa) or very long holding times (from 30 min to hours), which did not make the process suitable for industrial applications due to the required energy to achieve this level of pressure and/or holding processing times. Second, researchers used high pressure at the tested levels for pasteurization but in combination with high temperature, lower than retorting temperature (121 °C), but higher than 70 °C, together with high pressure. Results were favorable; even the most heat-resistant spores were inactivated with the use of pressure and heat together, opening a new era for food sterilization processes, which has been called pressure assisted thermal sterilization (PATS). Since then, many combinations with pressure have been investigated using hurdle technology, in an attempt to reduce processing times and reduce pressure levels, while at the same time incorporating new preservation factors. However, special care must be taken in account once the combination of hurdles is chosen, because the use of two or more preservation factors does not always produce a positive effect. For example, an additive effect is observable when two preservation factors are used together and the combined effect is the summation of both factors, which is a positive effect. A synergistic effect occurs when the two preservation factors are combined and the final effect is greater than an additive effect, because using both preservation factors together enhances the effect of each factor; most of the time, a synergistic effect is highly desirable. Finally, an antagonistic effect is observable when using two preservation factors together: the individual effects are even reduced in the final effect; this is considered as a negative effect in

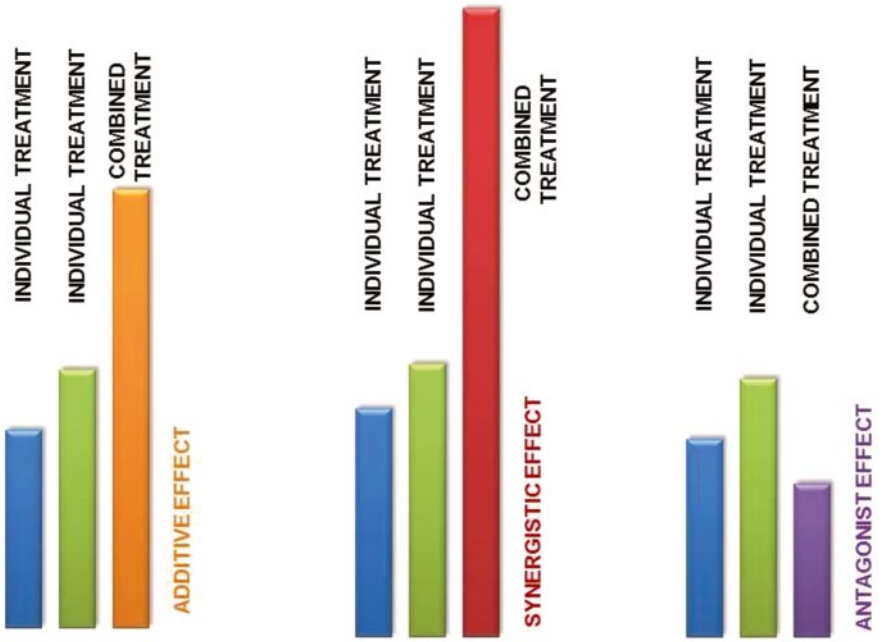

Fig. 11.1 Example of additive, synergistic, and antagonistic effect using hurdle technology

hurdle technology. These three examples are shown in Fig. 11.1. Most of these approaches have been successful in opening a new and huge field for the research and development of food products using HPP.

11.3 Pressure Assisted Thermal Processing (PATP)

The combination of pressure and high temperature is referred to as pressure assisted thermal processing (PATP). More recently, it has been referred to as pressure assisted thermal sterilization (PATS). The process of PATS basically focuses on the use of level of pressure traditionally used for high hydrostatic pressure (up to 700 MPa) and the use of high temperature (90–120 °C) (Ahn et al. 2007) for the inactivation of selected spores. Some researchers have applied lower pressure than 200 MPa combined with high temperature, while others have used high pressure (1 GPa) with mild thermal treatments (Reineke et al. 2011). The process has mainly been focused on inactivation of spores, which are microorganisms that are very resistant to high-pressure treatments. Several examples of microbial inactivation using PATS or combination with heat and other preservation factors have been reported in the literature; some of these examples are presented in Table 11.1.

Table 11.1 Examples of microbial inactivation using high pressure combined with other preservation factors

Microorganism	Medium of treatment	Treatment	Reference
Bacillus amyloliquefaciens, *B. sphaericus*, *Clostridium sporogenes*, *C. tyrobutylicum*, *Thermoanaerobacterium thermosaccharolyticum*	Deionized water	PATP	Ahn et al. (2007)
B. coagulans	Tomato juice	High pressure, high temperature	Daryaei and Balasubramaniam (2013)
B. stearothermophilus	Egg patties	PATP	Rajan et al. (2006)
B. stearothermophilus IFO12550	Glucose, NaCl, and ethanol solutions	High pressure plus temperature	Furukawa and Hayakawa (2000)
B. subtilis	Buffer solutions	HPTS	Reineke et al. (2013)
B. subtilis	Deionized water	HPTS plus ethanol	Zhang et al. (2012)
B. subtilis	Tris buffer, orange juice, and skimmed milk	High pressure plus temperature	Syed et al. (2012)
C. botulinum group I	Phosphate buffer	HPTS	Ramaswamy et al. (2013)
C. botulinum (proteolytic type A and nonproteolytic type B)	TPGY (trypticase-peptone-glucose-yeast extract)	High pressure, high temperature plus additives (lysozyme, L-alanine, L-aspartic acid, dipicolinic acid, sodium bicarbonate, and sodium lactate)	Reddy et al. (2010)
C. botulinum nonproteolytic type B	Phosphate buffer and crabmeat	High pressure and moderate to elevated high temperature	Reddy et al. (2006)
C. sporogenes ATCC 11437	Milk	HPS	Ramaswamy et al. (2010)
Listeria monocytogenes, *L. innocua*	Yogurt	High pressure plus mint essential oils	Akdemir Evrendilek and Balasubramaniam (2011)

PATP pressure assisted thermal processing, *HPTS* high-pressure thermal sterilization, *HPS* high-pressure sterilization

11.3.1 Microbial Inactivation

In an interesting study to test the resistance of anaerobic and aerobic microorganisms under PATS, spores of *Clostridium sporogenes*, *C. tyrobutylicum*, *Thermoanaerobacterium thermosaccharolyticum*, *Bacillus amyloliquefaciens*, and *B. sphaericus* were tested using a range of pressure from 0.1 to 700 MPa and temperature from 105 to 121 °C in deionized water. Results clearly showed the positive effect of pressure on spore inactivation, having between 7- and 8-log reduction in only 1 min when the pressure was set at 700 MPa and the temperature was about 121 °C (Ahn et al. 2007). Two strains of *T. thermosaccharolyticum* and *B. amyloliquefaciens* were shown to be the most resistant spores to PATS. In a further study with *B. amyloliquefaciens*, inactivation using PATS (700 MPa, 105 °C, 0, 1, 2, and 5 min) was enhanced when spores were treated with sucrose laurate ester (SLE). Experiments were conducted in deionized water, SLE solutions, and mashed carrots. SLE at 1 % combined with PATS showed the best synergistic effect against spore inactivation, with more than 7-log reduction (de Lamo-Castellví et al. 2010). In an attempt to inactivate spores of *B. amyloliquefaciens* only with pressure and heat, different pressurization rates and pressure pulses were tested in ionized water. Processing conditions were 600 MPa and 105 °C, from 0 to 5 min. Pressurization rates were 18.06 and 3.75 MPa/s; meanwhile, the effect of pulsing was tested using single and double pulses (1–3 min). Slow pressurization rate was more effective to achieve higher spore inactivation, and pulsing pressure using double pulses enhanced the treatment, going from 2.4 to 4-log reduction. The authors mention that the use of double pulsing during PATS enhances the lethality of the treatment because spores are injured during the first pulse, thus making them susceptible to the treatment, and being inactivated during the second pulse (Ratphitagsanti et al. 2009). Similar results have been observed in other technologies, such as pulsed electric fields or pulsed ultrasound, in which the microorganisms are weakened during pulsing.

PATS was also applied to inactivate spores of *B. stearothermophilus* in egg patties packaged in sterile pouches. Treatment at 700 MPa and 105 °C was able to inactivate 4-log reductions in only 5 min. When time was reduced to 100 s, using the same pressure and temperature, the inactivation was about 3 log, showing very little effect on inactivation, as the time was greater (Rajan et al. 2006).

Other bacterial spores of high importance in food science are those from *C. botulinum.* In most cases, the study of spores from *C. sporogenes* provides a good estimate of spore reduction with specific treatments, due to of the very high heat resistance of this strain. In one study conducted with these spores, milk (2 % fat) was inoculated with the spore strain and treated at very high pressure levels (700–900 MPa) using high temperature (80–100 °C) for about 32 min and pressure hold time and pressure pulsing. Although pressure pulsing reduced a small amount of spores, it was more consistent, and inactivation was achieved in small batches. In this study, the spores were more sensitive to the thermal effect rather than the pressure, under the tested conditions (Shao and Ramaswamy 2011). The same spores, *C. sporogenes*, were inoculated in ground beef and treated under 700, 800, and 900 MPa, using temperatures from 80 to 100 °C. These conditions were able to

achieve sterilization standards in the beef within a few minutes of treatment (less than 5 min), by taking advantage of the fast increase of temperature due to compression heating (Zhu et al. 2008).

PATS has not only been used to inactivate spores, the use of pressure and temperature has also been applied for other purposes to treat food products; in this case the combination of high pressure with heat becomes pressure assisted thermal processing (PATP), and one of the applications is to extend the shelf life of products beyond the regular period of storage. In a study with carrots, packaging materials were used to hold the carrots and to process them under 600 MPa, 110 °C during 10 min. Storage of the samples was done at 25 and 37 °C, and carrots showed a shelf life of 12 weeks due to reduction of the total plate count to less than the detection limit (10 cfu/g). Similar results have been reported for mashed carrots, tomato puree, mashed broccoli, and carrot pure (Ayvaz et al. 2012).

11.3.2 Enzyme Inactivation

High pressure has been used to study inactivation of enzymes in several fruit and vegetable products. The main objective has been the stabilization of the product during storage while taking advantage of pressure levels to reduce enzymatic activity. Several studies can be found elsewhere about the use of high pressure to inactivate target enzymes in food items such as polyphenol oxidase, pectin methylesterase, peroxidase, lipase, and lipoxygenase, among others (Hendrickx et al. 1998). However, most of these studies have been conducted at room temperature (21 °C), and even the increase of temperature during pressurization due to compression heating and final temperatures were not higher than 40 °C. Tested pressures have been from 100 up to 1000 MPa, and some reported processing times have been excessively long, i.e., as long as 200 min; even using these conditions, some enzymes have been activated rather than inactivated; other enzymes kept the original activity, even after the high-pressure treatment (Ludikhuyze et al. 2002). In some cases, the use of lower pressure (275 MPa) was more efficient to inactivate a percentage of the enzymatic activity rather than using higher pressure (414 MPa) using the same processing times (Bermúdez-Aguirre et al. 2011). Also, the use of pressurization can promote a reversible or irreversible reaction for enzyme inactivation (Boulekou et al. 2010), which must be considered and studied in accordance with the type of product.

Inactivation or activation of enzymes due to of the use of high pressure depends on several factors, and each enzyme has a different response to the treatment. Some factors that should be considered during inactivation are the treatment medium and the source of the enzyme, pressure level, processing time, presence of other food components, and temperature, among others. Temperature is considered very important to inactivate enzymes using high-pressure technology. In a study with pectin methylesterase on peach pulp, thermal treatment was shown to reduce enzymatic activity (10 min at 65 °C, 40 min at 60 °C), but when pressure was added, the

treatment was reduced to less than 5 min. A synergistic effect was observed between the range of pressure (100–600 MPa) and temperature (30–70 °C); however, an antagonistic effect was observed in the middle range of pressure and 70 °C, probably because the configuration of the enzyme under these conditions (Boulekou et al. 2010).

11.3.3 Other Studies

11.3.3.1 Vitamins and Nutritional Compounds

High pressure has been used in several products, and at the beginning, the most studied parameters were microbial and enzymatic inactivation; nowadays, research has been extended to other relevant elements in food products that might be altered by the treatment. Pressure assisted thermal processing has been studied in a number of nutritional and chemical compounds in selected food products. Valdez-Fragoso et al. (2011) studied, among others, the following compounds, the 5-methyltetrahydrofolic acid, folic acid, tetrahydrofolate, L-ascorbic acid, chlorophyll, α and β carotene, vitamin A acetate, retinol, and cyanidin-3-O-glucoside.

Studies in specific target products are next described in more detail. For example, the study of vitamins in pork has been analyzed when the product was subjected to high-pressure, high-temperature conditions. Thiamin and riboflavin were analyzed in model systems and in fresh and rehydrated pork fillets. Pressure at 600 MPa was combined with temperatures ranging from 25 to 100 °C to study the effect of high-pressure thermal sterilization. Results showed that vitamins are stable after pressurization in the real system, i.e., the pork fillets, but there is a decrease in vitamin content when the treatment is applied to solutions (model systems), so further research is required in this field (Butz et al. 2007).

β-Carotenes are also important compounds for the human diet and the use of thermal processing to treat certain foods can destroy them. PATP (500–700 MPa, 100 °C) was used to treat tomato juice and study the possible effect on lycopene and β-carotene. High pressure and thermal treatments alone were also conducted to compare the effects. Results showed that lycopene extractability was increased by 12 % in PATP tomato juice; however, all-trans β-carotene showed a reduction during processing from 60 up to 95 % (Gupta et al. 2011a). However, during storage (4, 25, and 37 °C), all-trans lycopene in tomato juice was very stable. Samples treated with pressure alone (700 MPa, 45 °C, 10 min, and PATP: 600 MPa, 100 °C, 10 min) retained the total lycopene and cis isomers during 52 weeks of storage. Color of pressurized samples was also better during storage compared with thermal treated and control samples (Gupta et al. 2010). Similar results were found during the processing of tomato puree (700 MPa, 90 °C, 30 s, one pulse), but reduction of spores of *B. stearothermophilus* (4.5 log) and reduction on the enzymatic activity of polygalacturonase and pectin methylesterase (99 %) were also detected. Lower viscosity but higher water binding capacity was observed in tomato puree after treatment (Krebbers et al. 2003).

Carotene content and color in carrots treated with PATP (500–700 MPa, 95–121 °C) showed positive results compared with conventional thermal processing. However, the effect of pressure was not very noticeable when the texture of PATP and thermal processed carrots was compared. The minor changes in color and carotene content might be due to pressure during processing that generates a faster increase of temperature because of compression heating and the expansion cooling during depressurization (Nguyen et al. 2007).

A study of CLA (conjugated linoleic acid) in milk has been receiving much attention in the last few years because of its health-promoting and disease-preventing effects. PATP was applied to milk using pressure from 100 to 600 MPa, temperature from 60 to 120 °C, and processing times from 0 to 14 min; the main aim was to study the possible degradation of CLA during treatment. Results showed that the use of low pressure (100 MPa) retained CLA content without important changes; more than 80 % of the compound was retained even with the longest processing time. However, increasing the pressure (600 MPa) and temperature (120 °C) during PATS generated a drastic degradation of CLA, retaining only 3.4 % of the nutrient, maybe because of the presence of metal ions in the milk (Martínez-Monteagudo et al. 2012).

Folates are compounds of great interest in nutrition, not only for good metabolic function but also because of their importance during pregnancy for the development of the neural tube in the fetus. However, some products that contain these nutrients are subjected to extreme conditions during thermal processing, generating degradation of these nutrients. High pressure processing (up to 800 MPa) was studied together with temperature (20–65 °C) in the degradation of folic acid and 5-methyltetrahydrofolic acid in model systems. Results showed that folic acid was more stable under high-pressure treatment than the treatment with 5-methyltetrahydrofolic acid, which showed its degradation as the pressure increased and became worse when temperature was higher than 40 °C (Nguyen et al. 2003).

A study of degradation of vitamin C due to the use of pressure combined with high temperature was carried out in strawberry and raspberry using 700 MPa and temperatures of 60, 90, and 100 °C as processing conditions. Results showed that both pressure and temperature were responsible for the degradation of vitamin C, with the oxidation of ascorbic acid to dehydroascorbic acid very slow compared with the further degradation of dehydroascorbic acid (Verbeyst et al. 2012).

11.3.3.2 Chemical Compounds

In another study, acrylamide and some Maillard reaction compounds were quantified after treating an asparagine-glucose model solution using pressure from 400 to 700 MPa and temperature from 100 to 115 °C during time intervals from 0 to 60 min. The effect of high pressure plus high temperature was studied on the concentration of acrylamide, reactants, hydroxymethylfurfural, organic acids, and melanoidins; results showed that pressure has a retarding effect on the formation of compounds during Maillard reaction (De Vleeschouwer et al. 2010).

One of the main concerns during thermal processing of milk is the formation of chemical compounds in the product that result in the well-known "cooked flavor" of milk and subsequent rejection by the consumer. Several novel technologies have been tested to pasteurize milk and reduce this problem. Pressure assisted thermal processing was used to inactivate some microorganisms in milk but at the same time to study the formation (or not) of chemical compounds of interest related to milk taste. Twenty-seven volatile compounds were analyzed in milk (3.25 % fat content) after treatment at 482, 586, 620, and 655 MPa, using temperatures from 45 to 75 °C during 1, 3, 5, and 10 min. Some of the chemical compounds that characterize "off-flavor" taste in milk and were significantly reduced in PATP milk were hexanal, heptanal, octanal, 2-heptanone, 2-octanone, and 2-nonanone. In general terms, PATP reduced or stopped the formation of those compounds related to off-flavor characteristics of thermal processed milk, thus being a good option for the dairy industry (Vázquez-Landaverde et al. 2007).

Other studies have been conducted to validate several technologies and to find heating patterns during processing, such as the use of chemical markers studied under PATS. The M-2 (4-hydroxy-5-methyl-3(2H) furanone), a by-product of the Maillard reaction, was studied under PATS (350 and 700 MPa, 105 °C) in whey protein gels. Results showed that heat affected the formation of the marker (which was increased); meanwhile, pressure had an opposite effect on the marker (decreasing the concentration). The experiment was also compared with thermal treatment and with high-pressure treatments alone. Results were contradictory, showing that this chemical marker is not suitable to study or validate PATS (Gupta et al. 2011b). However, in another attempt to find a chemical marker to validate the high-pressure, high-temperature process and map the temperature through the progress in the pressurization vessel and inside the product, an enzyme, xylanase B, produced by *Thermotoga maritima* (XTMB), was investigated as a possible sensor during PATS. This enzyme from a thermophilic microorganism was chosen because it meets the requirements for a sensor to map out changes in pressure-temperature processes, i.e., accuracy and reproducibility, heat stability at atmospheric pressure, and being very sensitive to temperature differences during the PATS process (Vervoort et al. 2011).

11.3.3.3 Texture of Foods

Indeed, one of the main characteristics of food is related with texture, and acceptability of a product depends, on a high proportion, on the texture of the food. Conventional thermal treatments affect the texture of the product, in addition to other sensory properties; thus, some studies have focused solely on the study of texture of products after being treated with PATP.

In one such study, foods were treated with PATP (600 MPa, 105 °C) and also treated with high pressure (600 MPa, 25 °C) and thermal treatment (105 °C) to compare the effect of the pressure treatment on the texture of the product. The treated foods were carrots, zucchini, apricot, red radish, and jicama, which were analyzed for texture, color, and sensory evaluation. Results showed that PATP was the least invasive treatment for maintaining texture and color of the products, actually

improving the texture in some of them. Jicama showed only minor changes, but the negative effect of thermal processing was observable in all of the treated foods (Nguyen et al. 2010).

11.3.3.4 Undesirable Chemical Reactions During PATS

The main goal of PATS is to substitute the regular sterilization process conducted in the traditional retort system. However, during the normal thermal process, certain undesirable physicochemical reactions take place and some compounds are formed. Among these compounds, there is a group of chemicals that are considered carcinogenic and known as food processing contaminants (FPCs), which are toxic compounds generated during food processing that represent a risk for human health. Some of these compounds are furan, monochloropropanediols, and their esters (MCPD/MCPD-esters). Sevenich et al. (2013) conducted a study comparing the formation of FPCs between a traditional retorting system and PATS in canned fish and ACE's buffer at 600 MPa (90–121 °C), analyzing the inactivation of spores of *B. amyloliquefaciens* or *G. stearothermophilus*. Results showed that spores of the first microorganism were more resistant to the action of PATS, but it could be almost inactivated (5 log) after 600 MPa, 115 °C, and 0.25 min in canned sardines and tuna in oil, using the conditions tested in that study. Also, the content of furan in the product was reduced between 71 % and 97 % using PATS, depending on the tested temperature. It is clear that PATS not only offers a good option for spore inactivation but also represents a choice for healthier and minimally processed food products.

11.4 High Pressure and the Combination of Other Preservation Factors

HPP is now being explored in combination with other preservation factors to increase its effectiveness in some areas such as microbial inactivation or improvement of the overall quality of food products. In the past, these additional preservation factors have been used alone with a specific objective, and they are now being used together to enhance the effect of high pressure. In Table 11.2, examples of these physical and chemical factors are listed while combined with high pressure to process selected food products.

11.4.1 High Pressure in Combination with Gases

The use of high pressure together with carbon dioxide (CO_2) was applied to orange juice to study the effect on cloud stability due to the presence of pectin methylesterase. Processing conditions involved the use of 600 MPa, 25 °C, and 130 s. Results showed that the use of carbon dioxide-assisted high pressure generated a stable

Table 11.2 Examples of some chemical and physical hurdles used in combination with high hydrostatic pressure

Chemical hurdle	Main objective	Physical hurdle	Main objective
• Bacteriocins	Microbial inactivation, extension on shelf-life	• Temperature	Microbial inactivation, reduction of enzymatic activity
• Carbon dioxide	Reduction of enzymatic activity	• Ohmic heating	Spore inactivation
• Essential oils	Microbial inactivation	• Pulsed electric fields	Spore inactivation
• Organic acids	Microbial inactivation		
• Sodium hydrogen carbonate	Improve of texture and palatability of meat		
• Sugars	Improve in texture and extension of shelf-life		
• Argon	Enhance spore inactivation		

product during storage for about 4 months when stored at 4 and 30 °C, having a residual activity of PME of about 20 %, similar to thermal treated juice. However, ascorbic acid content and volatiles in the product were retained in much higher proportion in the carbon dioxide-assisted high-pressure processed juice, rather than in the thermal treated product, because of the displacement of oxygen (Boff et al. 2003).

In a different study, high pressure (400–600 MPa) was combined with argon to inactivate *B. cereus* spores at 20 and 40 °C. Results showed that the combination of argon and high pressure was more effective at low temperature and low pressure because of hydrophobic hydration. This phenomenon occurs when a rare gas like argon is dissolved in water (under specific temperature and pressure conditions) and forms "iceberg-like" structures around solute molecules (Fujii et al. 2002).

11.4.2 High Pressure Combined with Natural and Artificial Compounds

The use of certain natural compounds for microbial inactivation, including bacteriocins, some vegetable extracts, and organic acids, is well known. In the case of high pressure, some of these compounds have been used together to achieve higher microbial inactivation without the use of heat, to minimize any possible thermal effect on the product. Other chemical compounds have been also tested together with high

pressure. Consumers looking for additive free foods may consider the use of minimum concentrations of chemicals together with innovative processes to be healthier.

During the processing of yogurt, use of high pressure achieved better characteristics in the final product because of the effect of pressure by itself, but combination with mint essential oil was also able to increase microbial reduction. *Listeria innocua* and *Listeria monocytogenes* were inoculated in a drinkable yogurt and treated at 600 MPa during 300 s; 5-log reduction was achieved after this treatment. When mint essential oil was added, inactivation was increased by at least one-log reduction, redefining new treatments as low as 100–300 MPa during 210 s (Akdemir Evrendilek and Balasubramaniam 2011).

Organic acids such as acetic, citric, and lactic acids were tested together with PATS (700 MPa, 105 °C) against spores of *B. amyloliquefaciens.* After 3 min of treatment, spores were inactivated regardless of the presence of organic acids; however, only 2 min was required to inactivate spores when citric or acetic acid was present in the medium of treatment. These organic acids were also added to carrot puree that was used as the treatment medium to study the possibility of spore recovery after processing and during storage (32 °C, 28 days), but the acids showed strongly negative results for spore recovery (Ratphitagsanti et al. 2010).

In another study, a comprehensive list of additives was tested against *Salmonella enteritidis* together with high-pressure treatment; some of these additives were natural compounds such as nisin or traditional preservatives such as sodium benzoate, which were used in concentrations from 0.03 % to 1 %. Pressure was tested from 100 to 400 MPa during 30 min. Results showed that all of the 30 food additives showed synergistic effect when used at 1 %. However, when used together with high pressure at 250 MPa during 30 min, minimum concentrations were required of C8-sugarester, C10-sugarester, and protamine (0.25 %, 0.125 %, and 0.06 %, respectively) to inactivate *Salmonella* cells, indicating that a minor amount of additive is required because of the effect of high pressure processing (Ogihara et al. 2009).

The inactivation of *L. monocytogenes* was studied on raw milk cheese processed with high pressure and bacteriocin-producing lactic acid bacteria (BP-LAB). Samples were treated at 300 or 500 MPa (10 °C) for 10 and 5 min, respectively. The produced bacteriocins were nisin, lacticin, TAB, and enterocin. Synergism between high pressure and bacteriocins was observed during ripening of the cheese, reducing considerably the counts of *Listeria* cells; very low microbial counts (0.1–0.7 log cfu/g) were observed during storage (60 days). Synergism between both preservation factors could be because sub-lethally injured cells are more sensitive to bacteriocins (Arqués et al. 2005). In this study, the use of middle pressure, such as 300 MPa, might not be totally effective for complete inactivation, but can damage cells for further action of bacteriocin.

Furthermore, considering the use of bacteriocins and high pressure, a study dealing with enterocin LM-2 was applied together with 200–400 MPa (10 min) in sliced cooked ham. Results showed the inhibition of microbial growth during refrigerated storage, inactivation of *L. monocytogenes* and *S. enteritidis*, and in general, a better sensory quality of the ham. The shelf life of the product was extended up to 90 days when the highest pressure was used together with 2560 AU/g of bacteriocin (Liu

et al. 2012). Also, different bacteriocins (enterocin A and B, sakacin K, pediocin AcH, and nisin) were combined with high pressure (400 MPa, 10 min, 17 °C) in a model meat system evaluating microbial inactivation of several species of *Salmonella*, *E. coli*, *Staphylococcus aureus*, *L. monocytogenes*, *Lactobacillus sakei*, and *Leuconostoc carnosum*. Results showed that nisin was effective against *E. coli* and *L. sakei*; meanwhile the bacteriocins sakacin K, both enterocin and pediocin, were able to reduce the counts of *Listeria*. The most resistant microorganism to the combination of bacteriocins and high pressure, in this study, was *S. aureus* (Garriga et al. 2002). In another meat product (dry-cured ham), two bacteriocins (nisin and pediocin) were individually tested against *E. coli* O157:H7 used together with 400 and 500 MPa during 10 min. Results showed an important reduction in microbial counts when pressure and bacteriocins were used together, as well as an important extension in the shelf life of the product (60 days) using the highest tested pressure (500 MPa) and nisin (De Alba et al. 2013).

A bacteriocin mixture (pediocin and nisin) was used together with high pressure to study changes on cell structure. *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 were used as target microorganisms, and 345 MPa, 5 min, and 25 °C were the tested conditions. Electron microscopy showed the collapse of cell membrane and cell wall as well as cell lysis (Kalchayanand et al. 2004).

In Japan, some studies using high hydrostatic pressure together with sodium hydrogen carbonate ($NaHCO_3$) have been conducted to treat different meats. Because sodium hydrogen carbonate has traditionally been used as a meat tenderizer, the main objective of these studies was to test the effect of the combination of this chemical compound and high pressure. Ohnuma et al. (2013) used $NaHCO_3$ and high pressure (100–500 MPa) to treat beef; the results showed an increase in the water content of the meat (70.1 %), decrease on hardness (>50 %), and reduction on whitening of the product. The reasons for these changes could be due to the myoglobin denaturation and modification in the myofibril structure of meat. In a similar study (Tabe et al. 2013), using chicken breast, $NaHCO_3$ was used as a pretreatment of the meat (soaking). Chicken is characterized by a lack of juiciness, and the idea of combining carbonate and high pressure was to increase its palatability. Samples were processed after soaking at 100–400 MPa and then heated (30 min, 80 °C). Results showed an increase in water content, making the chicken breast juicer, tenderer, and with good taste. Again, this result is related to the rupture of the myofibril structure, increasing the available space to hold water molecules. Finally, a third study on pork ham (Kim et al. 2013) was conducted using $NaHCO_3$ and high pressure (100–400 MPa) showing similar results to beef and pork regarding texture, with good acceptability during sensory studies. In this group of studies using sodium hydrogen carbonate and pressure, the effect of the combination of both factors is clear in reducing the hardness of the meat and improving texture and palatability. Furthermore, these results have been observed in poultry, pork, and beef products.

11.4.3 High Pressure and Osmotic Dehydration

The effect of high pressure (100–500 MPa, 10 min) in addition to osmotic dehydration (40 Brix) was studied on sliced strawberries during storage (14 days, 5 °C). Results showed that fruits became softer and with irregular shape after processing with pressure, and the polysaccharide content was also increased with the pressure. In addition, other parameters such as anthocyanins, flavonoids, total phenolic compounds, and total dietary fiber were reduced after processing. The increase of polysaccharides could be due to the inactivation of enzymes such as pectin methylesterase and polygalacturonase, which are related to the redistribution of polysaccharides. Decrease in anthocyanins in pressurized strawberries is related to the presence of oxidase enzymes which catalyze the degradation of certain pigments in fruits; meanwhile, the change in total phenolic compounds could be due to the solubilization of certain polymers during storage that react with phenolic compounds (Nuñez-Mancilla et al. 2013).

11.5 High Pressure and Other Novel Technologies

Certain novel technologies have been tested in isolation, with successful results; however, some researchers have been combining at least two of these technologies to study the possibility of enhancing microbial inactivation because of a synergistic effect and reduced processing times. The following combinations seem to have some future even though their implementation at the industrial level might be difficult and eventually nonpractical.

11.5.1 Pressure-Ohmic Thermal Sterilization (POTS)

A very interesting approach between a novel thermal technology, ohmic heating, and a nonthermal technology, high pressure, has been studied together under the name of pressure-ohmic thermal sterilization (POTS). Two very heat-resistant spores were tested with this combined technology: *B. amyloliquefaciens* and *Geobacillus stearothermophilus*. The media used were NaCl solution, green pea puree, carrot puree, and tomato juice. The treatment consisted of 50 V/cm (ohmic heating) and 600 MPa and 105°. Spores were inactivated in NaCl solution at 4.6 and 5.6 log for *B. amyloliquefaciens* and *G. stearothermophilus*, respectively, after 30 min. For tomato juice, only 10 min was required to achieve inactivation in the range of 3.1–4.8 log, respectively (Park et al. 2013).

11.5.2 Pulsed Electric Fields (PEF)

Pulsed electric fields have been reported as a promising technology for cold pasteurization of liquid products; however, spore inactivation using this technology is not feasible so far. Nevertheless, combining high hydrostatic pressure and pulsed electric fields can lead to notable spore inactivation. In a study conducted using spores of *Bacillus subtilis* in different chemical solutions such as 0.1 % NaCl solution and buffers, high pressure and pulsed electric fields were used alone and together to explore the spore death. High pressure was tested at 700 MPa and 55 °C; meanwhile, pulsed electric fields were conducted at 12 kV, 50 Hz, in a treatment chamber containing two acrylic resin pipes (55 mm length, 19 mm outside diameter for the inner pipe, inserted in the outer pipe with a gap of 3.5 mm) keeping the sample at 55 °C. Results showed that PEF by itself was not able to inactivate spores; but high pressure was able to reduce some spore counts. However, when the treatments were combined, two different trends were observed. First, when PEF was applied followed by high pressure, the reduction of spores was possible up to 7 log, especially in the buffer solution. However, when the treatment was initiated by high pressure followed by PEF, reduction was not observed, even though the reactivation of spores was reported. The efficacy of PEF followed by high pressure is related to the presence of cracks on the spore surface generated by the electric field and the subsequent rupture of the cell due to the elevated pressure. In the opposite case (high pressure followed by PEF), some spore inactivation was observed because of the elevated pressure, but when PEF was applied, the electric fields favored the spore germination and promoted the growth of H-spores. The H-spores were found during high-pressure treatment at low pH, in which the process replaced the ions of the spores (Ca^{+2}, Na^{+1}, Mg^{+2}, Mn^{+2}) by hydrogen (Sasagawa et al. 2006). It is interesting to see that even the order of the processes could not have a significant effect on cell inactivation; the mechanisms of inactivation need to be understood and taken into account in order to successfully combine two or more processes.

11.6 Estimation of Lethal and Sublethal Contributions to Microbial Inactivation in Combined Food Sterilization Processes

Pressure assisted thermal sterilization (PATS) and other combined food preservation processes rely on the application of at least two factors to achieve significant microbial inactivation, i.e., 5 log cycles or higher, while shortening processing times and/or improving the overall quality of the final product.

In the particular case of PATS, high pressure is applied during thermal treatment to increase food temperature instantaneously and uniformly throughout the bulk. Pressurization allows for shorter thermal processes that provide the same microbial lethality as conventional processes. Although high temperature can be considered

the main lethal factor in PATS, pressure increase might also affect the final microbial load. In principle, the combination of two factors such as temperature and pressure can result in synergistic, additive, null, or even antagonistic effects. The nature of the interaction can be assessed by isolating and quantifying the contribution of each factor to the efficacy of the combined process (Wouters et al. 2001). Experimental procedures to determine each contribution, when available, are difficult to perform. Therefore, Peleg et al. (2011) have suggested a mathematical method that allows determining the thermal and the nonthermal lethality associated with a PATS process. Although this mathematical model has been developed for pressure assisted thermal treatments, it can be expanded to any combined process to discriminate the effect of all of the relevant lethal and sublethal factors on overall microbial count reduction.

For PATS, this method involves obtaining a thermal profile during the pressurized experiment and using it to estimate the survival curve corresponding solely to the thermal contribution. The thermal survival curve is calculated based on kinetic parameters determined during conventional heating experiments. For a microbial population with inactivation kinetics that follows the Weibull-log logistic model (Peleg 2006) and the shape parameter is considered constant, i.e., $n(T)=n$, the thermal survival curve can be obtained by numerically solving the following differential equation for any given temperature profile:

$$\frac{d\log\left[S(t)\right]}{dt}=\frac{d\log\left[N(t)/N_0\right]}{dt}=-b\left[T(t)\right]n\left\{-\frac{\log\left[S(t)\right]}{b\left[T(t)\right]}\right\}^{\frac{n-1}{n}} \tag{11.1}$$

where $T(t)$ is the temperature profile, $S(t)$ the momentary survival ratio, $N(t)$ and N_0 the momentary and initial counts, respectively, and $b(T)$ the rate parameter. It should be noticed that the temperature dependence of b is characterized by the log logistic model:

$$b\left[T(t)\right]=\ln\left\{1+\exp\left[k\left(T(t)-T_c\right)\right]\right\} \tag{11.2}$$

where T_c corresponds to the onset of the lethal temperature regime and k is the approximate slope of $b(T)$ at $T\gg T_c$ (Peleg 2006).

The kinetic parameters required in Eqs. (11.1) and (11.2), namely, n, k, and T_c, can be determined experimentally from a set of isothermal or dynamic survival data (Peleg and Normand 2004). Alternatively, these parameters can also be extracted from a series of final survival ratios using the "three endpoints method" (Peleg et al. 2008; Corradini et al. 2008, 2009). This procedure does not require obtaining the whole survival curve of the inactivation process. It just needs three temperature profiles and their corresponding final survival ratio. It should be noticed that this procedure is limited to inactivation kinetics that can be described by up to three parameters, such as the Weibull-log logistic model.

Upon insertion of the parameters in Eq. (11.2) and subsequently in Eq. (11.1), the rate model can be solved for the corresponding temperature profile to obtain the survival curve due only to the thermal contribution.

If the experimental survival ratio at the end of the process is lower than the estimated one, it can be inferred that pressurization has a synergistic effect. Conversely, an experimental survival ratio higher than the estimated one for the thermal contribution can be correlated to an antagonistic effect on bacterial survival (see Fig. 11.2). Finally, if both final survival ratios are the same, the inactivation can be considered merely thermally driven and changes in the survival ratio cannot be attributed to the applied pressure.

PATS processes usually imply holding the temperature at a target level. Therefore, instead of using the difference in log survival counts to quantify the contribution of each factor of a combined process, the nonthermal or sub-lethal pressure effect could also be reported as the holding time at the target temperature of the PATS process, in minutes, for example. In order to calculate the holding time in a PATS process, a parameter that accounts for the length of time that the process is kept at the target temperature should be included in the mathematical expression that describes the temperature profile. Then, the purely thermal rate model equation (Eq. 11.1) has to be solved for the holding time parameter, t_{holding}. Since the final survival ratio used for this estimation corresponds to the actually recorded microbial reduction at the end of the pressure-assisted process, t_{holding} becomes a measure of the contribution of lethal effects, other than thermal, to the overall survival count. Longer holding times will account for synergistic effects, i.e., pressurization will improve the process

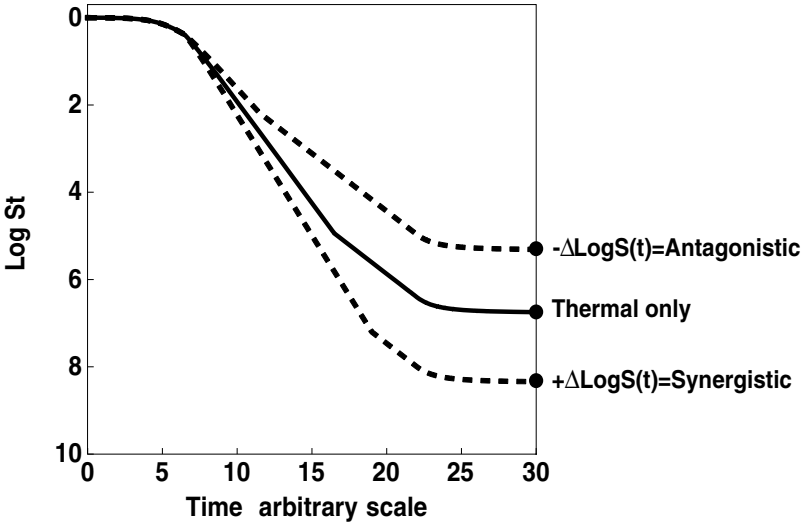

Fig. 11.2 Schematic view of how the pressure contribution can be quantified as synergistic or antagonistic in a PATS process

performance to beyond that expected from a purely thermal one, while shorter holding times will correspond to antagonistic ones. If this procedure is applied to a series of experiments with different holding times at the same and/or different pressure levels, a database that provides information on a treatment's efficacy in terms of the final survival ratio, but also on the absolute and relative contributions of the thermal and nonthermal effects, can be created.

There are several practical limitations in constructing combined inactivation models, such as the requirement of an extremely large database and the large number of parameters involved to characterize each lethal effect and their interactions, among others. Therefore, the methodology presented in this section implies a direct comparison of the actual experimental lethality level achieved, expressed as the final survival ratio, toward that estimated for a purely thermal process having the same temperature profile, providing a way to overcome those limitations and quantify each contribution.

11.7 Final Remarks

High pressure processing offers a huge field for research and development as new preservation factors are used together. Indeed, the sterilization process PATS is now well known, but there are still many areas concerning the process that need to be investigated. The development of better equipment for industry and better mathematical models to predict inactivation trends and, in general, the study of the final characteristics of products after sterilization including chemical profile are still ahead.

Also, as high pressure continues to be used with other novel technologies such as ohmic heating and pulsed electric fields, research should be conducted to test microbial counts, enzymatic stability, nutritional degradation, and the overall quality of products. High-pressure treatment also offers great opportunity for development of new products by taking advantage of its improvements in food quality together with other preservation factors.

One of the main challenges that food scientists and food engineers are facing right now is finding the right combination between high hydrostatic pressure and another hurdle that represents a viable alternative for food processing; this option should be feasible in terms of industry settings, time, money, energy, and ease of implementation.

Mathematical modeling must also be considered along with technology development to be able to describe, predict, and validate processes using, for now, two preservation factors and maybe in few years three or more factors at the same time, with minimum changes in the product, while ensuring the microbial safety and high quality that the consumer is looking for.

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Chapter 12
Modeling High-Pressure Processes: Equipment Design, Process Performance Evaluation, and Validation

Kai Knoerzer and Pablo Juliano

Abstract A common issue in high-pressure processing (HPP), both at low and high temperatures (also referred to as high-pressure thermal processing (HPTP)), is the nonuniformity of some aspects of the treatment. These issues are limited for low-temperature HPP but can be significant for HPTP, in particular with respect to non-uniformities in temperatures throughout the equipment, processing chamber, and food products. This chapter will highlight the advances that were made in recent years in modeling HPP and in particular HPTP. The importance of CFD simulations will be discussed and the underlying equations required to develop such models presented. The importance of material properties for the accuracy of the model predictions will be emphasized. Innovative approaches on expressing process uniformity and performance as well as predicting microbial and enzyme inactivation will be highlighted. Considerations and approaches on equipment scale-up and optimization will be presented.

Keywords CFD • Modeling • Simulation • High-pressure thermal • Sterilization • Pasteurization • Optimization • Scale-up • Process uniformity • Process performance

NotationLatin Letters

A	Relative activity or actual activity related to the initial activity (%)
A_0–A_6	Coefficients in Eq. (12.12)
b	Function of process temperature and/or pressure history
C_P	Isobaric heat capacity ($J \cdot kg^{-1} \cdot K^{-1}$)
$D_{ref} = D_{@T=T_{ref}}$	Decimal reduction time (min)
d_{wall}	Carrier wall thickness (m)

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F	Thermal death time (min)
g	Gravity constant (9.81 $m \cdot s^{-2}$)
h	Heat transfer coefficient ($W \cdot m^{-2} \cdot K^{-1}$)
$h_{carrier,int}$	Internal carrier height ($m \cdot$
H	Height (m)
k	First-order kinetic constant (s^{-1})
K	Inactivation rate constant (s^{-1})
k	Thermal conductivity ($W \cdot m^{-1} \cdot K^{-1}$)
k_C	Compression heating coefficient (Pa^{-1})
n	Function of process temperature and/or pressure history
n	Order of inactivation kinetic
N_0, N	Initial and final number of microbial spores
P	Pressure (Pa)
P_0	Atmospheric pressure (Pa)
Q	Volumetric compression heating rate ($J \cdot m^{-3} \cdot s^{-1}$)
r, R	Radius (radial direction, m)
$r_{carrier}$	Carrier radius (m)
R^2	Coefficient of determination
S	Survival ratio (N/N_0)
T	Temperature (K)
t	Time (s)
T_0	Initial temperature of sample (K)
T_c	Temperature after cooling (K)
T_f	Temperature after decompression (K)
t_f	Time after decompression (s)
t_{hold}	Pressure holding time
T_h	Preheating temperature (K)
t_p,$t_{process}$	Process time (s)
T_{p1}	Temperature after compression heating (K)
t_{p1}	Time after compression (s)
T_{p2}	Temperature at the end of holding time (K)
t_{p2}	Time after holding stage (s)
T_{ref}	Reference temperature (K)
t_s	Start time (s)
T_s	Initial temperature (K)
T_Δ	Temperature gradient (10 K)
T_{target}	Target temperature (K)
u,v,w	Velocity components in *x*-,*y*-, and *z*-direction ($m\ s^{-1}$)
V	Volume (m^3)
V_{usable}	Usable volume/load capacity (m^3)
v_{in}	Inlet velocity ($m\ s^{-1}$)
$\vec{V}$	Velocity vector (m/s)
x, *y*, *z*	Spatial directions
z_T	Thermal sensitivity (°C, K)

Greek Letters

α, α_p	Thermal expansion coefficient (K^{-1})
γ	Thermal diffusivity ($m^2\ s^{-1}$)
η	Dynamic viscosity (Pa s)
Λ	Dimensionless process uniformity parameter
ρ	Density ($kg \cdot m^{-3}$)

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
ASME	American Society of Mechanical Engineers
CFD	Computational fluid dynamics
cfu	Colony-forming units
HPHT	High pressure high temperature
HPP	High-pressure process
ITD	Integrated temperature distributor
NIST	National Institute of Standards and Technology
PATP	Pressure-assisted thermal processing
PATS	Pressure-assisted thermal sterilization
PE	Polyethylene
PEEK	Polyetheretherketone
PG	Propylene glycol
POM	Polyoxymethylene
PP	Polypropylene
PTFE	Polytetrafluoroethylene
ROI	Region of interest
UHMWPE	Ultrahigh-molecular-weight polyethylene
UHT	Ultrahigh temperature

Operators

d	Differential
f	General function
∂	Partial differential
Δ	Difference
∇	Gradient (nabla operator)
$\nabla\bullet$	Divergence

12.1 Importance of High-Pressure Process Modeling

Like most innovative food processing technologies, high-pressure processing (HPP) has a common challenge: to achieve sufficient uniformity of the process. This challenge is often already an issue at laboratory scale and it can become more pronounced when scaling up to pilot plants and, subsequently, to commercial equipment. Being able to predict process nonuniformities in terms of temperature, flow, and microbial and enzyme inactivation distribution is of great importance in understanding the performance of the process throughout the high-pressure vessel, to improve equipment design, and as an aid in the validation of the process for industrial uptake.

Although great progress has been made in the development of economically viable high-pressure applications, both the scientific community and the food industry recognized in the early 2000s that engineering fundamentals, including computational fluid-dynamic (CFD) models, were required to design, evaluate, optimize, and scale up high-pressure processes of foods (Hendrickx and Knorr 2001). CFD modeling can greatly assist in the characterization of temperature distribution, microbial inactivation distributions, and other quality changes as a result of temperature inhomogeneities. These models can also be applied in the (re)design and optimization of equipment and the determination of adequate processing conditions for optimum process/product performance.

A common issue in high-pressure processing, both at low and high temperature, is the nonuniformity of some aspects of the treatment. HPP generates pressure waves in liquids that travel at the speed of sound (sound in water travels at 1500 $m \cdot s^{-1}$). Therefore, pressure is commonly assumed to be transmitted instantaneously and uniformly. However, treatment nonuniformities can occur during HPP as a result of different compressibilities of the various substances in the food product, including trapped air (including headspace), but also by the food packaging material. In addition, if the purpose of the process is the inactivation of vegetative microorganisms, a nonuniform treatment can occur because some microorganisms are more resistant to pressure when embedded in a fat matrix. Foods with higher fat or oil content may, therefore, protect the microorganisms in some areas in the food where fat is contained.

In the case of processing that occurs above room temperature (initial temperature), nonuniform treatment is likely to be more pronounced. Here, in addition to pressure, temperature is an important process variable. Heterogeneous food materials exhibit differences in compression heating, and temperatures may not be uniformly distributed in the food products. Furthermore, the packaging material, the product carrier material, and the high-pressure steel vessel are not heated to the same extent as the food; therefore, temperature gradients are developed throughout the system, leading to heat flux from the products to the cooler areas (mainly the steel walls). These spatial temperature heterogeneities increase over the process time.

During high-pressure high-temperature treatment (HPHT), a preheating step is required for a product to achieve a certain initial temperature before pressurization. Although the preheated product heats up to sterilization temperatures uniformly

during compression, heat losses through the steel vessel walls can occur in certain designs. In addition, during pressure holding time, temperatures may further decrease in certain areas of the vessel. This can affect the extent and the ways in which spores and other microorganisms are inactivated, which could survive the process if temperature loss is not prevented. By coupling with adequate inactivation kinetic models, CFD modeling can assist in predicting the transient inactivation distribution throughout the vessel.

This chapter will summarize the principles of CFD as a predictive tool for the performance evaluation of HPP processes at low and high temperature. It will draw upon up-to-date models created to utilize flow, temperature, and inactivation distribution applied in vessel design, scale-up, and process optimization.

12.2 High-Pressure Processing Variables

A typical (batch) HPP system, applicable to any operating temperature, consists of (a) a cylindrical steel vessel (often wire wound) with two end closures; (b) a means of restraining end closures; (c) a low-pressure pump; (d) an intensifier for system compression, using liquid from the low-pressure pump to generate high-pressure process fluid; and (e) necessary system controls and instrumentation (Farkas and Hoover 2000). Additionally, a preheating system with fittings for a package carrier and a post-process package cooling system are required for processes operating at elevated temperatures. Stainless steel is preferred in the design of high-pressure chambers so that filtered (potable) water can be used as the compression fluid (Farkas and Hoover 2000).

Other system components include the compression fluid, a product carrier/basket, and the food to be processed (packed in flexible or semirigid containers). For HPP treatment, typical fluids used in pressure vessels include water, water with propylene glycol, edible oils, and water/edible oil emulsions (Meyer et al. 2000). Oils assist in pump lubrication and provide enhanced compression heating, particularly assisting in HPHT processes. For commercial food processing purposes, the use of potable water is the most recommended compression medium for maintaining the cleanliness of product packages (Farkas and Hoover 2000).

Packaging used for high-pressure treated foods, from institutional size to individual pouches, must accommodate more than a 12 % reduction in volume (up to 20 % or more, depending on product compressibility, headspace, and pressure level) and be able to return to its original volume, without loss of seal integrity and barrier properties (Caner et al. 2004; Farkas and Hoover 2000). Until now, identification of suitable packages that can survive pressure-assisted sterilization with the combined high-pressure and high-temperature conditions, i.e., that retain seal and overall integrity, as well as adequate barrier properties against oxygen and water vapor, remains a challenge. Packaging materials tested at HPHT conditions have been reviewed elsewhere (Fleckenstein et al. 2014; Juliano et al. 2010; Koutchma et al. 2009). For additional details on pressure effects on packaging material, readers are encouraged to consult Chap. 5 of this book.

Processed foods may differ in composition and, therefore, in their mechanical, structural, and thermophysical properties, such as viscosity, density, porosity, tortuosity, compressibility, specific heat capacity, thermal conductivity, and thermal expansivity, affecting compression heating and heat transfer during processing. A common problem is the headspace initially contained in the pouches or the air released and water vapor developed as a result of preheating. For this reason, and to avoid degradative reactions during storage, degassing and a minimum headspace are recommended.

As discussed in earlier chapters, the HPP process consists of (a) filling packages and sealing and placing them into large containers, (b) placing containers into the vessel and sealing the vessel, (c) filling the vessel with water, (d) pressurizing by water injection, (e) holding at target pressure, (f) releasing pressure, and (g) removing products and refrigeration for chilled products.

If an elevated temperature is used during the process, the following additional steps are considered (prime letters follow the steps described above): (a′) placing the packages into an insulating carrier, (b′) preheating the carrier and its contents outside the vessel, (b″) transferring the preheated carrier into the vessel and equilibrating up to an initial temperature, and (g′) removing the carrier from the vessel and cooling the packages. In this case, the temperature history inside a processed food is determined by six main process time intervals (Barbosa-Cánovas and Juliano 2007; Juliano 2006) (Fig. 12.1): (1) preheating product to a target temperature T_h,

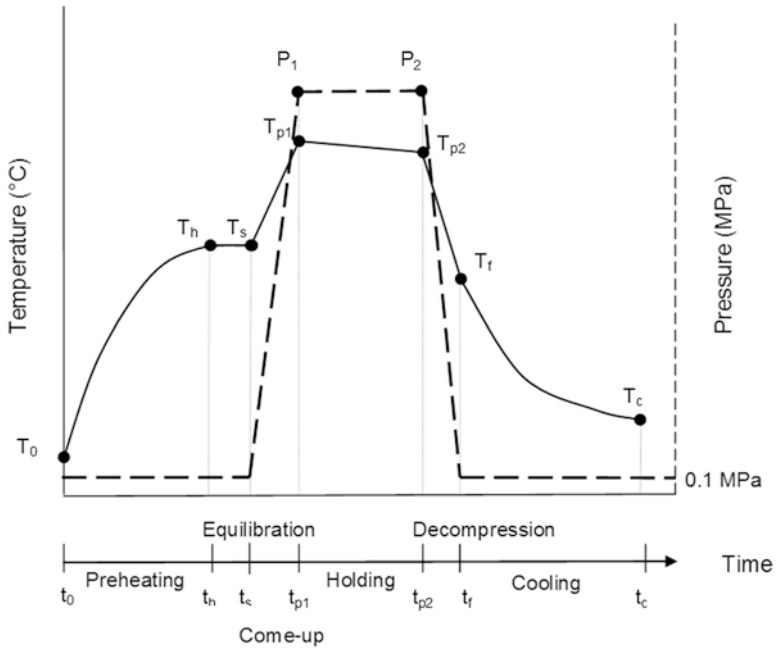

Fig. 12.1 Typical temperature profile of a pressure-assisted thermal process (the reader is referred to the Notation section in this chapter for explanation of the symbols used in this graph)

(2) equilibration of product to initial temperature T_s, (3) temperature increase of product to T_{p1} due to compression heating, (4) cooling product to T_{p2} due to heat loss, (5) decrease of product temperature to T_f during decompression (decompression cooling), and (6) cooling of product to T_c.

The critical process variables can be divided into preheating and HPP process variables. Preheating variables minimally include temperature (heating fluid and product) and heating time, whereas HPP variables include pressure (level, compression rate, and decompression rate), temperature (chamber, fluid filling the vessel, incoming pressure fluid, carrier, and product), and time. The temperature of the cooling fluid and product after processing, as well as cooling time, also needs to be taken into account. Factors during preheating, equilibration, pressure come-up, pressure holding, decompression, and cooling affecting these variables have been summarized in detail (Juliano et al. 2008).

The selected preheating method can affect initial temperature distribution within and between individual packages and potentially provide nonuniform microbial inactivation after HPP. Water baths, steam, steam injection in water, or dielectric (microwave) heating have been suggested as preheating alternatives (Juliano et al. 2006b). Faster preheating methods potentially provide less uniformity and, thus, may require a longer time for equilibration to achieve temperature uniformity.

Fluids such as air and water, as well as food packaging/polymeric solid materials, undergo compression heating when pressurized above room temperature (Ting et al. 2002). A rise between 20 and 40 °C has been observed in foods and packaging materials during high-pressure treatment at 600 MPa, depending on initial product temperature and the compressed material. In fact, the extent of compression heating varies according to the composition of the food, which in some cases is similar to that of water at different pressure-temperature combinations (Balasubramanian and Balasubramaniam 2003; Ting et al. 2002). Few researchers have reported data on compression heating rates for food products at high-temperature conditions. Compression heating of water is dependent on initial temperature (T_s) and has been reported as 3.0, 4.0, 4.6, and 5.3 °C/100 MPa at initial temperatures, 20, 60, 75, and 90 °C, respectively (Balasubramaniam et al. 2004; Farkas and Hoover 2000). The almost linear increase in temperature due to compression in (a) water, (b) orange juice, and (c) a glycol-water mixture (compression heating fluid) up to 90 °C and 600 MPa has also been studied (Ardia et al. 2004; Knoerzer et al. 2010b).

On the other hand, compression heating of steel is almost zero, maintaining the core of the vessel wall near the initial temperature (de Heij et al. 2003; Ting et al. 2002). Therefore, insulation or internal heating in the steel walls is necessary to avoid, or at least minimize, the development of a thermal gradient during pressurization and holding times (Denys et al. 2000b; Otero and Sanz 2003) as a result of heat loss toward the chamber wall and subsequent cooling down of the fluid/sample system.

Figure 12.1 shows a typical temperature profile during HPHT treatment, indicating the cooling experienced in the holding process. Loss of heat is reflected in the difference between initial and final temperature during holding time ($T_{p2} < T_{p1}$) and temperature at the beginning and end of the pressurization-depressurization period

($T_f < T_s$). In this case, "cold spots" may be located close to the vessel wall as a result of heat flow from the pressure-heated product and pressure fluid next to the vessel wall. Moreover, during compression a certain amount of liquid (which may be preheated to an insufficient extent) enters the vessel (about 17 % of the vessel volume at a pressure of 600 MPa).

For improved thermal insulation, i.e., to prevent heat loss through the steel wall, a material with low thermal conductivity (less than 1 W/m/K) could be used as part of the pressure vessel design (de Heij et al. 2003; Van Schepdael et al. 2003), for example, in the form of an internal polymeric liner. Another alternative is to use a polymeric carrier in which products are preheated to the initial temperature and then carried into the vessel. Materials suggested for this application are polyoxymethylene (POM), polyetheretherketone (PEEK), polytetrafluoroethylene (PTFE), polypropylene (PP), or ultrahigh-molecular-weight polyethylene (UHMWPE). Knoerzer et al. (2010c) measured temperatures of selected polymers during pressurization up to 750 MPa (at initial temperatures up to 90 °C) and demonstrated that PP and polyethylene (PE) exhibit higher compression heating than water within the entire pressure-temperature range, whereas PTFE has higher compression heating only up to 500 MPa. The authors did not report a linear compression heating rate value of the polymers due to the logarithmic nature of curves, as opposed to the near linearity observed in fluids at elevated temperature. Other authors have reported an average compression heating rate of 4.5 °C/100 MPa on PP; however, the conditions were not clearly reported (Schauwecker et al. 2002).

In order to maintain temperature, some HPHT installations can utilize an internal cylindrical heater or "furnace" located inside the chamber near the vessel wall, surrounding the preheated polymeric basket carrying the food packages. The furnace temperature is usually set higher than the initial target temperature to prevent potential heat loss through the carrier into the vessel walls.

12.3 Governing Equations of High-Pressure Processing

12.3.1 Heat Transfer

Increase in temperature due to pressurization, or compression heating, depends on the change of the thermal expansion coefficient α_p, density ρ (kg/m^3), and specific heat capacity C_p of both food and liquid during compression, as summarized in Eq. (12.1). Thermodynamic derivation of the compression heating equation has been covered in earlier literature (Ardia et al. 2004; Juliano et al. 2008; Knoerzer et al. 2007).

$$\frac{dT}{dP} = \frac{T\alpha_p}{\rho C_p} \tag{12.1}$$

In a high-pressure system (insulated or not), heat can be diffused throughout the vessel boundaries. Then, the balance in Eq. (12.2) can be expressed using terms for unsteady-state heat conduction during the pressure come-up step by using Fourier's law of heat diffusion, with heat generation term Q (Datta 2001; Davies et al. 1999).

$$\underbrace{\frac{\partial}{\partial t}(\rho C_p T)}_{\text{Rate of accumulation}} = \underbrace{\nabla \cdot (k \nabla T)}_{\text{Fourier's Law of unsteady heat conduction}} + \underbrace{Q}_{\text{Compression heating}} \tag{12.2}$$

where k is the thermal conductivity of the respective material in the modeled domain. In this case, convective currents within the pressurizing fluid are assumed negligible and are not included in this balance. From Eqs. (12.1) and (12.2), assuming adiabatic conditions during compression, the compression heating rate Q can be expressed as

$$Q = T\alpha_p \frac{dP}{dt} \tag{12.3}$$

where	
$Q > 0$ at $\forall t > t_s$	(pressure come-up step)
$Q = 0$ at $\forall t$, $t_{p1} < t < t_{p2}$	(pressure holding step)
$Q < 0$ at $\forall t$, $t_{p2} < t < t_f$	(pressure release step)

Hence, Eq. (12.2) also applies to the representation of all high-pressure processing steps. Thermal properties in the energy balance shown in Eq. (12.2) can be rearranged so that the thermal diffusivity γ is part of the unsteady heat conduction term:

$$\frac{\partial T}{\partial t} = \frac{T\alpha_p}{\rho C_p}\frac{\partial P}{\partial t} + \gamma \nabla^2 T \tag{12.4}$$

Thermal diffusivity is directly proportional to thermal conductivity at a given density and specific heat capacity, i.e.,

$$\gamma = \frac{k}{\rho \cdot C_p} \tag{12.5}$$

In conventional heat transfer calculations, thermal conductivity is often assumed to be independent of temperature (Holdsworth 1997). However, it is questionable whether this assumption can be made for certain thermal properties in food materials and other polymeric materials, as their dependency on temperature and pressure is unknown. In fact, Yen et al. (1991) reported a 50 % increase in thermal conductivity of molten polypropylene at a pressure level of 200 MPa, which may well be similar for solid materials.

12.3.2 Fundamental Equations of Fluid Motion

The thermo- and fluid-dynamic behavior of the pressure medium influences temperature distribution and is described by conservation equations of mass, momentum, and energy (Hartmann et al. 2003). These equations account for convection currents inside the vessel and are expressed as follows:

- Mass conservation or equation of continuity (Chen 2006):

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{V}) = 0 \tag{12.6}$$

where $\vec{V}$ is the velocity vector. This equation assumes that the fluid is initially at rest. Density increases with increasing pressure; therefore, the first term in Eq. (12.4) becomes nonzero. Since the left side must be equal to zero, the fluid velocity $\vec{V}$ must adopt nonzero values at pressure increments, thus enforcing a fluid motion.

- Energy conservation (Chen 2006):

$$\frac{\partial (\rho C_p T)}{\partial t} + \nabla \cdot (\rho \vec{V} C_p T) = \frac{\partial P}{\partial t} + \nabla \cdot (k \nabla T) \tag{12.7}$$

- Momentum conservation or Navier–Stokes equation of motion (Chen 2006):

$$\rho[\frac{\partial \vec{V}}{\partial t} + (\vec{V} \cdot \nabla)\ \vec{V}] = -\nabla P + \nabla \cdot (\eta \cdot \nabla \vec{V}) + \rho g \tag{12.8}$$

where η represents the viscosity of the compressed fluid and g represents the gravity constant.

These partial differential equations are solved simultaneously or, as referred to in the literature, "coupled" by using CFD software (Juliano et al. 2008; Knoerzer et al. 2007). These equations can also be coupled with other differential equations representing inactivation or degradation kinetics, thus accounting for turbulent flow conditions (i.e., representing a means of describing the changes in viscosity and thermal conductivity through the presence of turbulent eddies).

12.3.3 Boundary Conditions

The complete computational domain is defined by the sample, medium, carrier, and pressure vessel setup (also referred to as subdomains) forming the high-pressure system (Fig. 12.2). The initial conditions of a system can be defined in its subdomains. Provided the system is initially at thermal equilibrium (i.e., uniform temperature distribution), heat retention in the product during pressure holding is

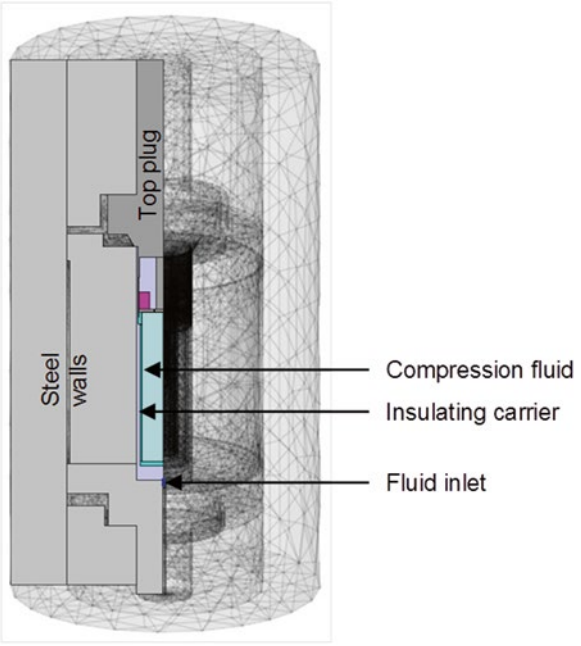

Fig. 12.2 Computational domain of a rotation-symmetric high-pressure vessel for CFD modeling. The model comprises steel components of the vessel, insulated carrier (polypropylene), compression medium domain, and fluid inlet (adapted from Knoerzer and Chapman (2011))

limited by the sample's mass, thermal diffusivity, heat transfer coefficient at the sample boundary, and temperature gradient developed due to heat loss from the compression fluid to the cooler vessel walls that do not undergo significant compression heating. Boundary conditions represent the thermal and/or flow behavior at the system's external or internal boundaries. These conditions need to be defined when constructing the model in the CFD software packages.

The most commonly applied boundary conditions in HPP modeling are symmetry boundary, inflow velocity boundary, pressure boundary, and boundaries between different subdomains:

- Symmetry boundary is defined for an axis-symmetric model, also referred to as a pseudo-three-dimensional model, and is computationally less demanding than a three-dimensional model. This is only possible in a vertical system comprising of mainly axis-symmetric features, e.g., by assuming that the compression fluid enters from the geometrical center of the bottom part of the vessel (Fig. 12.2). [This assumption is not valid for horizontal vessels, since convective motion caused by density differences, i.e., gravity/buoyancy, is not axis-symmetric; therefore, a full three-dimensional model is required.]
- Inflow velocity boundary applies to indirect pressure systems, i.e., systems using an external high-pressure intensifier pump, where the inlet tube allows the entrance of pressure fluid entrance from the pump.

- Pressure boundary is a condition that includes an expression for pressure increase during compression (due to incoming fluid), a constant pressure for holding time, and a pressure decrease during decompression due to fluid exiting the system.
- Other boundary conditions include heat transfer between liquid and solid subdomains (i.e., compression fluid, food, packages, and carrier and steel vessel walls). Continuity of heat flux at the vessel wall can be assumed between all fluid-solid and solid-solid boundaries. If laminar flow conditions exist, fluid velocity at the chamber walls can be assumed to be zero due to fluid adhesion to the walls (no slip condition).

Equations for these boundary conditions are detailed elsewhere (Juliano et al. 2008; Knoerzer et al. 2010a). Other boundary conditions have been applied to specific models, such as the use of time-dependent vessel wall temperatures (due to heat transferred into the steel mass) (Hartmann et al. 2004) and the description of boundaries for turbulent flow conditions inside the vessel (i.e., logarithmic wall function) (Juliano et al. 2009; Knoerzer et al. 2007).

12.3.4 Physical Properties of Foods and Other Materials

In order to reach good prediction accuracy, CFD models require thermophysical properties to be included as a function of pressure and temperature. For food and other polymeric materials, thermal expansivity, specific heat, density, viscosity, and thermal conductivity at high-pressure and high-temperature conditions are mostly unknown. However, some properties have been published for water, oils and alcohols, and insulating polymeric materials (Harvey et al. 1996; Knoerzer et al. 2010b, c). Information on the dependency of thermophysical properties for food and other materials with pressure and temperature can be found in Chap. 2, as well as in other references (Juliano et al. 2008; Knoerzer et al. 2010b, c).

The abovementioned thermal and transport properties of water were extracted from the NIST/ASME database (Harvey et al. 1996) to assess their variation with pressure (Juliano et al. 2008) at an initial temperature of 75 °C.

Thermal expansion coefficient α and specific heat capacity C_p have been shown to be more sensitive to temperature than pressure, whereas density ρ has been shown to be more affected by pressure change. During come-up time, α gradually decreased by 28 % when reaching 700 MPa and 105 °C, in comparison to its value at atmospheric pressure and temperature 75 °C. Similarly, C_p was reduced by 12 % and ρ was increased by 15–18 % (Juliano et al. 2008).

Thermal conductivity k has shown an increase of 44 % at 700 MPa and 105 °C, at a similar rate as temperature increases, conducting heat 1.4 times faster than water at atmospheric pressure and a temperature of 75 °C. Similarly, thermal diffusivity increased as water was pressurized, following the same trend as thermal conductivity and the increase in temperature due to compression. Zhu et al. (2007)

also found an increase in thermal diffusivity with increased pressure, not only in water but also in potatoes and cheese. The term in Eq. (12.4) containing the ratio $\alpha_p/(C_p.\rho)$, or k_C, as referred to in Knoerzer et al. (2010b, c), has been shown to be less variable with pressure than the conduction term containing the thermal diffusivity (Juliano et al. 2008) for water at initial temperatures of 75 °C. Knoerzer et al. (2010b, c) reported that k_C, and therefore compression heating, of glycol–water mixtures and some insulating polymers, is mainly dependent on pressure, whereas water and PTFE also show pronounced dependence on temperature.

Viscosity showed a relatively small increase with increasing pressure (6 %) to 700 MPa and 105 °C, behaving similarly to atmospheric pressure while showing significant decrease (25 %) if heated from 75 to 99 °C at atmospheric pressure. If cooling down would occur during holding time at 700 MPa (e.g., from target 105 °C to the chamber temperature of 75 °C), properties (ρ, C_p, α, γ) would show only small variations (1–6 %), except for the viscosity which would increase by 39 % (Juliano et al. 2008a).

The heat transfer coefficient h is used to quantify the transfer rate of heat by convection from a liquid to the surface of the food and pouch. This enables evaluation of the effectiveness of heat transfer in processes through the examination of overall resistances participating in the system under study. Particularly, in HPHT processing this proportionality coefficient is used to calculate the heat transferred through a certain area between the food package and the surrounding fluid driven by the temperature gradient. Heat transfer coefficients can be extracted from empirical relations between dimensionless Nusselt, Prandtl, and Reynolds numbers according to laminar or turbulent conditions (Perry 1997). However, not much information has been published on the validity of empirical equations, as applied to extracting heat transfer coefficients at high-pressure conditions. Different regions inside the vessel may require a particular equation (including dimensionless numbers) at the boundary to represent accurate heat transfer coefficients. For more discussion on in situ properties of food materials under pressure, readers can consult Chap. 6.

12.4 Prediction of Process Uniformity at Low Temperature

CFD, a computer-aided analysis of fluid conservation laws (mass, momentum, energy), is a convenient numerical modeling approach for the prediction of temperature and flow during high-pressure processes in two or three dimensions. This analysis allows the simulation of systems (solid and semisolid structures) that interact with fluid, by solving the governing equations for fluid flow and heat transfer (Eqs. (12.6), (12.7), and (12.8)). These partial differential equations describing the entire processing system are transformed into a set of equations and solved numerically to approximate the exact solution (Nicolaï et al. 2001). The underlying methods for the numerical analysis include finite-volume, finite-element, and finite-difference methods described elsewhere (Juliano et al. 2008). These discretization methods provide a solution for a “discrete” number of points (on a

computational grid), where conservation equations are applied. In all cases, such grids are tailored to provide a "mesh-independent" solution for the numerical approximation of the governing equations.

Several authors have conducted extensive research into developing discrete CFD models to predict transient temperature and flow distributions, uniformity, and loss of heat generated during compression through high-pressure vessel walls during all processing steps (Denys et al. 2000a, b; Hartmann et al. 2003; Hartmann et al. 2004; Hartmann and Delgado 2002a, 2003a). Some models include solid materials (Juliano et al. 2009; Knoerzer et al. 2007; Otero et al. 2007), whereas other models predict temperature distribution in three dimensions (Ghani and Farid 2007) and at HPHT conditions (Juliano et al. 2009; Knoerzer et al. 2007). Due to the vertical configuration of the modeled laboratory and pilot-scale vessels, some simulations include a vertical pressure fluid inlet near the center bottom (Hartmann 2002; Hartmann et al. 2004; Hartmann and Delgado 2002a, 2003a, b; Knoerzer et al. 2007; Otero et al. 2007). Thus, in most cases, 2D cross sections were used as the computational domain, similar to the one shown in Fig. 12.2, due to rotation symmetry at the central axis.

Examples described in this section use the finite-element and finite-volume methods to develop CFD models. It will be shown how the CFD models were developed and applied to evaluate the effects of varying inlet velocities, presence of packages, vessel size, carrier configuration, and model inputs on temperature and flow distribution during and after HPP processing.

12.4.1 Flow and Temperature Distribution

In indirect high-pressure systems, a certain amount of fluid is pumped into the already filled vessel, further increasing the amount of fluid that reaches the target pressure level. Velocity at the inlet, where the pressure fluid is pumped into the vessel, determines pressure come-up time. It has been found that the velocities used in a micro 4 mL vessel modeled by Hartmann (2002) provided laminar flow at the inlet, whereas a 35 L pilot-scale vessel modeled by Knoerzer et al. (2007) gave turbulent conditions at the inlet region, as will be shown later.

One study at low-temperature conditions (Hartmann and Delgado 2002a) analyzed the thermo- and fluid-dynamic effects of pressurizing fluid (water) in a high-pressure vessel by numerical simulations. Temperature and fluid velocity profiles were modeled in a 4 mL chamber, pressurizing up to 500 MPa with an initial temperature of 15 °C and pressure holding time of 200 s at three inflow velocities (2, 4, and 8 mm/s). Process simulations were carried out using the finite-volume method in the commercial software package CFX 4.4 (Ansys Inc., Canonsburg, PA, USA; originally developed by the United Kingdom Atomic Energy Authority, AEA Technologies). The velocity profile was maintained constant at the inlet cross section for each case, giving a parabolic velocity profile further downstream in the inlet tube. In the inlet tube, velocity was zero at the surrounding wall (due to the "no slip" boundary condition), reaching a maximum value at its central axis.

The flow field obtained inside the microvessel, a low-temperature vessel, was governed by forced convection. Within a close region around the inlet, the entering fluid underwent a strong deceleration, resulting in a nonuniform temperature distribution. Temperature gradients caused an inhomogeneous density distribution that generated a buoyancy-induced fluid motion (natural convection due to the gravitational field). Very low inlet velocities resulted in near-isothermal conditions inside the vessel, i.e., no temperature rise during to compression. However, faster compression rate resulted in a temperature increase, which was close to the maximum adiabatic conditions.

Validation was performed with a temperature probe, which was placed in the central plane near the wall. Numerical values were found to be within an error range of 0.7 K of the experimental values throughout the whole process, which was attributed to the uncertainty of the manual positioning of the temperature probe (±1 mm) and temporal resolution of the thermocouple (approx. 2 s), and thus concluded to be in good agreement with the experimental data.

Knoerzer et al. (2007) used the finite-element method (COMSOL Multiphysics, COMSOL AB, Stockholm, Sweden) to model a much higher inlet velocity of 5.7 m/s velocity for a 35 L pilot-scale vessel filled only with water, corresponding to a Reynolds number of 60,000 and creating turbulent flow in the vessel's bottom region. In this case, the pronounced turbulent region with arising eddies (and, therefore, increased thermal conductivity, also referred to as turbulent thermal conductivity), provided significant cooling during pressure come-up and holding steps. Thus, opposite from what was observed by Hartmann (2002) in a small vessel at laminar conditions, higher inlet velocities (at turbulent conditions) in the larger vessel still hindered the system from achieving temperatures expected for an adiabatic system through compression heating.

Hartmann and Delgado (2003b) developed models of different-sized vessels (microscale (0.8 L), pilot scale (6.3 L), and semi-industrial scale (50.3 L)) to study the effect of scale on temperature distribution. These models included five packages in each vessel containing an enzyme solution equally distributed through the chamber height, operating at 550 MPa and 40 °C initial temperature. More heat retention was found in packages contained in the larger 50.3 L vessel, resulting in an average temperature difference per package of around 7 K compared with the 0.8 L vessel and 4 K compared with the 6.3 L vessel. Lower temperatures were mainly attributed to the incoming "cold" pressure medium, which was less influential in a larger-scale vessel with bigger packages.

12.4.2 Microbial and Enzyme Inactivation Distributions

CFD software packages can not only assist in calculating temperature evolution and distribution but also allow the coupling of the governing fluid-dynamic equations with kinetic inactivation or degradation equations to predict transient microbial and enzyme inactivation distribution and provide a two- or three-dimensional prediction of the safety and quality of the treated food product.

Such model coupling can be done either internally (solved simultaneously within the software package) or externally (by converting the transient temperature profile predicted by the CFD model into distributions of the extent of inactivation).

An equation describing the temporal and spatial enzyme activity or microbial inactivation distribution (Eq. (12.9)) can be used to determine the inactivation distribution or relative retention throughout the vessel volume at different times (Hartmann et al. 2003):

$$\frac{\partial A}{\partial t} + u\frac{\partial A}{\partial x} + v\frac{\partial A}{\partial y} + w\frac{\partial A}{\partial z} = K(P,T)A \tag{12.9}$$

where A is the relative enzyme activity or microbial load (actual value related to initial value); $K(P,T)$ is the inactivation rate constant; and u, v, and w are the components of the fluid velocity vector in the x-, y-, and z-directions, respectively. The left-hand side contains the coupling between A and the flow field, i.e., the velocity of the solution. The right-hand side represents the coupling of A with the temperature distribution and pressure.

For example, Hartmann et al. (2003) studied the influence of packaging material on heat transfer and inactivation of *E. coli* suspended in UHT (ultrahigh-temperature-treated) milk. In this case, the inactivation equation (Eq. (12.9)) included an inactivation rate constant for *E. coli*. The study mainly discussed inactivation effects during the holding period at 400 MPa and 20 °C (fluid temperature inside vessel) or 30 °C (at vessel wall). Hartmann et al. (2003) validated their simulations by comparing numerical simulation results with experimental data obtained for *E. coli* in UHT milk at an initial concentration of 10^8 cfu/mL. In this case, validation was performed with inactivation data obtained in a small 0.2 L vessel. They also compared numerical and experimental inactivation results with predictions from a two-parameter model (pressure and temperature), describing inactivation with an ordinary differential equation (Hinrichs 2000); the model was valid between 5 and 40 °C for a pressure range 300–500 MPa. This numerical simulation, including dynamic effects, showed good agreement with the experimental data and the two-parameter model. Differences between the numerical simulation and experimental results were attributed to the assumption of adiabatic pressurization set for the numerical model, resulting in a temperature overshoot of about 10 °C.

12.5 Prediction of Process Uniformity at High Temperature

HPHT processing, also referred to as pressure-assisted thermal processing (PATP), has received significant focus in the area of pressure-assisted thermal sterilization (PATS), which is an emerging preservation method for the development of shelf-stable low-acid food products. HPHT involves combining pressures up to 800 MPa with moderate initial chamber and product temperatures up to 90 °C.

PATS takes advantage of the compression heating developed in the product and pressure-transmitting fluid, as well as the high pressure, to eliminate spore-forming bacteria (Barbosa-Canovas and Juliano 2008; Margosch 2005; Matser et al. 2004) (see also Chaps. 11 and 28). For instance, pressurization temperatures of 90–116 °C, combined with pressures of 500–700 MPa, have been used to inactivate a number of strains of *C. botulinum* spores (Farkas and Hoover 2000; Margosch et al. 2004). Other researchers have shown that certain bacterial endospores (*C. sporogenes*, *B. stearothermophilus*, *B. licheniformis*, *B. cereus*, *and B. subtilis*) in selected matrices like phosphate buffer, beef, vegetable cream, and tomato puree (Balasubramanian and Balasubramaniam 2003; Gola et al. 1996; Krebbers et al. 2003; Meyer et al. 2000; Raso et al. 1998; Rovere et al. 1998) can be eliminated after short-time exposure to temperatures and pressures above 100 °C and 700 MPa, respectively. In February 2009, the US Food and Drug Administration accepted a petition for the commercial production of a PATS-processed mashed potato-based product. This is the first process of this kind that has been filed and accepted (in a 35 L high-pressure sterilization vessel).

Several HPHT combinations have been proposed for spore inactivation (Barbosa-Cánovas and Juliano 2008; de Heij et al. 2003; Leadley 2005), among which the application of pressures around 600 MPa with short holding times (5 min or less) seems to be an appropriate balance between economical processing for industrial purposes and food safety. Shorter processing times could increase productivity and equipment lifetime and reduce maintenance costs. Furthermore, a shorter thermal pressure process may result in a product with increased quality retention, making the process potentially more attractive than conventional in-container sterilization (i.e., canning). This result would particularly be the case for obtaining high-quality foods in large containers. In fact, in several HPHT studies, the process has been shown to produce foods with higher nutrients, color, and flavor, along with other improved sensory attributes (Barbosa-Cánovas and Juliano 2008; Juliano et al. 2007; Juliano et al. 2006a,b ; Krebbers et al. 2002; Matser et al. 2004).

Thermal processing of foods requires evaluation of temperature and flow distributions involved at both equipment and product levels to assess process performance. This information from HPHT processes can be utilized to predict safety level achieved (i.e., extent of *C. botulinum* inactivation), product shelf stability (i.e., inactivation of spoilage organisms and enzymes), and anticipated product quality (i.e., "cook" level and nutrition). Mass, momentum, and energy transfer models, coupled with microbial inactivation models and/or predictive models for product quality, can greatly assist in evaluating process performance and benefits.

A high-pressure system designed for sterilization conditions must be able to achieve high pressures of at least 600 MPa and chamber temperatures equal to or greater than 90 °C. This can be accomplished by building a pressure chamber of appropriate materials and designing a high-pressure pumping system with fast pressure come-up. Current sterilization systems range from laboratory scale (0.02–1.5 L) to pilot scale (2–55 L). Concepts for industrial systems (up to 150 L) have been designed but are not available yet for purchase. Existing types of equipment consist

of various configurations (Balasubramaniam et al. 2004) that offer different levels of compression heat retention and efficiency.

To achieve sterilization during pressurization, all treated foods must at least reach the target compression temperature for a certain time during pressure holding (i.e., an F_0 of at least 2.8 min to ensure product safety). To achieve this goal, a number of variables must be controlled from the start. The following section provides an overview of the application of CFD models to predict uniformity of temperature and achieved sterility in HPHT systems.

12.5.1 Flow and Temperature Distribution

CFD models can determine how a number of variables in the HPHT process affect heat generation and transfer and, therefore, temperature evolution throughout the vessel (Juliano et al. 2008). These models can be utilized to improve process and equipment design. For example, compression heat retained in the vessel can be maximized by determining the optimum thickness of internal product carrier walls. Performance of the process at different scales, from small laboratory scale vessels to industrial-size scales (i.e., 300 L to 600 L capacity vessels), can also be compared. Once CFD models are validated against measured temperature data, output distributions from these models can assist regulatory authorities to approve the commercial use of the process.

A cylindrical carrier, used to preheat the product to the initial target temperature and transport the packages into a (pilot-scale) vessel, can act as a barrier to the flow of incoming colder fluid into the package area. In particular, carriers made of solid polymeric materials may prevent heat loss to the vessel walls and to the top and bottom areas of the pressure vessel.

Knoerzer et al. (2007) simulated the temperature distribution obtained after placing two types of food carriers inside an Avure 35 L pilot-scale high-pressure sterilization system (Quintus Food Press Type 35 L-600, Avure Technologies, Kent, WA, USA), i.e., a metal composite carrier (developed in the original design of the vessel to operate with a furnace) and a PTFE (polytetrafluoroethylene) carrier. They simulated all processing steps in the 35 L vessel filled with water pressurized to 600 MPa and 285 s pressure holding (at an initial temperature of 90 °C). To validate the simulated temperatures, a 3×3 thermocouple array placed in an axis-symmetric plane was set up in randomized form in several runs. An example of the modeling domain and temperature distribution is shown in Fig. 12.3. The models included a compression heating term from Eq. (12.3) and thermal properties such as density, specific heat, and thermal expansivity as functions of temperature and pressure.

The simulations showed significant cooling (to about 40 °C) below the carrier due to the colder turbulent incoming fluid and demonstrated that both carriers provided a barrier against cooling (Fig. 12.3). However, the lower region inside the metal carrier was colder and, therefore, an uneven temperature distribution was

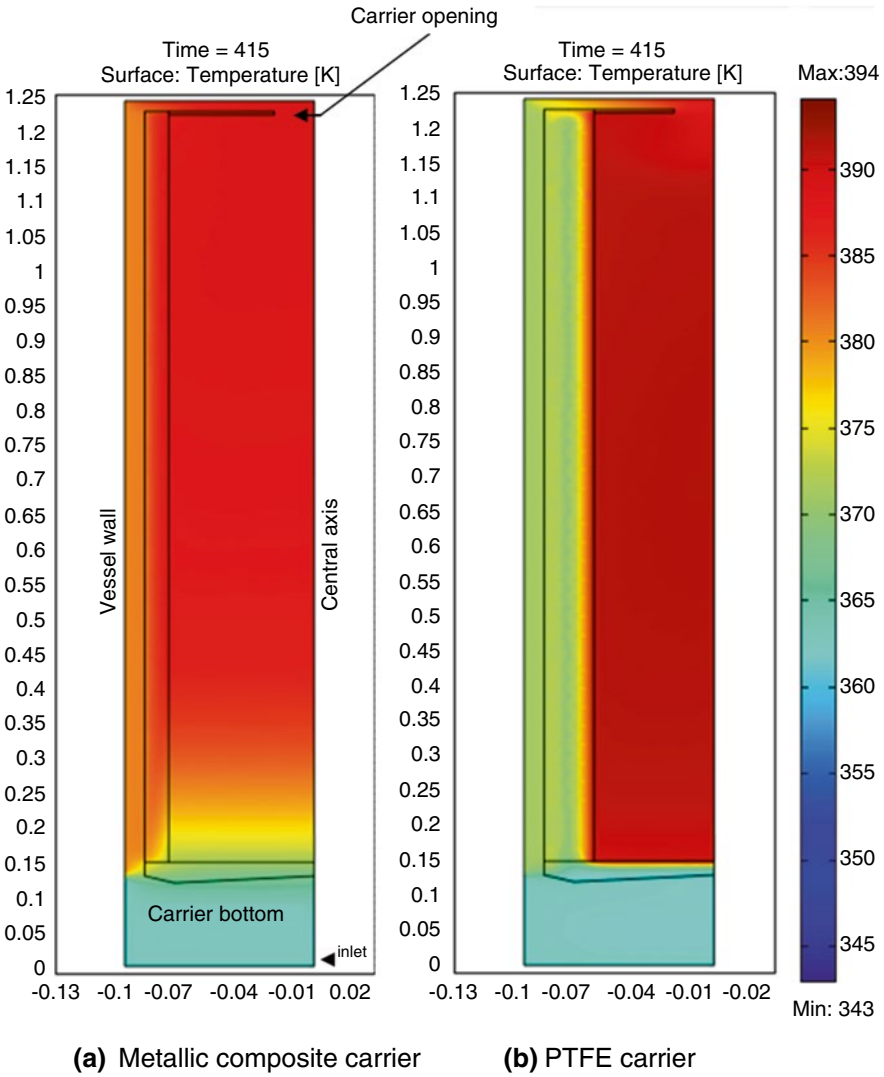

Fig. 12.3 Thermal profile of two CFD models of a 35 L vessel at the end of pressure holding time (415 s) at 600 MPa including: (**a**) a metal composite carrier and (**b**) a PTFE carrier (adapted from Knoerzer et al. 2007)

observed and validated. An excellent correlation ($R^2 = 0.97$) was obtained by comparing predicted and measured values in a parity plot, which included all locations of the thermocouples at each time step throughout the process.

On the other hand, the carrier made of PTFE was able to retain most of the compression heat generated even during pressure holding time throughout its entire

volume, providing better insulation than the metal carrier (Fig. 12.3) at the end of pressure holding. The axis-symmetric model was further developed (Juliano et al. 2009) by including steel vessel walls, a vessel lid, and cylindrical packages containing a "water-like" solid. The initial temperature of the vessel lid was considered to be lower, as in the actual process no heating source was included in the lid. As expected, this addition lowered the temperature at the upper region of the vessel and increased the natural convection.

Our group has compared the temperature distribution predicted by a CFD model representing to scale the Avure 35 L sterilization vessel with a model representing a Stansted ISO-LAB FPG11501 High Pressure 3.6 L unit (Stansted Fluid Power Ltd., Stansted, Essex, UK), both with compression fluid entering at the bottom of the vessel and containing a PTFE carrier with an opening at the top, operating at the same process conditions (initial temperature 90 °C and final pressure of 600 MPa, come-up time 130 s, holding time 315 s, decompression time 15 s) (Fig. 12.4). The predictions show the higher heat retention provided by the 35 L unit and a more pronounced loss of heat in the 3.6 L vessel during pressurization (Fig. 12.4). In this case, the model assumes no compression heating of PTFE at the carrier region, which, if included, may assist in reducing the heat loss rate inside the 3.6 L vessel (Fig. 12.5).

In a recent work, Knoerzer and Chapman (2011) investigated the effect of variations in model inputs for compression fluid properties and process conditions

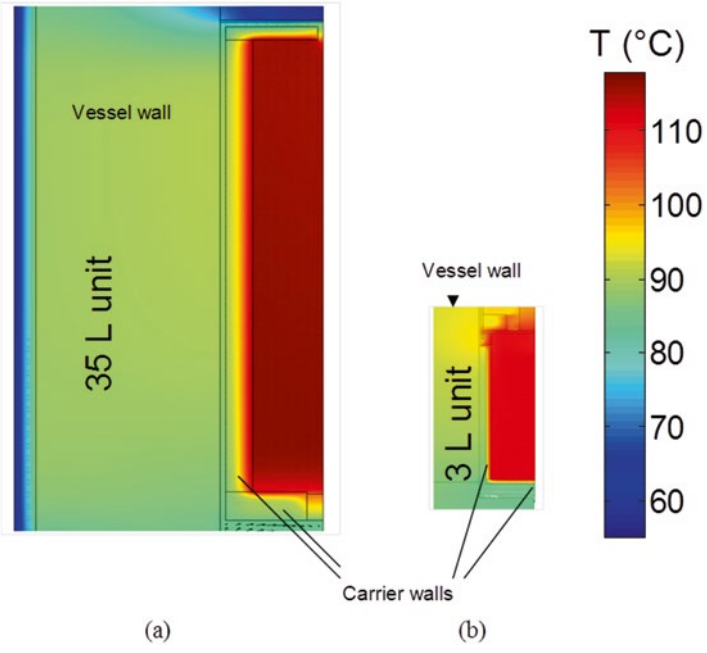

Fig. 12.4 Comparison of the temperature distribution predicted by CFD models of two high-pressure sterilization vessels including a PTFE carrier at the end of 315 s holding time (initial temperature 90 °C and 600 MPa): (**a**) a 35 L vessel and (**b**) a 3 L vessel (not to scale)

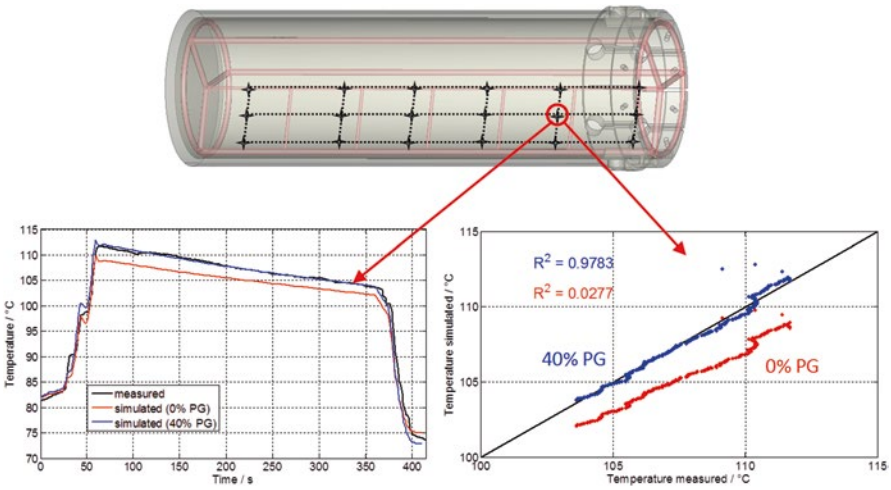

Fig. 12.5 Predicted temperature profiles and parity plot (predicted vs. measured temperatures) during pressure holding stage at the top location (location 1) inside the empty product carrier from simulations assuming different pressure-transmitting fluids (adapted from Knoerzer and Chapman (2011))

(i.e., the applied pressure profiles) on the prediction accuracy of a CFD model for the 3.6 L Stansted system. They found good agreement between simulated and measured temperature distributions when accurate compression heating coefficients for the compressed materials and actual pressure profiles (providing data for pressure evolution, fluid inlet velocity, and time-temperature profiles) were used as inputs for the model. Inaccurate approximations of these values and conditions resulted in much less useful models, highlighting the importance of attention to detail in input data for CFD models of high-pressure processing, particularly in the still early stages of development of the high-pressure high-temperature technology.

The authors reported that representative pressure profiles can be readily obtained from the logged pressure data of the high-pressure units. Until recently, the compression heating properties, particularly as functions of pressure and temperature, were, except for water, unknown. Published work by Knoerzer et al. (2010b, c), however, includes reports on a new methodology to measure these properties for any given fluid and solid and derives equations for compression heating properties as a function of pressure and temperature.

Although it was highlighted that the process conditions implemented in the models ought to be as close to actual process conditions as possible, the authors acknowledge that it is obviously not feasible to perform modeling after the actual process has been completed, as that would defeat the purpose of modeling. However, it was recommended that representative, averaged close-to-reality process conditions (e.g., the distinctive pressure profiles characterizing the mechanics of the various types of HP units) be obtained wherever possible prior to model development.

12.5.2 Microbial Spore Inactivation Distribution

As mentioned, models able to predict microbial (spore) inactivation can be coupled with the fluid-dynamic and heat transfer equations contained in the models as described in the earlier section. For example, Knoerzer et al. (2007) evaluated *C. botulinum* spore inactivation distribution inside a vessel, as shown in Fig. 12.6 for three scenarios: (a) vessel without carrier, filled with only water, (b) vessel with metal carrier, and (c) vessel with PTFE carrier. The temperature component of the model output was used to calculate the F-value, which was transformed into inactivation values by using Eq. (12.10):

$$F = \int_{t}^{0} 10^{\frac{T(t)-T_{\text{ref}}}{z_{\text{T}}}} \mathrm{d}t = D_{@T=T_{\text{ref}}} \cdot \log S \tag{12.10}$$

where F is the thermal death time, i.e., the equivalent time the material would be held at a T_{ref} of 121.1 °C to achieve the same microbial inactivation of the process, z_{T} is the temperature increment that changes the rate of the process by a factor of 10, S is the survival ratio N/N_0 (N and N_0 are the final and initial number of spores), and $D_{@T=T_{\text{ref}}}$ is the time at T_{ref} to lower the number of spores by a factor of 10. The traditionalfirst-order inactivation kinetics can also be expressed as

$$\log S(t) = -10^{\frac{T(t)-T_{\text{ref}}}{z_{\text{T}}}} \cdot \frac{t}{D_{@T=T_{\text{ref}}}} \tag{12.11}$$

In the vessel without a carrier, inactivation distribution yielded less than 1 log reduction, while inhomogeneous distribution was seen in the metal carrier (between 2 and 9 log reductions). For the insulated PTFE carrier, almost 95 % of the carrier's volume showed more than 12 (up to 14) log reductions in *C. botulinum* spores (Fig. 12.6).

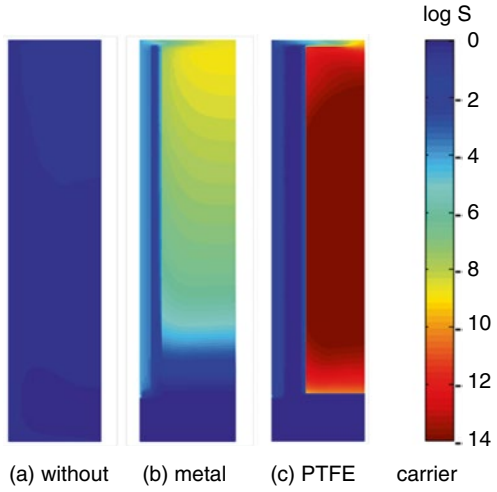

Fig. 12.6 Predicted distribution of extent of *C. botulinum* inactivation inside a 35 L pilot-scale high-pressure vessel in three scenarios: (**a**) vessel without carrier, (**b**) vessel including a metal composite carrier, and (**c**) vessel including a Teflon (PTFE) carrier (adapted from Knoerzer et al. 2007)

Based on the increased extent of inactivation observed when using an insulated carrier, this type of model can be of great assistance in finding an optimum carrier design that produces thermal uniformity and uniform spore inactivation throughout the carrier, as well as optimum processing times.

A more detailed CFD model, as described in Fig. 12.7, was later on developed (Juliano et al. 2009) as a platform to compare the performance of known predictive *C. botulinum* inactivation models for estimating spore inactivation distribution. In addition to the previously considered linear kinetic model including the *F*-value (Eq. (12.11)), the Weibullian model (Eq. (12.12)), an *n*-th order model (Eq. (12.13)), and a combination of the *n*-th order model and the log-linear model were selected.

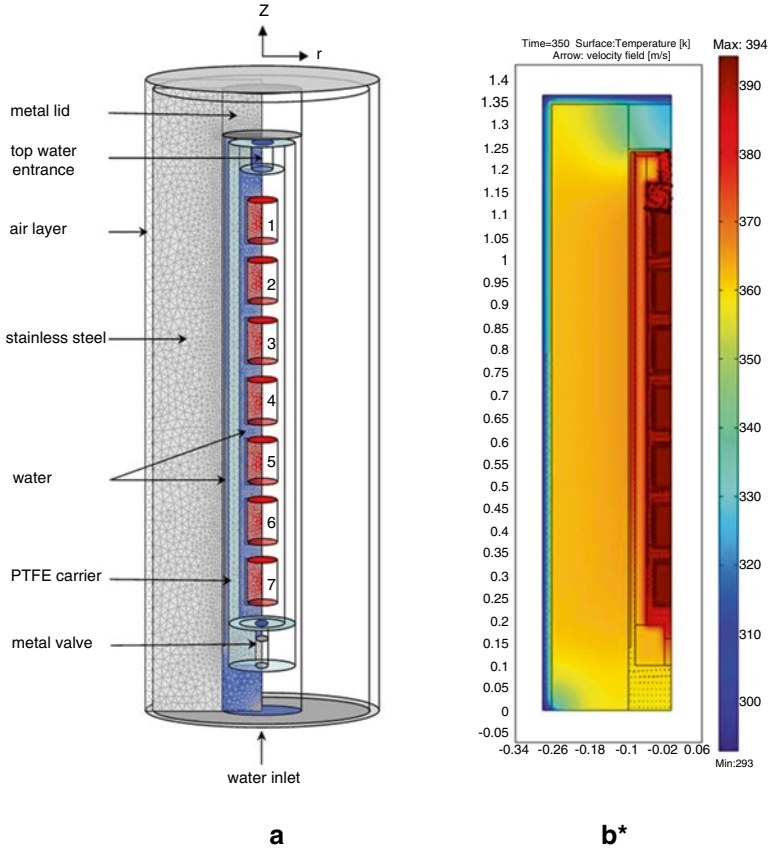

Fig. 12.7 CFD model of a 35 L vessel including carrier, packages, steel walls, and metal lid: (**a**) computational domains of the model structure and (**b**) thermal and flow profile in the vessel 350 s after process start at 600 MPa (adapted from Juliano et al. 2009). Conditions simulated include: starting temperature and pressurization rate to a final pressure of 600 MPa and a holding time of 315 s. *arrows* proportional to the maximum velocity at a specific time

The Weibullian model can be expressed as

$$\log S(t) = -b[T] \cdot t^{n'[T]} \tag{12.12}$$

where $b(T)$ and $n'(T)$ are temperature-dependent parameters. Assuming that spore inactivation is only affected by temperature, these parameters can be expressed as function of the thermal history before, during, and after pressurization. Furthermore, inactivation of a selected thermo-baroresistant *C. botulinum* strain has been expressed as a function of both temperature and pressure in an n-th order model (Margosch et al. 2006):

$$\frac{dN(t)}{dt} = -k_i(T,P) \cdot N^n \tag{12.13}$$

A reaction order of 1.35 was found by fitting k to curves obtained at different pressure-temperature combinations of 70–120 °C and 600–1400 MPa as well as ambient pressure. Single values of each constant at each combination were condensed into:

$$k_i'(T,P) = e^{A_0 + A_1 \cdot P + A_2 \cdot T + A_3 \cdot P^2 + A_4 \cdot T^2 + A_5 \cdot P \cdot T + A_6 \cdot P \cdot T^2} \tag{12.14}$$

where $k_i' = k_i'(T,P) = k_i \cdot N_0^{n-1}$ (Margosch et al. 2006).

The rationale for comparing the performance of these inactivation models is the questionable validity of the traditional F_0 method, which assumes first-order reaction kinetics for the inactivation of *C. botulinum* and the linearity of the D-value curves as a function of temperature (constant z_T) (Peleg 2006). Furthermore, an HPHT process most likely requires an expression that accounts for the possible combined effect of pressure and temperature.

The kinetic models were expressed in the form of an ordinary differential equation (Eq. (12.15)) which were externally coupled with the CFD model temperature output through the MATLAB routine described by Knoerzer et al. (2007), which allowed predicting inactivation for non-isothermal scenarios.

$$\frac{\mathrm{d}[\log S(t)]}{\mathrm{d}t} = f[\log S(t)] \tag{12.15}$$

where f is a generic function of $\log S(t)$. The routine fitted the temperature data to give a function $T(t)$ in all locations inside the vessel and was subsequently substituted in the differential equation, which was then solved for the total time of the process. The output of this routine was capable of providing: (a) log $S(t)$ vs. t plots at different locations, (b) log $S(x,y)$ distributions at specific times, and (c) transient log $S(x,y,t)$ animations.

Table 12.1 summarizes the microbial inactivation models used (traditional first-order log-linear kinetics model (A), Weibullian model (B), n-th order kinetics model (C), combined log-linear/n-th order model (D)) expressed in Eq. (Eq. 12.17) and

Table 12.1 Description of selected *C. botulinum* inactivation models and their parameter values for comparison in a CFD model platform of a 35 L high-pressure system

Model	$\frac{\mathrm{d}[\log S(t)]}{\mathrm{d}t}$	Parameters for *Clostridium botulinum*
A—linear kinetics	$-\frac{10^{\frac{T(t)-T_{ref}}{z_r}}}{D_{@T=T_{ref}}}$	$T_{ref} = 121.1$ °C $z_T = 10$ °C $D_{@T=T_{ref}} = 12.6$ s
B—Weibull distribution	$-b[T(t)]\cdot t^{n'[T(t)]}\cdot\{-\frac{\log S(t)}{b[T(t)]}\}^{\frac{n'[T(t)]-1}{n'[T(t)]}}$	$b[T(t)] = \ln\{1+e^{0.3\{T(t)-102.3\}}\}$ $n'[T(t)] = 0.325+\frac{0.425}{1+e^{0.0994\{T(t)-101\}}}$
C—*n*-th order kinetics	$-10^{(n-1)\cdot\log S(t)}\cdot\frac{k_i'[T(t),P(t)]}{\ln(10)}$	$k_i'[T(t),P(t)] = e^{A_0+A_1\cdot P+A_2\cdot T^2+A_3\cdot P^2+A_4\cdot T^2+A_5\cdot P\cdot T+A_6\cdot P\cdot T^2}$ where $n = 1.35$ $A_0 = 2.465$; $A_1 = -0.023$; $A_2 = -0.149$; $A_3 = 2.259\times10^{-5}$; $A_4 = 1.462\times10^{-3}$; $A_5 = 1.798\times10^{-4}$; $A_6 = -1.806\times10^{-7}$
D—combined linear *n*-th order	if[$T\le T_c, -\frac{10^{\frac{T(t)-T_{ref}}{z_r}}}{D_{@T=T_{ref}}}$, $-10^{(n-1)\cdot\log S(t)}\cdot\frac{k_i'[T(t),P(t)]}{\ln(10)}$]	Refer to models A and C $T_c = 100$ °C is the critical temperature when model A switches to model C

adapted from Juliano et al. 2009
A: (Pflug, 1987)
B: (Campanella and Peleg, 2001)
C: (Margosch et al. 2006)

includes the respective parameter values for *Clostridium botulinum* spores. The log-linear first-order and Weibullian models originated from *C. botulinum* inactivation data at near-atmospheric conditions and, therefore, only accounted for the temperature variation. On the other hand, the *n*-th order model was obtained from *C. botulinum* inactivation data corresponding to several combinations of temperature and pressure (Margosch et al. 2006) and, thus, included terms for both temperature and pressure. Considering that the n-th order model was only valid above 100 °C in the pressure range 0.1–600 MPa, a combined discrete model was tested, which solved for log-linear kinetics at temperatures equal to or lower than 100 °C and for *n*-th order kinetics at temperatures greater than 100 °C (Table 12.1).

The distribution of *C. botulinum* inactivation log reduction predicted by each model from the CFD platform for the 35 L vessel is shown in Fig. 12.8. The final

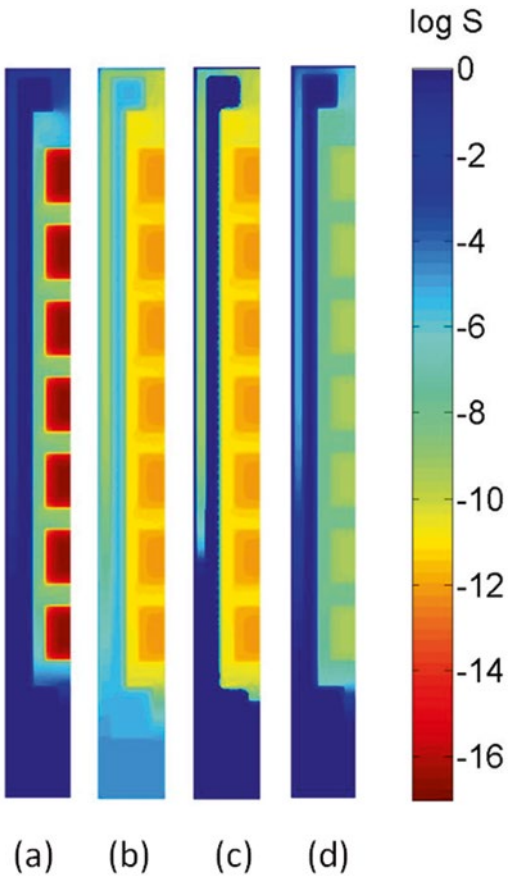

Fig. 12.8 Predicted distribution of *C. botulinum* log reduction extent according to four selected kinetic inactivation models using a CFD model platform for a high-pressure 35 L sterilization system: (**a**) traditional log-linear kinetic model, (**b**) *n*-th order kinetic model, (**c**) combined discrete log-linear and *n*-th order kinetic model, and (**d**) Weibullian distribution model (adapted from Juliano et al. 2009)

inactivation calculated inside each package when using model A (log-linear kinetics) was 16.5 log reductions; model C (*n*-th order kinetics) and model D (*n*-th order and combined log-linear/*n*-th order) achieved approximately 12.0 log reductions; model B (Weibullian) achieved only around 9.4 log reductions at the end of the process. Hence, the conventional thermal processing kinetics (not accounting for the combined effects with pressure) required shorter holding times to achieve a 12D reduction of *C. botulinum* spores compared to the other models. The inactivation distribution predicted by the Weibullian model and the *n*-th order models inside the vessel was found to be more uniform compared to the log-linear kinetics model (Fig. 12.8).

Differences in inactivation and more uniform distribution provided by models B, C, and D (Table 12.1) can be explained by (1) the tailing of curves given by the model parameters and (2) the fact that only data from different *C. botulinum* strains were available and used in each model. Furthermore, the Weibullian model (B) only accounted for the temperature variation, not pressure, which also affected the outcome. This result showed how the CFD platform became quite useful in evaluating and comparing the inactivation extent and uniformity provided by different

C. botulinum inactivation models in the same system. Inactivation models used in different food media and accounting for the heat transfer effect of the selected package during the preheating step, to be developed in the future, could be evaluated through such CFD platforms. These CFD platforms can then be used as an aid for regulatory filing of the technology as well as in process and equipment design.

A similar exercise was conducted for the 3.6 L Stansted unit with the Weibullian and log-linear (first-order) *C. botulinum* spore inactivation models (data not published). Experimental trials were conducted by pressurizing different strains of proteolytic *C. botulinum* spores, and the level obtained was compared with that provided by the model. It was found that some strains followed the log-linear predictions better, while others closely followed the Weibullian model predictions. This finding reemphasizes the importance of utilizing predictive models specifically developed for the strain (or strains) of interest; otherwise, prediction accuracy for microbial spore inactivation is questionable.

12.6 Process Performance Parameters

In order to evaluate the performance of a high-pressure process and make comparisons to others, a dimensionless parameter able to represent process uniformity (e.g., temperature uniformity and magnitude) during treatment inside the processing vessel is more convenient. It is desirable that this process performance parameter can assist in (a) providing the extent of inactivation (e.g., of microorganisms, spores, and enzymes) achieved throughout the volume of prepackaged food contained in the vessel, (b) predicting the quality degradation as a result of temperature (pressure) use, and (c) facilitating HPHT process design and validation. Aspects of the HPHT process include thermal process evaluation based on container size and shape, food composition, equipment modification, and optimization, scale-up studies, energy use, modification and optimization of process conditions, etc. (Juliano et al. 2008). The following section will describe two performance parameters which have been able to characterize the process uniformity (Hartmann and Delgado 2003b; Knoerzer et al. 2010a).

12.6.1 Process Uniformity Λ

Hartmann and Delgado (2003b) conducted a proportional scale-up study through CFD modeling but, in this case, modeled the use of five packages containing enzyme solution in 0.8, 6.3, and 50.3 L vessels. Equation (12.9) was used for predicting the inactivation of *B. subtilis* α-amylase. Process uniformity Λ was defined as the ratio of the minimum (A_{ave_min}) and maximum (A_{ave_max}) average activity retention per package in all five packages:

$$\Lambda = \frac{A_{ave\_min}}{A_{ave\_max}} \tag{12.16}$$

Table 12.2 Process uniformity for simulated vessel volumes and heat transfer coefficients (adapted from Hartmann et al. 2003)

		h_{pp} (W/m^2.K)		
Λ (-)		1 x 10^{-4}	1 x 10^{-3}	1 x 10^{-2}
Vessel volume (L)	0.8 L	0.86	0.84	0.84
	6.3 L	0.90	0.78	0.74
	50.3 L	0.97	0.81	0.69

Table 12.2 describes the uniformity values obtained for each vessel volume while varying the heat transfer coefficient h_{pp} at the boundary of each package. Process uniformity for the 0.8 L vessel did not depend on h_{pp}, whereas the other larger vessels were greatly affected. The lowest heat transfer coefficients provide more inactivation uniformity between packages because heat is retained better inside the packages for a major part of the process. For instance, a uniformity of 0.97 for the 50.3 L vessel indicates that heat is retained inside the package throughout most of the process, leading to a high degree of inactivation and uniformity.

This type of measure of process uniformity is useful for comparing whole pouches but gives no information on uniformity inside the pouches.

12.6.2 Integrated Temperature Distributor

To overcome this limitation, and in order to conveniently evaluate a process with respect to temperature performance, a parameter that accounts not only for temperature profiles in discrete locations (i.e., a package, a carrier, or the whole vessel) but also for temperature variations across the entire volume has been proposed (Knoerzer et al. 2010a). The so-called integrated temperature distributor (ITD) value is as an expression for process performance that represents the temperature distributions in relation to a target temperature during HPHT throughout the entire vessel volume.

12.6.2.1 Concept and Determination Method

Commercial thermal processing is currently characterized by using the *F*-value (thermal death time, Eq. (12.8)) to relate the temperature history of a process to a reference temperature for target bacterial spore inactivation (e.g., *C. botulinum*; *B. stearothermophilus*). In this case z_T (°C) represents the thermal resistance constant using the following model:

$$z_T = -\frac{(T - T_{ref})}{(\log D_{ref} - \log D)} \tag{12.17}$$

where the reference decimal reduction time D_{ref} (min) is at a reference temperature T_{ref} (°C), within the range of temperatures T (°C) used to generate experimental data. The decimal reduction time D (min) of the target microorganism at a given temperature is expressed as

$$D = \frac{t}{\log N_{ref} - \log N} \tag{12.18}$$

where N is the number of survivors (typically colony-forming units (cfu) per gram or mL) at time t and N_{ref} is the initial number of microorganisms (Ball 1943).

As stated previously, the F-value model has been questioned due to the assumption of the linearity of the inactivation kinetics of target bacterial spores to very low numbers as well as the linearization of temperature dependence of the D-value to obtain the z_T value (Peleg 2006). In the case of HPHT processing, a microbial kinetic expression depending on pressure and temperature would be required to provide an accurate representation of the sterility achieved. Moreover, the F-value provides a representation of the level of sterility achieved at a single point in the sterilization unit, not accounting for temperature gradients throughout the unit and carrier or package volumes.

Therefore, the ITD value has been developed as an expression to evaluate thermal process performance in an HPHT process throughout the vessel without requiring inputs from microbial kinetic parameters and only accounting for temperature and time data:

$$ITD = \frac{\int_{r_{min}}^{r_{max}} \int_{z_{min}}^{z_{max}} 10^{\frac{\int_0^{t_{process}} T(t)\,dt}{t_{process}} - T_{target}} dr\,dz}{(r_{max} - r_{min}) \cdot (z_{max} - z_{min})} \tag{12.19}$$

where r_{min}, r_{max}, z_{min}, and z_{max} cover the region of interest (which may be the entire vessel volume, carrier, or only product region), $t_{process}$ is the process time of interest (in this case, the holding time where most of the heat loss is expected), T_Δ is a temperature gradient of 10 K, and T_{target} is the targeted hold temperature of the process.

The ITD is made dimensionless by relating the integrated temperature profiles in each location to the process (holding) time and T_{target} and the surface (or volume, depending on the availability of 2D or 3D temperature data) integral to the area (or volume) of the region of interest (ROI). A flowchart for determination of the ITD value is shown in Fig. 12.9. Being dimensionless, this expression is universally applicable to any equipment size and process time, making it a convenient tool for comparisons between different types and scales of equipment and processes.

Depending on whether the calculation is applied to an axis-symmetric or full 3D temperature distribution, the ITD value represents either the pseudo-volumetric 2D or volumetric 3D thermal process performance. The following conclusions can be

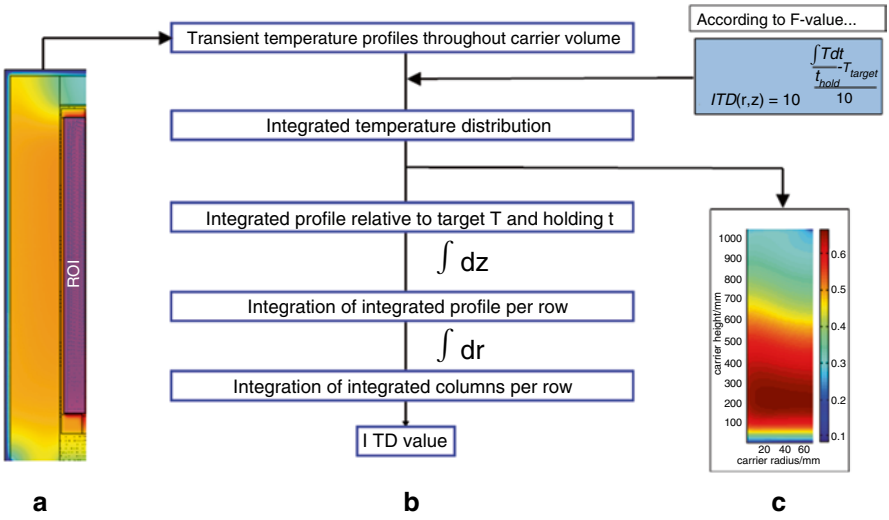

Fig. 12.9 ITD value determination in HP process (at discrete time): (**a**) depiction of region of interest (*ROI*, *purple*), (**b**) flowchart of MATLAB routine for ITD determination, and (**c**) example output of an integrated temperature distribution at a target temperature equal to the maximum adiabatic heating temperature for this process and a pressure holding time of 300 s (adapted from Knoerzer et al. 2010a)

drawn from the calculated ITD value (in combination with the integrated temperature distribution plot):

- ITD = 1: perfect target temperature uniformity with respect to process time and distribution
- ITD < 1: underprocessing in some or all parts of the carrier volume
- ITD > 1: overprocessing in some or all parts of the carrier volume

12.6.2.2 Example of Utilizing Modeling to Determine Optimum Insulating Carrier Wall Thickness

The ITD expression has been successfully utilized for predicting the optimum thickness of a PTFE carrier to be used inside the 35 L Avure vessel (Knoerzer et al. 2010a) such that the best compromise between heat retention and usable volume could be determined. The usable volume V_{Usable} (Eq. 12.20) was increased by decreasing the wall thickness values d_{wall}, with a load capacity between 1.3 and 26.3 L:

$$V_{\text{Usable}} = \pi \cdot (r_{\text{carrier}} - d_{\text{wall}})^2 \cdot h_{\text{carrier\_int}} \tag{12.20}$$

where r_{carrier} is the carrier's external radius, d_{wall} is the carrier wall thickness, and $h_{\text{carrier\_int}}$ is the carrier's internal height.

By following the procedure shown in Fig. 12.9, a carrier thickness value for maximum temperature performance (7 mm) was determined, as well as a compromise value for maximization of usable volume and temperature performance (4 mm). In either case, a significant decrease from the wall thickness of the PTFE carrier originally built for the 35 L vessel (d_{wall} = 28 mm) was determined, suggesting an increase from 12 L capacity to 22–24 L capacity while maintaining ITD values close to 0.9 (normalized to the maximum ITD value of the scenarios investigated). Future trials including compression heating values for polymeric materials and thermal conductivities as a function of temperature and pressure will further improve the accuracy of these models.

12.7 Outlook

This chapter has described the application of CFD in the prediction of temperature distribution and flow for the design and characterization of HPP processes. The main reasons for developing these models are to (a) aid in the design of processing units utilizing thermo-fluid-dynamic models, (b) provide understanding about how these units are to be adjusted to the process line (to make the process more effective and efficient), (c) prove that these processes can consistently deliver safe food products, and (d) provide safety, efficiency, and sustainability in the processing lines.

It was shown how CFD models can include all parts of the equipment, including packages and product carrier, while at the same time representing all steps of the process. CFD models allowed demonstration of how the inlet temperature fluid can influence temperature distribution inside the chamber and how a product carrier can act as a thermal insulator. The importance of the thermophysical properties of all materials involved in the modeled scenario as a function of temperature and pressure for improved model accuracy was highlighted. Although these property functions have been determined for water and other liquids as well as some insulating plastics, more research is needed to characterize other food, packaging, or carrier materials.

CFD models provide the possibility of coupling fundamental equations of fluid motion with kinetic models to determine the extent of microbial or enzyme inactivation or any other process throughout products inside the vessel as well as its impact on other quality parameters. Parameters such as the ITD value can characterize inactivation performance throughout a volumetric section of the vessel contents and assist in design and process characterization.

One challenge that lies ahead is the accuracy of validation tools for these models. Thermocouples have assisted in measuring temperature profiles at specific locations. However, thermocouple systems in many HPHT systems tend to fail through their closure connections, and, therefore, comprehensive validation experiments become time consuming and, in some cases, may lack accuracy. Wireless temperature loggers such as the “Thermo-Egg” (Knoerzer et al. 2010d) have recently been developed and such loggers show promise of accurate results while allowing

simultaneous temperature measurement at several vessel points. These Thermo-Eggs are mainly useful for measuring temperatures in bulk liquids as their thermal mass is too high to be used inside packs and pouches with limited volume, where they can act as heat sinks and therefore give readings significantly lower to what would be achieved without their presence. Another possibility of investigating temperature distribution is the use of pressure-temperature-time indicators (Grauwet et al. 2010, 2012; Vervoort et al. 2011).

Measuring thermophysical properties of foods, process fluids, packaging, and construction materials in situ under pressure and elevated temperatures will further enhance the accuracy and usefulness of CFD models. This outlook also applies to the process of interest (e.g., microbial inactivation, enzyme inactivation, or biochemical and chemical transformations). Validated kinetic models are needed to couple these to the outputs of the CFD models to predict performance. Conversely, microbial validation has also encountered challenges due to the sometimes erratic behavior of microbial spores during preheating and sterilization.

Spore inactivation models considering the preheating stage, which provide conditions for spore germination, will aid in accurate validation of CFD-assisted sterility predictions. As CFD software packages become more user friendly, it is foreseen that such models will be more frequently utilized for the characterization and design of HPP/HPHT processes. As pH measurement in situ is becoming possible, it will also be possible to model changes in pH throughout the sample, which may have an impact on process performance or product quality.

The next generation of CFD models may, in addition to the quality attributes of the products, also link to an economic module to alternate between process scenarios of pressure, time, and temperature utilized. To date, no such model can predict as an output the overall quality, nutrition, and safety of the product. Very few kinetic models are available which can express *C. botulinum* inactivation during HPHT as a function of pressure, temperature, and time in different food matrices.

In some cases, the link of preprocessing to microbial inactivation and quality outputs after processing is unexplored. For example, kinetic models utilized for high-pressure thermal sterilization processes ignore the fact that the food package requires a preheating step at temperatures between 70 and 90 °C, where spore germination can be triggered. The main issue here is that different preheating rates will depend on various parameters (preheating system, packaging material, package size, and product thermal properties) and, therefore, impact the kinetics of germination. Hence, kinetics will be needed to describe the inactivation of spores as a function of preheating rate, preheating temperature, hold time during preheating, and process variables in the high-pressure process. These variables are extremely important when establishing final processing conditions based on microbial outputs.

The same concerns may apply to evaluation of product quality and nutrient retention. More kinetic models representing texture and color change (e.g., Leadley et al. 2008), loss or gain of nutrients during processing, and change in structural and chemical parameters that may control the shelf life of the material are needed as functions of pressure and temperature. When the kinetics of different specified attributes that make a final product have been determined, CFD models will be able to

provide the full range of attributes describing the product as an outcome of the process. This determination will then enable selection of the most appropriate process parameters for the material involved and the desired final outcome or intended use for the product.

The solid food packages are generally assumed not to undergo deformation. However, it is well known that there is 15–20 % compression of liquids and, therefore, a certain deformation of solids under those conditions, which depends on the food materials contained in the packages. The hydrostatic deformation processes occurring during high-pressure processing have not yet been fully quantified in both food structures and other materials contained in the vessel during the process.

Models able to represent the rate of deformation of packages and contained foods, as well as carriers, inside the vessel will provide further understanding and accuracy for the following:

- Heat transfer phenomena at package level and for different food materials, as well as situations where the vessel is operated at full capacity
- Predictive capacity of the vessel, which then will enable maximizing the usable volume and provide information for improving process economics

In order to obtain this information, structural deformation kinetics at the mesoscale (millimeter-scale) level would suffice. In this case, compositional variations should be coupled with continuum mechanics. However, we believe that minimal useful information to characterize the industrial process can be gathered if the structural changes at the microscale level are determined. Furthermore, an assumption previously made is that chemical changes will not affect heat transfer. We believe this assumption to be valid in most situations, and, therefore, there is no need to detail the model further, at least from the thermo-fluid-dynamic point of view.

Most or all other phenomena occurring during HPP can be better explained from CFD modeling at the microscale level. For example, insights into the formation of solid structures due to enhanced gelation processes during HPP or the shrinkage of porous materials can be obtained and incorporated into the models.

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Chapter 13
High-Pressure Processing Uniformity

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Abstract As pressure is uniform and residence time is fixed, studying nonuniformity of high-pressure process variables comes down to studying temperature uniformity. This book chapter starts from the definition of adiabatic heat of compression to demonstrate that compression heat differences are a basis for the development of temperature heterogeneities in a high-pressure reactor. Since kinetics of change of target attributes of high pressure processing can be temperature dependent, temperature differences might result in process impact differences. There is a need for methods which enable insight in temperature gradients inside the high-pressure reactor. Since direct monitoring of the temperature as a function of time and space under pressure is today still a technical challenge at industrial scale, two alternative methods for temperature documentation under high-pressure conditions are described from principle to application. Finally, this chapter puts forward strategies to improve temperature uniformity under high-pressure processing conditions.

Keywords High pressure processing • Temperature uniformity • Computational thermal fluid dynamic modeling • Pressure-temperature-time indicator • Process uniformity

13.1 Introduction

The perception of the uniformity of a high-pressure process has greatly changed over time. When high pressure processing pioneered at research level (Hite 1899), and even when it was introduced at an industrial scale in the 1990s, based on the Pascal principle, it was assumed to be a "uniform processing technique" in which uniform pressure could be applied independently from product size, geometry, and composition. At that time, only the effect of pressure and time on the stress responses

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of biological material (e.g., food) by high pressure processing was taken into account. Since 2000, the potential effect of temperature on these process-induced changes is recognized which changed the perception of the uniformity of high pressure processing to "a processing technique in which pressure acts uniformly, but temperature might be a non-uniform process parameter resulting in a non-uniform process impact field" (Denys et al. 2000). Recently, even the uniformity of the local pressure response has been questioned (Minerich and Labuza 2003; Hartmann et al. 2006). In general, it can be argued that if the nonuniformity of pressure response exists only in a short time frame of treatment compared to the time frame of the treatment, or the pressure response difference is very limited, nonuniformity of the treatment impact caused by pressure is very unlikely to occur (Grauwet et al. 2012; Van der Plancken et al. 2012). Based on this, in the following, the pressure response will be assumed uniform in high pressure processing of food materials which can be homogenously compressed. Consequently, a verification of uniformity of the high-pressure process variables may be reduced to a study of temperature uniformity.

This book chapter starts from the definition of adiabatic heat of compression to demonstrate that compression heat differences are a basis for the development of temperature heterogeneities in a high-pressure reactor (Sect. 13.1). Since the kinetics of change of target attributes of high pressure processing can be temperature dependent (Van der Plancken et al. 2012), temperature differences might result in process impact differences. There is a need for methods which enable insight in temperature gradients inside the high-pressure reactor. Since direct monitoring of the temperature as a function of time and space under pressure is today still a technical challenge at industrial scale, in Sect. 13.2, two alternative methods for temperature documentation under high-pressure conditions are described. The potential of these two methods to map temperature uniformity in high-pressure vessels is exemplified in Sect. 13.3. Finally, this chapter puts forward strategies to improve temperature uniformity under high-pressure processing conditions.

13.2 Differences in Compression Heat as a Basis for Temperature Nonuniformity

13.2.1 Definition of Adiabatic Heat of Compression

The conversion of the work of compression into internal energy results in a temperature increase of compressible materials. Conversely, the temperature will drop during decompression. Under adiabatic conditions, this temperature change is termed "adiabatic heat of compression" and can be defined by Eq. (13.1) (e.g., Zemansky 1957):

$$\frac{\mathrm{d}T}{\mathrm{d}p} = \frac{T\alpha_{(T,p)}}{\rho_{(T,p)}c_{\mathrm{p}(T,p)}} \tag{13.1}$$

In Eq. (13.1), α represents the volumetric thermal expansion coefficient (K^{-1}), ρ the density (kg/m^3), c_p the specific heat (J/kg K) at a particular temperature T (K), and pressure p (Pa). In this equation, the thermophysical properties as well as their pressure and temperature dependencies are component dependent. Consequently, during the same high-pressure treatment, the compression heat of distinct materials equilibrated at the same starting temperature can be different. In reality, due to heat exchange between different components and the environment, adiabatic conditions are unlikely to occur. Therefore in the following, the temperature increase linked to a pressure increase in compressible material will be termed "compression heat."

13.2.2 Compression Heat of Water

Water receives special interest because high moisture foods are good candidate of high-pressure processed products. Furthermore, water is usually employed as the pressure transmitting fluid in food industry. Bridgman (1912) started characterizing the behavior of water under isothermal compression. Today, the behavior of free, stable, fluid water is well described and can be fully predicted using the software implementation of the National Institute of Standards and Technology (NIST) which includes the formulation of water thermodynamic properties made in 1995 of the International Association for the Properties of Water and Steam (IAPWS). In this way, compression heating values (°C/100 MPa) of water of 3.0, 3.7, and 5.0 °C can be determined for initial temperatures of 25, 50, and 90 °C, respectively. However, while the use of properties of free water as an approximation to those of real food systems was suggested to be considered with caution (Otero and Sanz 2003), other authors proved that foods with high water content (>70 %) displayed a comparable compression heat as water (Rasanayagam et al. 2003; Patazca et al. 2007; Buzrul et al. 2008). Chapter 6 summarizes heat of compression as well as various other in situ thermophysical properties of food and packaging material.

13.2.3 Compression Heat of Multicomponent Food Systems

Food systems are multicomponent food systems with water, carbohydrates, proteins, and fats as major components. Some authors studied the compression heat of selected foods directly (Rasanayagam et al. 2003; Patazca et al. 2007; Buzrul et al. 2008). Others tried to build a mixture model to predict the compression heat of multicomponent systems based on the compression heat of the individual components and their abundance (Rasanayagam et al. 2003). However, this model is only applicable if the interaction between the different components does not affect compression heat. As shown above, some studies have been performed on the compression heat of fats and oils. However, insight in compression heating values of proteins, carbohydrates, or other food components is scarce.

13.2.4 Compression Heat of Food Product Basket/Container

Today, commercial-scale high-pressure pasteurization units use polymeric product carriers. Upon implementation of high-pressure, high-temperature processing, polymeric carriers become even more important because of their thermal insulation capacities (de Heij et al. 2003). In addition, the carrier can facilitate product handling, since high-pressure, high-temperature products are heated before entering the vessel and are still hot when (un)loaded from/to the vessel. Knoerzer et al. (2010a) studied the adiabatic compression heat of different insulating materials (polytetrafluoroethylene (PTFE), polypropylene (PP), high-density polyethylene (HDPE)) over a broad range of initial temperatures (5–90 °C) and pressures (100–750 MPa). Carrier materials undergo compression heating. At relevant pressures for high-pressure processing (500–700 MPa) and 25 °C, the compression heat increases from PTFE (3.7 °C/100 MPa) over PP (4.4 °C/100 MPa) to HDPE (5.3 °C/100 MPa).

13.3 Methods for Temperature Documentation Under High-Pressure Processing Conditions

As described above, the compression heat is component dependent and can be distinctly different between materials present in a high-pressure vessel (e.g., 3–9 °C/100 MPa). During the holding time or even during the pressure buildup period, based on the law of heat transfer, heat will be exchanged as a function of time and space.

Direct measurement of the temperature seems to be the most straightforward method to gain insight in temperature gradients inside a high-pressure reactor. However, at industrial scale, several hurdles are associated with it. To completely demonstrate temperature fields, temperature sensors should be positioned across the whole volume of the vessel. At lab scale, the pressure-temperature history of samples is obtained by the use of one pressure sensor and one or more temperature sensors placed as close as possible to the sample. Wired thermocouples type K and J are frequently used. Because of the high pressures involved, this requires special attention to the sealing of the thermocouple passage through the vessel wall. Wireless systems currently make their appearance (Knoerzer et al. 2010b). However, the systems developed are large and some of them act as heat sinks. It is important that the temperature measuring devices do not affect the free movement of the flow (e.g., the pressure medium, food product) inside the vessel, influencing the temperature gradient itself.

Two alternative methods for temperature documentation have been described in literature: (1) computational thermal fluid dynamic modeling (CTFD) for simulation of temperature fields and (2) use of pressure-temperature-time indicators (pTTIs) for experimental detection of temperature differences at distinct points. In the following, the concept of both methods is shortly described, completed with a description of their potential limitations (Grauwet et al. 2012).

13.3.1 Computational Thermal Fluid Dynamic (CTFD) Modeling

Mathematical modeling and numerical simulation show great advantage compared to experiments as they can deliver reliable, arbitrary space and time accurate resolving temperature fields in the complete vessel (Delgado et al. 2008). In numerical simulations, high-pressure processing uniformity can be evaluated regarding flow fields, temperature fields, and, based on experimentally determined kinetics, the resulting process impact on food quality or safety (Rauh et al. 2009). For that purpose, specifically adapted mathematical balance equations based on the governing equations of thermofluid dynamics have to be solved. For a description of the conservation equations (mass, momentum, energy, transport) and their integration, the reader is referred to the review of Delgado et al. (2008). In addition, the thermofluid dynamical properties of the treated products have to be known for the calculation. Products with high moisture content and some relevant pressure transmitting media have been shown to behave thermofluid dynamically similar to water with an increased viscosity. However, as often not enough detailed data are available in the relevant pressure and temperature range, mathematically modeling the thermophysical properties of all materials present in the vessel (pressure, transmitting medium, food, packaging material, etc.) is often a challenge (Denys and Hendrickx 1999; Otero and Sanz 2003; Torrecilla et al. 2004, 2005; Werner et al. 2007). Validation of the mathematical models and numerical simulations has been done based on comparison with experimental temperature measurements and velocity fields applying measurement techniques specially developed for high-pressure conditions (Song et al. 2008a, b, 2009, 2010) and analytical considerations (Rauh and Delgado 2010, 2011). CTFD modeling demands high computational power and is case dependent, thus requiring reevaluation if, for example, the load of the pressure vessel, the pressure medium, or the vessel design is changed (Delgado et al. 2008). For more details on CTFD modeling in the context of high pressure processing, the reader is refereed to Chap. 12.

13.3.2 Pressure-Temperature-Time Indicators (pTTIs)

By analogy with temperature-time integrator (TTI) discussed in the field of thermal processing (Hendrickx et al. 1995; Van Loey, Hendrickx, De Cordt, Haentjens, Tobback 1996), a pressure-temperature-time indicator (pTTI) can be defined as "a small, wireless device that shows a pressure, temperature and time dependent, easily and accurately measurable, irreversible read-out to the high pressure treatment" (Van der Plancken et al. 2008).

Denys and co-workers (2000) pioneered with the idea that such indicator systems could be used as sensors to obtain insight in temperature gradients occurring in high-pressure reactors. More in general, they postulated that "studying the read-out after

treatment of pressure, temperature, time-sensitive indicators positioned at different positions in a high pressure vessel, the non-uniformity of process variables can be detected, without the need of a monitored or simulated pressure, temperature-history." In a review paper, Van der Plancken et al. (2008) summarized the then status of the development of pTTIs. However, hitherto, no single system has been described that can sensitively and accurately detect temperature heterogeneities in high-pressure reactors under industrially relevant high-pressure processing conditions.

Grauwet and co-workers filled this gap by developing protein-based, extrinsic, isolated pTTIs for specific application windows (i.e., range of pressure, temperature, time combinations) to be used as user-friendly, broadly employable systems (e.g., independent of the equipment) detecting temperature nonuniformity in high-pressure reactors (Grauwet et al. 2009, 2010a, b, c, 2011). In total, four indicators were developed using a five-step approach (Grauwet et al. 2009). In practice, the indicators developed are small, flexible microtubes (250 μL) filled with a buffer solution of a biological active protein, which activities after treatment can be easily evaluated spectrophotometrically.

Mapping temperature uniformity by pTTIs has also its limitations. (1) Indicators are characterized by a specific application window. The pTTI can only be used under processing conditions for which the reaction rate constant is high enough to detect a significant difference but not too high to render a detectable readout. (2) Indicators are characterized by a specific temperature sensitivity. Consequently, uniform readouts evaluated at different coordinates in a high-pressure vessel do not necessarily prove that no temperature differences existed. Put differently, nonuniform readout zones do not necessarily cover all nonuniform temperature zones. In food processing, there is only need to gain insight in those temperature differences in the relevant processing domain which will have an effect on the process impact on the food product. Consequently and if possible, it is important to link the temperature sensitivity of the targets to the temperature sensitivity of the indicator applied (Grauwet et al. 2011). To this end, kinetic data on the target attributes are needed. (3) In theory, pTTIs can be positioned over the whole volume of the pressure vessel. In practice, they will only integrate the pressure-temperature history evaluated at that specific point. (4) The pTTIs are actually pressure-temperature-time integrators. They integrate the combined effect of pressure, temperature, and time. Consequently, one readout (e.g., 50 % residual activity) can be the result of different histories: for one particular pressure profile, strong inactivation levels can be, for example, obtained by subjecting the integrator to high temperatures for a short period or to lower temperatures for a longer period of time. In conclusion, it is impossible to state that a certain absolute temperature has been exceeded or not. However, if kinetic information of the indicators is available, minimally detectable temperature differences can be calculated (Grauwet et al. 2010a, b, c).

In spite of the limitations, it can be stated that extrinsic pTTIs are cheap (<0.10 EUR/sensor), small, and broadly employable systems (i.e., can be used independent from the vessel load or the equipment design) which enable fast and easy detection of temperature differences at distinct points in high-pressure vessels.

13.4 Temperature Uniformity Mapping

As stated briefly above, CTFD modeling and pTTIs are interesting methods for temperature documentation under high-pressure processing conditions. In the following sections, results published on temperature uniformity mapping of different scale and different vessel design (plunger/injection; horizontal/vertical vessel orientation) high-pressure equipment using those methods are compared: (1) in a small-scale (0.5 L), vertically oriented high-pressure vessel, in which pressure is generated through injection of pressure transmitting medium at the bottom of the vessel and (2) in a large-scale (55 L), horizontally oriented high-pressure vessel in which pressure is generated through injection of pressure transmitting medium from both axial ends of the high-pressure vessel. In these examples, processing conditions are characterized by industrially relevant pressure levels (500–600 MPa) and initial temperatures up to 50 °C.

13.4.1 Uniformity of a Small-Scale, Vertically Oriented High-Pressure Unit

By positioning indicator tubes at six different axial and radial positions in a lab-scale, vertically oriented high-pressure vessel, Grauwet et al. (2009) mapped the temperature uniformity of the vessel under different high-pressure pasteurization conditions. Significantly different readouts were evaluated depending on the treatment position of the indicator tube. Given the fact that the kinetics of the indicator system were characterized by a positive pressure-temperature-time dependency and given the uniformity of pressure and the fact that residence time is fixed under high pressure processing (batch process), lower residual activities detected at particular positions in comparison to other positions should be attributed to harsher temperature histories that must have occurred at these points. Interpreting the pTTI readouts after treatment at different coordinates enabled localization of low- and high-impact zones and consequently low- and high-temperature zones. Indicators located more closely to the vessel bottom and more closely to the wall were less inactivated. For this specific experimental setup, the first observation can be attributed to the stream of pressure transmitting medium injected from the bottom of the vessel (forced convection) and the downward-directed flow of cooled pressure medium close to the vessel wall (free convection). Forced and/or free convection phenomena have been simulated by, for example, Hartmann et al. (2003), Ghani and Farid (2007), and Rauh et al. (2009). The following paragraph describing numerical simulation of the thermofluid dynamic phenomena explains these effects in more detail. The second observation, regarding the high readout at the wall, can probably be explained by the pronounced transfer of compression heat during compression and pressure holding, from the center of the pressure vessel to the colder vessel wall; this was identified as a conduction phenomenon by simulations by, for example, Denys et al. (2000), Otero and Sanz (2003), and Otero et al. (2007).

As explained above, numerical simulations enable closer insight in thermofluid dynamical uniformity in terms of origin of temperature gradients, documentation of the complete temperature field, and the prediction of the consequences for process uniformity. Rauh et al. (2009) investigated the thermofluid dynamical uniformity in water-based foods (liquid and high viscous) directly filled in a cylindrical, vertically oriented high-pressure vessel. These products were modeled with either properties of pure water or properties of water with an artificially increased viscosity. In the simulations, the vessel walls were replaced by corresponding thermal and fluid dynamical boundary conditions. In order to gain insight in the effect of initial temperature, product viscosity, and thermal boundary conditions on the temperature uniformity distribution in a high-pressure vessel, four different parameter settings were studied: (i) reference process with an identical initial and boundary temperature of 50 °C; (ii) process with an identical initial and boundary temperature of 20 °C; (iii) initial and boundary conditions equal to (i), but convection is suppressed by an increased viscosity ($\eta = 2 \times 10^4\ \eta_{water}$); and (iv) initial temperature of 50 °C and boundary temperature is assumed to be equal to the temperature the product reaches after compression under adiabatic conditions starting at 50 °C (i.e., 76 °C). Figure 13.1 depicts dimensionless temperature fields of the four processes (i)–(iv) in a vertical cutting plane of the vessel at the end of the pressure holding phase ($t^* = t/t_{process} = 0.99$). The dimensionless temperature is defined as $T^* = (T - T_i)/(T_{ad} - T_i)$ with T temperature, T_i initial temperature, and T_{ad} temperature after pressure increase up to target pressure under adiabatic conditions starting from T_i. The results of the simulations show that in short-time high-pressure treatments with a high-pressure buildup rate of 400 MPa/s (industrial conditions, 150–200 MPa/min) for the given parameter settings, the temperature of the bulk liquid rises as much as

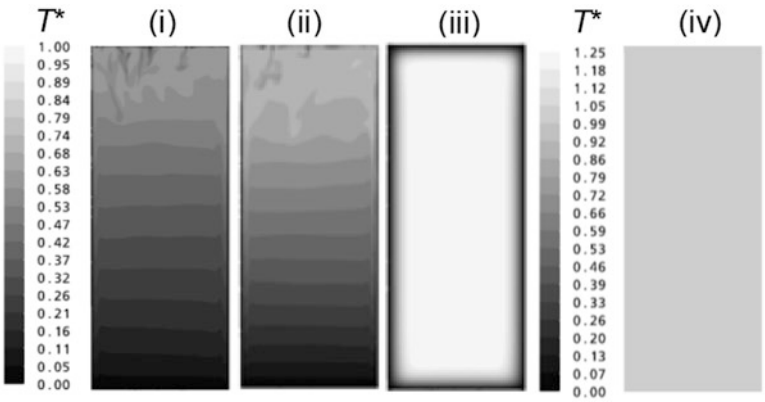

Fig. 13.1 Dimensionless temperature field T^* ($T^* = (T - T_i)/(T_{ad} - T_i)$) at end of pressure holding time ($t^* = t/t_{process} = 0.99$) for HPP of water-based foods up to 600 MPa (400 MPa/s) with (i) $T_i = 50$ °C (η_{water}), (ii) $T_i = 20$ °C (η_{water}), (iii) $T_i = 50$ °C ($\eta = 2 \times 10^4\ \eta_{water}$), and (iv) $T_i = 50$ °C (η_{water}). (i–iii) Boundary conditions equal to initial conditions ($=T_i$); (iv) boundary conditions equal to adiabatic conditions ($=T_{ad}$). (Reprinted from Rauh et al. (2009) with permission from Elsevier)

under adiabatic conditions (Rauh et al. 2009). Consequently, in processes with equal initial temperature and boundary temperatures, the wall temperature will be colder in comparison to the center of the vessel after pressure buildup. By normalization, the temperature in processes (i) to (iii) is scaled from zero to one. As in process (iv), wall temperature is set to T_{ad}, heat transfer between wall and fluid and work of compression leads to fluid temperatures at the walls greater than T_{ad}. Therefore, values between zero and greater than one exist. Thermal stratification as well as unstable layering at the top surface can be detected in Fig. 13.1i and ii. Due to heat transfer between walls and fluid, a temperature and density layer develops in the vicinity of the walls and grows into the bulk liquid. Since, at the vertical walls the gravity vector is perpendicular to the density gradient, free convection sets in; cold fluid flows down at the lateral wall. For continuity reasons, fluid flows upward in the center of the pressure chamber. Until the end of the holding phase, thermal stratification in vertical direction with rising temperature from bottom to top develops. At the top surface of the vessel, unstable temperature stratification arises with colder liquid above warmer liquid. This can leads to the down-flow of cold liquid into the warmer bulk liquid (Rauh et al. 2006). Comparing processes (i) and (ii), the layering is less developed in (ii) than in (i) due to higher viscosity resulting from lower absolute temperature and smaller driving temperature gradient at the walls (ii). Process (iii) has the same thermal initial and boundary conditions than (i), but 2×10^4 times higher viscosity. The high viscosity suppresses convective flow; thus, heat transfer is restricted to conduction throughout the process resulting in harsher temperature conditions. Thermal stratification through convection does not take place. However, a continuous cooldown of the vessel content from the walls occurs. In process (iv), heat transfer and resulting free convection are reduced by decreasing the temperature gradient at the wall-liquid interface. Until the end of the pressure holding phase, no temperature nonuniformities seem to be present. From Fig. 13.1 it can be concluded that the temperature distribution at $t^* = 0.99$ is most uniform in process (iv).

13.4.2 Uniformity of a Large-Scale, Horizontally Oriented High-Pressure Unit

By evaluating the readouts of indicator systems after treatment at more than 30 positions over the whole vessel volume, the temperature uniformity of an industrial-scale, horizontally oriented high-pressure vessel was studied under different high-pressure pasteurization conditions (Grauwet et al. 2010a, b, c) (Fig. 13.2). The vessel was studied in a filled condition (vessel filled with product dummies). For a particular radial position, no significantly different indicator readouts after treatment as a function of different axial sections were reported (A–F; L; R in Fig. 13.2). However, in a particular axial section, different indicator readouts could be detected. As an example, in Fig. 13.2, the readouts of indicator systems treated at different distances from the vessel wall are depicted. The strongest level of enzyme

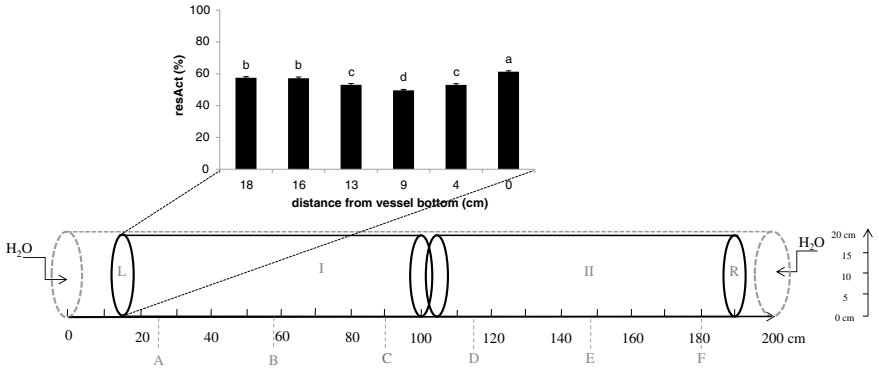

Fig. 13.2 Residual α-amylase activities (resAct, %) of α-amylase-based indicator system after treatment during 3 min holding time at 600 MPa (150 MPa/min) as a function of different distances from the vessel bottom of a horizontally oriented HP vessel. Means indicated with the same particular letter are not significantly different ($\alpha = 0.05$) (according to Grauwet et al. (2010a))

inactivation was detected for indicators treated at 9 cm distance from the vessel bottom in comparison to the other positions. pTTIs treated at the vessel bottom were characterized by the highest residual activities. This can be explained by temperature uniformity mapping of this vessel by CTFD modeling (Grauwet et al. 2012). During the holding phase, a temperature layering with decreasing temperature from top to bottom is observed. In analogy to the vertical vessel, the cooling down fluid at the walls is flowing downward toward the bottom and is leading to the observed effect (Rauh et al. 2009).

Based on the two examples of temperature uniformity mapping given above, it can be stated both CTFD modeling and pTTIs enable insight in temperature heterogeneities occurring under high-pressure processing conditions and that they resulted in detection of the same low- and high-temperature zones. In addition, from these examples, it becomes clear that the localization of these different temperature zones can be influenced by the vessel dimension, orientation, boundary condition, pressure generation system, pressure medium, etc. of the equipment used. Moreover, the effect of the vessel load should not be forgotten (Delgado et al. 2008).

13.5 Strategies to Improve Temperature Uniformity

Different strategies have been searched for to improve the temperature uniformity in a high-pressure reactor during processing. Many of these insights have been gained, thanks to CTFD modeling and simulations. In the following, a distinction is made between recommendations to improve the temperature uniformity (1) within the vessel content (i.e., between product, pressure medium, product container, packaging material, etc.) and (2) between vessel content and wall.

13.5.1 Recommendations to Improve Temperature Uniformity Within the Vessel Content

In general, to minimize heat transfer in the vessel content, selecting components with comparable compression heat behavior is advisable. In addition, to reduce development of heat differences between medium and product characterized by a similar compression heat, both components should be equilibrated at the same initial temperature.

As discussed in Fig. 13.1, the flow and temperature field induced during indirect pressurization (injection system) influence considerably the temperature distribution at the beginning of the holding time: injection of pressure transmitting medium for compression purpose introduces cold liquid into the vessel if the pipe or pressure pump is not heated (de Heij et al. 2003). This induced thermal heterogeneity can be large and can be avoided by a moving plunger reducing directly the vessel volume (Hartmann and Delgado, 2002b).

Only recently insight has been obtained in the compression heat of the product baskets used for accessible loading and unloading the products from the high-pressure vessel. Based on the simulation work of Knoerzer et al. (2010a), it seems that carrier materials can be selected with compression heat similar to water to prevent thermal diffusion in the vessel content. This group compared the compression heat of the carriers to the compression heat of water when pressure was built up from the same initial temperature (T_i). Since the T_i dependency of the carrier material compression heat is different from that of water, at low T_i (e.g., 10 °C, 750 MPa), the compression heat of water and polytetrafluoroethylene (PTFE) were most comparable. At intermediate T_i (e.g., 50 °C, 750 MPa) and high T_i (e.g., 90 °C, 750 MPa), the same could be stated for polypropylene (PP) and high-density polyethylene (HDPE), respectively.

In batch high pressure processing, food products are packed before treatment. Currently, no open literature is available on the compression heat of food packaging materials. However, some researcher suggested heat barrier properties of packaging materials based on simulations (Hartmann and Delgado 2002a, 2003).

13.5.2 Recommendations to Improve Temperature Uniformity Between Vessel Content and Wall

Heat exchange through the vessel wall is described as the major source of temperature gradients in a high-pressure vessel. Many suggestions have been made to enable isothermal conditions between vessel and wall. These suggestions can be divided into two categories: (1) strategies to promote heat transfer between vessel content and wall, resulting in a homogenous vessel content temperature equal to T_i, and (2) strategies to prevent or reduce heat transfer between vessel content and wall, to obtain uniform temperature approaching the process temperature (T_p) as much as possible.

It has been proposed to equip the vessel with a stirrer stimulating the heat exchange. However, this would be technically too difficult under pressures higher than 300 MPa (Otero et al. 2000). Furthermore, a slow pressure buildup rate readily allowing transfer of the compression heat to the high-pressure vessel wall during pressure buildup has been put forward (Van der Plancken et al. 2008). Nevertheless, in commercial applications, a controlled, slow pressure buildup rate is not straightforward since it is difficult to control. In addition, this slow pressure buildup is not interesting from an economical point of view (Otero and Sanz 2003; de Heij et al. 2003). Otero et al. (2007) simulated the effect of the vessel filling ratio on the temperature heterogeneity. When the filling ratio is reduced, thermal re-equilibrium is reached sooner, albeit reducing the economical relevance of the process. Hartmann et al. (2004) noted that the temperature compensation time scale reduces when the viscosity of liquids is lower. This finding has been reconfirmed in Fig. 13.1. In general, it should be remarked that the temperature compensation time increases in proportion to the scale of the high-pressure equipment used (Hartmann and Delgado 2003).

Heat transfer in the pressure buildup phase between vessel content and wall will be minimized by increasing the high-pressure buildup rate. In this way, maximal (i.e., adiabatic-like conditions) compression heating is obtained (Hoogland et al. 2001).

The use of an isolating container or liner on the vessel wall can be used to retard or prevent heat transfer between vessel and wall (de Heij et al. 2002, 2005; Hartmann et al. 2004; Knoerzer et al. 2007, 2010a; Juliano et al. 2009). Insulation of the vessel wall with a metallic PTFE and poly-oxy-methylene (POM) carrier has been described (Knoerzer et al. 2007; Shao et al. 2008; Juliano et al. 2009; Ramaswamy et al. 2009). All carriers reduced the heat exchange between vessel wall and load. However, only using the PTFE or POM carrier, quasi-isothermal conditions (less than 3 °C temperature difference measured at one position in function of time) could be reached for more than 4.5 min. However, insulator capacities are limited: eventually, the carrier and its content will equilibrate to the temperature of the vessel wall.

Thermal conductivity of packaging material (polypropylene) has been simulated as a function of material thickness. Depending on the thickness, the packaging material can represent a heat barrier that ensures almost adiabatic conditions (Hartmann and Delgado 2002a, 2003).

Next, control of both the vessel content temperature and the vessel wall temperature has been suggested. In particular, if the vessel load upon compression reaches the same temperature as the vessel wall, reduced gradients, near isothermal conditions, and thus uniform impact on the food will be achieved (Rauh et al. 2009). Koutchma et al. (2005) conducted experiments using a high-pressure system with an internal heater to maintain stable, high temperatures. Margosch et al. (2006) used an external heating-cooling block and heated the small vessel at the same rate as the sample temperature increase by compression heating. Ahn et al. (2007) controlled temperature by immersing the high-pressure chamber in a temperature controlled bath. These three methods, involving gradual increase of the vessel wall temperature during buildup, were powerful on lab scale but infeasible on a pilot or industrial

scale, because of thermal load issues. Equipments in which the wall is set at the process temperature from the start of the process and the temperature of the incoming medium is set at the required initial temperature offer better possibilities for scale-up. In literature, this protocol has been termed isothermal endpoint pressure treatment procedure (Guan et al. 2005; Landfeld et al. 2011). Nevertheless, the life span of a high-pressure vessel wall is inversely correlated to its temperature. Knoerzer et al. (2010a) reported carrier materials with compression heating higher than the water-like vessel content. In this situation, the carrier itself acts as an internal heater preventing heat loss through the vessel wall. It needs to be mentioned that in the two latter situations, the vessel wall can become the warmest zone.

13.6 Conclusion

In high pressure processing, pressure, temperature, and time are the process variables to take into account. Given the uniformity of hydrostatic pressure in compressible materials and the fact that time is fixed in a batch process, temperature differences are the only possible source of process impact heterogeneities. Differences in compression heating and the effect of medium injection are the major causes for temperature nonuniformity. There is a need for methods which enable documentation of temperature under high pressure processing. Since direct monitoring of the complete temperature profile in a high-pressure vessel is technically still a challenge at industrial level, CTFD modeling and the use of pTTIs have been developed as alternative methods. CTFD modeling can be a method to compare the temperature distribution of different equipment designs in combination with different pressure media, food packages, isolating materials, etc. before the high-pressure equipment is actually built. In this way, the most uniform configuration could already be selected. In addition, the thermofluid dynamical characterization of the high-pressure vessel by CTFD modeling enables definition of critical vessel positions that have to be checked with pTTIs to validate the process impact. When the most uniform configuration has been selected and has been constructed, pTTIs can be a fast and easy way to document and validate the high-pressure process in terms of temperature uniformity during actual processing.

To fully document the consequences of process variable nonuniformities (e.g., temperature history) for process impact nonuniformity, the importance of kinetic information of relevant food safety and quality target attributes under a range of pressure, temperature, and time conditions should not be forgotten. Given the low temperature sensitivity of reactions under the reduced process temperatures in the high-pressure pasteurization processing window (400–600 MPa; initial temperature 5–25 °C; 1–15 min holding time), the consequence of temperature gradients under high-pressure pasteurization might be invisible from the process impact point of view. This statement might be less straightforward for high-pressure sterilization conditions (500–800 MPa; process temperature 90–120 °C; 1–10 min holding time) in which high process temperatures are necessary to reach the targeted safety impact and in which reactions become clearly temperature dependent.

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Part IV
Microbial Safety of Pressure Treated Foods

Chapter 14
Microbiological Aspects of High-Pressure Processing of Food: Inactivation of Microbial Vegetative Cells and Spores

Hossein Daryaei, Ahmed E. Yousef, and V.M. Balasubramaniam

Abstract High-pressure processing (HPP) of food utilizes elevated pressures with or without combination of heat to inactivate harmful pathogens and spoilage microorganisms in their vegetative or spore state. Since the treatment reduces thermal impact, pressure-treated products have better organoleptic attributes. The importance of identifying a relevant surrogate organism for high-pressure pasteurization and sterilization studies is highlighted. Process- and product-related factors influencing the antimicrobial efficacy of pressure treatment are reviewed.

Keywords High pressure • Microbial safety • Vegetative bacteria • Spore • Kinetic model • Mechanism

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14.1 Introduction

The worldwide increasing demand for safe and high-quality foods with extended shelf life and without artificial preservatives has prompted food processors to investigate several alternative food preservation techniques. These include advanced thermal processing (ohmic heating, microwave heating, and radio-frequency heating) and "nonthermal" food preservation methods such as high-pressure processing, pulsed electric field processing, irradiation, ultrasound treatment, ultraviolet processing, ozone treatment, and cold plasma treatment. Among various nonthermal technologies, high-pressure processing (HPP) has been of specific interest to food manufacturers. Nowadays, many HPP-treated foods are available in the market worldwide. Optimized HPP can ensure the microbial safety of foods while retaining their fresh-like characteristics and nutritional attributes. Different aspects of the HPP technology including microbial inactivation and food safety have been reported by Anon (2000), Mañas and Pagán (2005), Patterson (2005), Rastogi et al. (2007), Black et al. (2011), and Daryaei and Balasubramaniam (2012). This chapter covers the antimicrobial efficacy of HPP during preparation of pressure-pasteurized and pressure-sterilized products. In addition, processing and product parameters influencing antimicrobial efficacy will be presented. Underlying mechanisms of inactivation of bacteria by HPP technologies and potential injury and recovery of the bacteria during extended storage also will be summarized. Chapter 15 will cover the impact of pressure treatment on viruses.

14.2 Antimicrobial Efficacy

14.2.1 Microbial Characteristics

Microbial inactivation by HPP is affected by the microorganisms' type (bacteria, yeasts, and molds), form (vegetative cells or spores, Gram positive or negative), genus, species, strain, and growth phase (Balci and Wilbey 1999; Mañas and Pagán 2005). Various microorganisms and viruses show different sensitivity against physical stresses caused by HPP. Table 14.1 compares antimicrobial efficacy of HPP and thermal processing. The sensitivity may even vary between strains of the same species and with the stage of the growth cycle at which the microorganisms are subjected to treatment. Resistance of microorganisms to both HPP and conventional thermal treatment is similar in many aspects. For example, Gram-positive bacteria are generally more resistant than Gram-negative bacteria, and spores are more resistant than vegetative cells to both heat and physical stresses caused by pressure treatment (Cheftel 1992; Smelt 1998; Patterson 1999). It has been suggested that the complex structure of the cell envelope in Gram-negative bacteria makes them significantly susceptible to physical changes caused by HPP (Shigehisa et al. 1991; Patterson and Kilpatrick 1998). However, a number of microorganisms, such as

Table 14.1 Comparison of the characteristics of high-pressure processing (HPP) with conventional thermal processing

Processing factors	Thermal processing	HPP
Process parameters affecting microbial inactivation	Time and temperature	Pressure, time, and temperature
Product parameters affecting microbial inactivation	pH, composition, and water activity	pH, composition, and water activity
Batch or continuous	Either	Batch/semicontinuous
Process in package	Yes	Yes (flexible packages, vacuum preferred)
Chemical changes in foods	Many	Minimal
Physical changes in foods	Many	Minimal (e.g., gelation, loss of turbidity)
Enzyme inactivation	Yes—most	Varies depending on the process conditions
Microbial resistance[a]	S>G^+>G^-, Y & M	S>G^+>G^-, Y & M
Pasteurization	Yes	Yes
Sterilization (spore inactivation)	Yes	Yes—in combination with heat. Extended shelf life (ESL) foods distributed under refrigeration is also possible
Shelf life extension	Yes	Yes

[a]*S* spores, *Y & M* yeasts and molds, G^+ Gram-positive vegetative bacteria, G^- Gram-negative vegetative bacteria

Escherichia coli, Salmonella enterica, and *Staphylococcus aureus* that are known to possess relatively low heat resistance, some of their strains have shown extreme resistance to HPP (Patterson et al. 1995; Erkmen and Karatas 1997; Patterson and Kilpatrick 1998; Linton et al. 2001).

Cells of the stationary phase are more resistant than the exponential-phase cells to adverse physical changes caused by high-pressure treatment (Mackey et al. 1995; Benito et al. 1999; McClements et al. 2001; Mañas and Mackey 2004). Mackey et al. (1995) reported a 1.3 log reduction in the viable numbers of *Listeria monocytogenes* cells at the stationary phase by treatment at 400 MPa for 10 min, whereas the exponential-phase cells were reduced by more than 7.0 logs. The difference in pressure resistance between exponential- and stationary-phase cells may vary widely depending on the growth temperature (Casadei et al. 2002). Casadei et al. (2002) reported a decrease in the pressure resistance of the exponential-phase cells of *E. coli* NCTC 8164 with increasing growth temperature from 10 °C up to 45 °C. In contrast, the pressure resistance of the stationary-phase cells increased with increasing growth temperature, reaching a maximum at 30 to 37 °C before decreasing at 45 °C.

The pressure resistance of bacteria may be affected by the morphology of the cells. The most sensitive bacteria are rod-shaped and the most resistant ones are spherical-shaped (Ludwig and Schreck 1997); however, there are some exceptions. For example, Casal and Gómez (1999) found that lactococci inoculated into 10 % (w/v) reconstituted skim milk were more sensitive than lactobacilli, prepared under similar conditions, to pressures of 100–350 MPa.

Yeasts and molds are generally considered to be sensitive to HPP. Yeasts are especially more sensitive than prokaryotic cells. Yeasts are single-celled fungi and generally not associated with foodborne diseases but are important in spoilage, especially in acidic foods. The sensitivity of yeasts to HPP makes this treatment effective for controlling yeast spoilage and extending shelf life of acidic foods such as fruit-based products.

14.2.2 Role of Process Parameters in Antimicrobial Efficacy

Equipment process parameters (such as pressure, temperature, treatment time, process come-up time) play important roles in governing antimicrobial efficacy of HPP. The HPP efficacy is dependent on the pressure level, duration of pressurization (holding or exposure time), and process temperature (Smelt et al. 2002). The pressure holding time should not include the pressure come-up time (the time required to increase the pressure of the sample from an atmospheric pressure to the target process pressure) or depressurization time (Farkas and Hoover 2000; Balasubramaniam et al. 2004). The temperature of the food is usually increased by approximately 3 °C per each 100 MPa increase when the food is subjected to high-pressure treatment at ambient temperatures (~25 °C). This temperature increase is known as "heat of compression" which is generated within the material due to compressive work against intermolecular forces (see Chap. 6). The temperature increase can even reach to 9 °C per 100 MPa increase if the food contains a significant amount of fat (Rasanayagam et al. 2003). Please see Chap. 6 for more discussion on the impact of pressure treatment on relevant food properties.

It is possible to enhance the lethality of HPP against microorganisms by increasing the process temperature, since this may lead to a higher degree of damage (denaturation) of proteins (Sonoike et al. 1992; Patterson and Kilpatrick 1998; Alpas et al. 2000). Patterson and Kilpatrick (1998) achieved 6.0 and 5.0 log reductions of *E. coli* O157:H7 NCTC 12079 in poultry meat and ultra-high-temperature-treated (UHT) milk, respectively, by simultaneous application of high-pressure treatment at 400 MPa and 50 °C for 15 min. Similarly, they observed 5.0 and 6.0 log reductions of *S. aureus* NCTC 10652 in poultry meat and UHT milk, respectively, by treatment at 500 MPa and 50 °C for 15 min. In contrast, a <1.0 log reduction of these bacteria was achieved by the application of pressure or heat alone. High-pressure treatment at chilled temperatures (below 20 to 30 °C) may also inactivate microbial cells faster, possibly due to the reduced membrane fluidity (Casadei et al. 2002).

14.2.3 Pasteurization Effects

Various researchers demonstrated the feasibility of using pressure treatment at ambient or chilling temperatures to provide "pasteurization" type effects on a variety of food materials. Mussa and Ramaswamy (1997) achieved reductions of 3.5

and 4.0 logs in raw whole milk microflora by treatment at 350 MPa for 20 and 32 min, respectively. The latter sample had shelf lives of 25, 18, and 12 days when stored at 0, 5, and 10 °C, respectively. Trujillo et al. (1999) and Buffa et al. (2001) found that treatment at 500 MPa (20 °C, 15 min) was as efficient as thermal pasteurization (72 °C, 15 s) in reducing the microflora of goat's milk including total bacterial count, psychrotrophs, lactobacilli, *Enterobacteriaceae*, and *Micrococcaceae*. Drake et al. (1997) found a comparable microbiological quality (coliforms, psychrotrophs, and total counts) in pasteurized and pressurized milks. Styles et al. (1991) showed that the decimal reduction time (*D*-value) of *L. monocytogenes* Scott A serotype 4b inoculated in UHT and raw milk was 13.2 and 9.2 min, respectively, when treated at 340 MPa at 23 °C. Although HPP may have the potential to complement traditional milk pasteurization under specific conditions, care should be taken to consider the risks associated with pressure resistance of the traditional milk pathogens, i.e., *Mycobacterium tuberculosis* and *Coxiella burnetii* (Cerf and Condron 2006). Tables 14.2–14.5 show the inactivation of different microorganisms in various substrates by HPP or combined pressure-heat treatment, including vegetative bacteria (Table 14.2), yeasts, and molds (Table 14.3).

14.2.4 Sterilization Effects

Pressure treatment at ambient temperature may not be effective in killing bacterial spores. However, combining pressure with heat can effectively inactivate spores. Pressure-assisted thermal processing (PATP) has emerged as one of the viable alternatives to conventional thermal sterilization for producing sterile shelf-stable low-acid foods. The technology utilizes a combination of high pressure and heat to inactivate bacterial spores in food matrices. In this method, the temperature of a preheated food product is elevated up to 121 °C by the means of pressure (usually between 500 and 700 MPa), and then the temperature and pressure are maintained for a short duration (Margosch et al. 2004a; Rajan et al. 2006a; Ahn et al. 2007). The rapid increase in the product temperature, as well as the rapid cooling upon decompression, helps preserve product quality attributes including color, flavor, texture, and nutritional values to a higher extent than is possible with traditional thermal retorting (Ting et al. 2002; Rajan et al. 2006a). Spore inactivation by PATP may be influenced by the target pressure, process temperature, and pressure holding time (Tables 14.4 and 14.5). Researchers have also shown that the lethality of combined pressure-heat treatment against bacterial spores can be enhanced by applying pulsed pressurization (Hayakawa et al. 1994; Margosch et al. 2004b; Rajan et al. 2006a; Ahn and Balasubramaniam 2007a; Ratphitagsanti et al. 2009). More recently Nguyen et al. (2013) proposed an integrated process lethality model that considered lethal effects of both pressure and heat on spore inactivation. In general, the combined pressure–heat treatment enhanced process lethality and reduced the process time compared to conventional heat treatment. However, at elevated temperatures (>110 to 115 °C), thermal effects became a dominant factor over the pressure effect. The term Extended Shelf Life (ESL) refers to foods that have received mild processing

Table 14.2 Inactivation of vegetative bacteria, in different media, under different high-pressure processing conditions

Bacteria	Medium	Process conditions	Inactivation (log_{10} reduction)	Reference
Campylobacter jejuni	Pork slurry	300 MPa/25 °C/10 min	6.0	Shigehisa et al. (1991)
C. jejuni	Chicken purée	375 MPa/25 °C/10 min	6.0	Solomon and Hoover (2004)
Citrobacter freundii	Minced meat	280 MPa/20 °C/20 min	5.0	Carlez et al. (1993)
Escherichia coli O157:H7	UHT milk	600 MPa/20 °C/15 min	≤2.0	Patterson et al. (1995)
E. coli O157:H7	Poultry meat	600 MPa/20 °C/15 min	3.0	Patterson et al. (1995)
E. coli O157:H7	Milk	400 MPa/50 °C/15 min	5.0	Patterson et al. (1995), Patterson and Kilpatrick (1998)
Lactobacillus sakei	Cooked ham	500 MPa/40 °C/10 min	4.0	Hugas et al. (2002)
Lactobacillus viridescens	Ham	500 MPa/25 °C/5 min	4.0	Park et al. (2001)
Lactococcus	Fresh lactic curd cheese	300–600 MPa/≤22 °C/5 min	≤7.0	Daryaei et al. (2006, 2008)
Listeria monocytogenes	Ultra-high-temperature processed milk	375 MPa/20 °C/15 min	<1.0	Patterson et al. (1995)
L. monocytogenes	Poultry meat	375 MPa/20 °C/15 min	2.0	Patterson et al. (1995)
Pseudomonas fluorescens	Minced beef	200 MPa/20 °C/20 min	5.0	Carlez et al. (1993)
Salmonella Enterica	Navel and Valencia orange juices	600 MPa/20 °C/1 min	≤7.0	Bull et al. (2004)
Salmonella enterica serovars (*S.* Senftenberg 775W, a heat-resistant strain, and *S.* Typhimurium ATCC7136, a heat-sensitive strain)	Phosphate buffer (pH 7.0) and strained chicken baby food	340 MPa/23 °C/10 min	3.0–4.0 (Senftenberg), and 2.0 (Typhimurium)	Metrick et al. (1989)
Staphylococcus aureus	UHT milk	600 MPa/20 °C/15 min	≤2.0	Patterson et al. (1995)
S. aureus	Poultry meat	600 MPa/20 °C/15 min	3.0	Patterson et al. (1995)
S. carnosus	Cooked ham	500 MPa/40 °C/10 min	1.29	Hugas et al. (2002)
Vibrio parahaemolyticus O3:K6	Oysters	300 MPa/10 °C/3 min	5.0	Cook (2003)

Table 14.3 Inactivation of yeasts and molds, in different media, under different high-pressure processing conditions

Fungi	Medium	Process conditions	Inactivation ($\log_{10}$ reduction)	Reference
Byssochlamys nivea (ascospores)	Grape juice (a_w 0.97)	700 MPa/70 °C/30 min	4.0	Butz et al. (1996)
B. nivea (ascospores)	Bilberry jam (a_w 0.84)	700 MPa/70 °C/30 min	<1.0	Butz et al. (1996)
Saccharomyces cerevisiae (vegetative cells)	Pork slurry	300 MPa/25 °C/10 min	2.0	Shigehisa et al. (1991)
Zygosaccharomyces bailii (vegetative cells)	Apple, orange, pineapple, cranberry, and grape juices	300 MPa/5 min	5.0	Raso et al. (1998a)
Z. bailii (ascospores)	Apple, orange, pineapple, cranberry, and grape juices	300 MPa/5 min	0.5–1.0	Raso et al. (1998a)

and have a shelf life under refrigerated storage that is greater than that afforded by minimal processes such as pasteurization (Marth, 1998). Currently very limited studies systematically investigated the conditions required to demonstrate the antimicrobial efficacy of combined pressure-thermal treatments to produce ESL products. More research will help the successful introduction of ESL type products in the market.

When reviewing the published data on pressure pasteurization and sterilization (Tables 14.2–14.5), often it is difficult to compare the results of experiments produced in different laboratories. Several factors make the comparison difficult including the lack of sufficient description of methodologies as well as variations in preparing inocula, enumerating microorganisms, and treating samples. Due to the emerging nature of the novel processing technologies such as HPP, different laboratories may utilize equipment designed by different vendors. Thus it is important to provide an adequate description of the equipment such as treatment chamber dimensions, material of construction, wall thickness, heating and cooling system, power specification, data acquisition system, and any other pertinent information necessary to reproduce the results. It is important to document thermal conditions and temperature distribution within the processed volume (Balasubramaniam et al. 2004). Kinetic parameters and models can be used to compare the impact of different process conditions on reduction of microbial populations. The variation in parameters such as come-up time and processing time leads to different results. It is essential to specify the location of the thermocouple sensor in relation to the sample tested (Balasubramanian and Balasubramaniam 2003).

Pressure-ohmic thermal processing (POTP) is a relatively new high-pressure-based technology for preserving shelf-stable low-acid foods and extended shelf life foods (Balasubramaniam et al., 2012). POTP involves sequential or simultaneous application of elevated pressure and ohmic heating to sterilize low-acid foods. The

Table 14.4 Inactivation of spores of pathogenic bacteria, in different media, under different high-pressure processing conditions

Bacterial spores	Medium	Process conditions	Inactivation (log_{10} reduction)	Reference
Bacillus cereus LMG 6910	UHT skim milk	400 MPa/60 °C/30 min	>6.0	Van Opstal et al. (2004)
B. cereus (strains NZ 3, NZ 4, NZ 5, NZ 6, and NZ 7)	9.5 % reconstituted skim milk	600 MPa/72 °C (initial temperature)/1 min	3.6–6.1	Scurrah et al. (2006)
B. cereus NIZO LB5	10 % reconstituted skim milk + 500 IU/ml nisin	500 MPa/40 °C/5 min (cycled twice)	5.9	Black et al. (2008)
Clostridium botulinum nonproteolytic type B (strains ATCC 25765 and TMW 2.518)	Mashed carrots (pH 5.15)	600 MPa/80 °C/1 s	>5.5	Margosch et al. (2004a)
C. botulinum nonproteolytic type B (strains 2-B, 17-B, and KAP9-B)	Sorensen phosphate buffer (0.067 M, pH 7.0), and crabmeat blend (pH 7.2–7.4)	827 MPa/75 °C/20–30 min	>6.0	Reddy et al. (2006)
C. botulinum proteolytic type A (strain ATCC 19397)	Mashed carrots (pH 5.15)	600 MPa/80 °C/12 min	>5.0	Margosch et al. (2004a)
C. botulinum proteolytic type A (strains BS-A and 62-A)	Sorensen phosphate buffer (0.067 M, pH 7.0)	827 MPa/75 °C/20 min	2.0 (BS-A) and 3.0 (62-A)	Reddy et al. (2003)
C. botulinum proteolytic type A (strains BS-A and 62-A)	Crabmeat blend (pH 7.2–7.4)	827 MPa/75 °C/15 min	3.2 (BS-A) and 2.7 (62-A)	Reddy et al. (2003)
C. botulinum proteolytic type B (strain TMW 2.357)	Mashed carrots (pH 5.15)	600 MPa/80 °C/60 min	<3.0	Margosch et al. (2004a)

technology synergistically combines the heat of compression effects of elevated pressures along with ohmic heating to minimize thermal exposure effects on product quality (Park et al. 2013, 2014). POTP treatment (600 MPa, 105 °C, 50 V/cm) inactivated *B. amyloliquefaciens* and *Geobacillus stearothermophilus* spores suspended in 0.1 % NaCl at pH 5.0 by 3.0 and 4.1 log during 10-min treatment, respectively. Increasing acidity of the food matrices accelerated the inactivation of both spores.

Table 14.5 Inactivation of spores of non-pathogenic bacteria, in different media, under different high-pressure processing conditions

Bacterial spores	Medium	Process conditions	Inactivation (log_{10} reduction)	Reference
Alicyclobacillus acidoterrestris DMS 2498	Orange juice	700 MPa/80 °C (initial temperature)/20 min	6.0	Ardia et al. (2003)
Bacillus amyloliquefaciens TMW 2.479 Fad 82	Mashed carrots (pH 5.15)	800 MPa/80 °C/16 min	2.0	Margosch et al. (2004a)
Bacillus coagulans ATCC 7050	Tomato juice (pH 4.5)+1 % sucrose laurate	392 MPa/45 °C/10 min	5.0	Shearer et al. (2000)
B. coagulans	Potage (pH 7)	100 MPa/ 85 °C/12 h	4.0	Islam et al. (2006)
B. coagulans	Reconstituted skim milk (9.5 %)	600 MPa/95 °C/1 min	3.0	Scurrah et al. (2006)
Bacillus licheniformis TMW 2.492	Mashed carrots (pH 5.15)	600 or 800 MPa/80 °C/16 min	>7.0	Margosch et al. (2004a)
Bacillus subtilis 168	Milk+monolaurin at a concentration as low as 0.001 %	392 MPa/45 °C/10 min	3.0	Shearer et al. (2000)
B. subtilis (laboratory strains)	Mashed carrots (pH 5.15)	800 MPa/70 °C/1 min	>6.0	Margosch et al. (2004a)
Clostridium sporogenes	Chicken breast	680 MPa/80 °C/20 min	2.0	Crawford et al. (1996)
C. sporogenes NCIMB 8053	Distilled water	600 MPa/60 °C/30 min	<1.0	Mills et al. (1998)
C. sporogenes PA 3679	Scrambled egg patties	688 MPa/121 °C/3 min	6.0	Koutchma et al. (2005)

(continued)

Table 14.5 (continued)

Bacterial spores	Medium	Process conditions	Inactivation (log_{10} reduction)	Reference
C. sporogenes ATCC 7955	Deionized water	700 MPa/105 °C/1 min	3.80–6.31	Ahn and Balasubramaniam (2007a)
Geobacillus stearothermophilus IFO 12550	1 mg/ml of bovine serum albumin (BSA), ovalbumin, and β-lactoglobulin	800 MPa/60 °C/60 min	>3.8	Hayakawa et al. (1994)
G. stearothermophilus IFO 12550	1 mg/ml of bovine serum albumin (BSA), ovalbumin, and β-lactoglobulin	600 MPa/70 °C/6 pulses (5 min each)	>6.0	Hayakawa et al. (1994)
G. stearothermophilus ATCC 7953	Mashed broccoli	600 MPa/120 °C/20 min	>6.0	Ananta et al. (2001)
G. stearothermophilus ATCC 7953	Cocoa mass	600 MPa/90 °C/60 min	6.0	Ananta et al. (2001)
G. stearothermophilus ATCC 7953	Egg patties	700 MPa/105 °C/5 min	4.0	Rajan et al. (2006b)
Thermoanaerobacterium thermosaccharolyticum ATCC 27384	Deionized water	700 MPa/105 °C/5 min	5.0	Ahn et al. (2007)
T. thermosaccharolyticum ATCC 27384	Deionized water	700 MPa/121 °C/1 min	>8.0	Ahn et al. (2007)

This indicates that POTP spore inactivation is influenced by pressure and thermal effects (Park et al. 2014). This feasibility study was carried out using laboratory-scale equipment, and more studies using pilot-scale equipment are needed to understand advantages, limitations, and potential application of POTP technology.

14.2.5 Influence of Product Characteristics on Antimicrobial Efficacy

Antimicrobial efficacy of HPP can be significantly influenced by the food composition. Different levels of microbial inactivation may be achieved when different substrates are used. As a general rule, rich media exert a protective effect on bacteria against inactivation by HPP, similar to that observed for heat treatments (Mañas and Pagán 2005). The protective effect of various food matrices on HPP inactivation of microorganisms, usually referred to as "baroprotective effect," has repeatedly been reported in the literature. A treatment at 375 MPa for 30 min and 20 °C resulted in a 6.0 log reduction in *E. coli* O157:H7 population in phosphate buffer (pH 7.0) but only a 2.5 log reduction in poultry meat and a 1.8 log reduction in milk (Patterson et al. 1995). García-Graells et al. (1999) found that the level of pressure-induced inactivation of *E. coli* MG1655 was 7.0 logs in phosphate buffer treated at 400 MPa but only 3.0 logs in milk at 700 MPa (20 °C, 15 min).

Proteins, carbohydrates, lipids, and minerals may exert a baroprotective effect. The baroprotective effects of bovine serum albumin (BSA), glucose, and olive oil on *L. monocytogenes* strains in model food systems have been demonstrated by Simpson and Gilmour (1997). A strong baroprotective effect of milk constituents on *L. monocytogenes* 4a KUEN 136 and *L. innocua* 4202 has been reported by Dogan and Erkmen (2004) and Black et al. (2007a), respectively. Black et al. (2007a) attributed this effect to a combination of HPP-induced solubilization of colloidal calcium phosphate (CCP) with a concomitant increase in the buffering capacity of milk and stabilization of the cell membrane by divalent cations such as Ca^{2+} and Mg^{2+}.

14.2.6 Importance of Considering Water Activity

Reducing water activity (a_w) of the medium by addition of solutes such as sugars and salts at high concentrations may exert a baroprotective effect on microorganisms. It has been suggested that the reduced water activity may result in cell shrinkage and thickening of the cell membrane, thus reducing the cell size and membrane permeability and fluidity (Knorr 1993, 1994; Oxen and Knorr 1993; Palou et al. 1997; Takahashi et al. 1993; Molina-Höppner et al. 2004). Oxen and Knorr (1993) found better survival of the yeast *Rhodotorula rubra* when treated at room temperature with 200–400 MPa for 15 min, by reducing the water activity of the medium from 0.98–1.0 to 0.94–0.96 through addition of sucrose, glucose, fructose, or

sodium chloride to the suspending medium. A similar baroprotective effect of reduced water activity was reported by Takahashi et al. (1993) for *E. coli* and *S. cerevisiae*. Reducing water activity may also increase pressure resistance of bacterial spores, possibly by increasing the osmotic dehydration of the spore core (Cheftel 1995; Raso et al. 1998b).

14.2.7 Role of Acidity in Antimicrobial Efficacy of Pressure-Treated Samples

The pH of suspending media is an important factor that can affect microbial inactivation by HPP. Similar to thermal processing, a greater microbial inactivation may be achieved by HPP when reducing the pH of treatment medium. The reduction in pH may also result in the inability of sublethally injured cells to recover during subsequent storage. Mackey et al. (1995) reported a progressive increase in the sensitivity of *L. monocytogenes* to pressure treatment by reducing the pH of the suspending medium. Stewart et al. (1997) found that by changing the pH of 0.1 M phosphate-buffered saline (PBS) from 6.0 to 4.0, an additional 3.0 log reduction in *L. monocytogenes* CA population during treatment with 353 MPa and 45 °C for 10 min. Alpas et al. (2000) reported a significantly enhanced inactivation of eight foodborne pathogenic bacterial strains (*S. aureus* 485 and 765, *L. monocytogenes* CA and OH, *E. coli* O157:H7 933 and 931, *Salmonella* Enteritidis FDA and *Salmonella Typhimurium* E21274) at 345 MPa (25–35 °C, 5 min) by reducing the pH of cell suspensions from 6.5 to 5.5 and to 4.5. Adding 1 % lactic acid or 2.1 % citric acid to the suspending medium increased the pressure-induced inactivation by 1.2–3.9 logs at pH 4.5.

Bacterial spore inactivation by combined pressure-heat treatment may also be enhanced at acidic pH. Roberts and Hoover (1996) reported an additional 1.5 log reduction of the *Bacillus coagulans* ATCC 7050 spores in citrate/phosphate buffer when pH was lowered from 7.0 to 4.0 during pressurization at 400 MPa and 45 °C. Stewart et al. (2000) found a 2.5 log reduction in the population of *Clostridium sporogenes* PA 3679 spores by treatment at 400 MPa and 25 °C for 30 min at pH 4.0, while only a <0.5 log reduction was achieved by the same treatment at pH 7.0.

The decrease in pH of suspending media may not always enhance the inactivation of microorganisms by HPP. Ogawa et al. (1990) reported that the pressure-induced inactivation of yeasts and molds, inoculated in Satsuma mandarin juice, was not affected by the pH (2.5–4.5) or the type of organic acids (citric, tartaric, lactic, or acetic acid) used for juice acidification before treatment at 100–600 MPa. Wuytack and Michiels (2001) found that the inactivation of *B. subtilis* spores at 100 and 600 MPa and 40 °C was not influenced by pH (over a pH range of 3.0–8.0). However, a higher level of spore inactivation was achieved when spores were first pressure-treated at neutral pH and then exposed for 1 h to low pH.

14.3 Choice of Microorganisms for Technology Validation

For successful commercial introduction of novel technologies, it is important to understand the impact of technology on most resistant pathogens of public health concern surviving the treatments. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) has redefined the term "pasteurization" to be applicable to foods processed by not only heat, but also alternative technologies. On this basis, pasteurization is defined as any process, treatment, or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage (NACMCF 2006). The target microorganisms may vary depending on the type of food to be challenged. For example, a precooked prepackaged deli meat product might be challenged with *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* (NACMCF 2006, 2010). *Listeria monocytogenes* might be used as the target microorganism if researcher is attempting to determine growth or inactivation due to recontamination with this organism in a ready-to-eat product (NACMCF 2010). The common pathogenic bacteria of public health concern, including *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and *Vibrio parahaemolyticus,* are frequently used as target microorganisms to validate the efficacy of HPP as an alternative pasteurization technique (NACMCF 2006). Nonpathogenic surrogate microorganisms (with similar or higher resistance than that of the target pathogen) may be used in challenge studies to validate the efficiency of a treatment if working with pathogens would have practical challenges. *Listeria innocua* and *E. coli* K12 are commonly used as nonpathogenic surrogates to *L. monocytogenes* and *E. coli* O157:H7, respectively (Farkas and Hoover 2000; NACMCF 2010).

It appears that pathogenic *Clostridium botulinum* type A and B spores are among the most resistant to combined pressure-thermal sterilization process. Research is also underway for identification of suitable nonpathogenic bacterial spores that could be potentially used as surrogates to *C. botulinum* spores for validation of the efficacy of PATP treatments of low-acid foods. Certain strains of *C. sporogenes* (PA3679) and *B. amyloliquefaciens* are among the nonpathogenic pressure-heat-resistant spores (Rajan et al. 2006a; NACMCF 2010; Wan 2014). More recently, Reddy et al. (2013) reported that *C. botulinum* type A strains and *C. sporogenes* PA3679, tested in *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) buffer (0.05 mol L^{-1}, pH 7.0) at 105 °C and 700 or 750 MPa, exhibited higher pressure-thermal resistance than that of *B. amyloliquefaciens* TMW 2.479 Fad 82 spores. It should be noted that the pressure-thermal resistance of bacterial spores does not necessarily correlate with their heat-only resistance (Margosch et al. 2004a; Koutchma et al. 2005; Margosch et al. 2006; Bull et al. 2009; Olivier et al. 2011). More research is needed to identify suitable surrogate organisms for combined pressure-thermal sterilization process.

The regulations related to high-pressure processing, especially for the purpose of sterilization of low-acid shelf-stable foods, are still evolving. These products are

required to be processed under good manufacturing practice conditions and relevant commodity-specific regulations (Rastogi et al. 2007). It is important that the food-contact surfaces should be made from the FDA-approved list of materials. Most of the HPP-pasteurized food products such as guacamole, juices, meat and oysters are currently distributed under refrigerated conditions. Potential temperature abuse during product handling and distribution should be taken into account. In 2009, the FDA issued a no-objection letter to an industrial petition for the use of PATP to produce a shelf-stable low-acid food product (IFT News 2009) (relevant validation procedures are summarized in Chap. 29). More recently, in July 2015, the FDA issued no objection to a second industrial petition for a pressure-enhanced sterilization process (PES) for commercial production of multicomponent shelf-stable food. However, no commercial PATP-treated shelf-stable low-acid food is currently available in the market.

14.4 Kinetic Models for Microbial Inactivation

The first-order kinetic model has traditionally been used to calculate thermal inactivation parameters (Juneja 2000; Juneja et al. 2001). Similarly, microbial inactivation by nonthermal technologies, such as HPP, is often described by first-order linear kinetic models. However, due to the complexity of the inactivation of microorganisms by HPP, the first-order model may not be always suitable to describe this behavior. The occurrence of shoulders and tails has been reported for microbial inactivation by HPP. Shoulders could result from the occurrence of sublethal injury, cell clumping, multitarget inactivation, or activation of dormant spores (Heldman and Newsome 2003; Mañas and Pagán 2005). The presence of subpopulations of different resistance may result in the tailing phenomenon (Peleg and Cole 1998; Benito et al. 1999; Heldman and Newsome 2003; Tay et al. 2003; Mañas and Pagán 2005). Various models have been proposed to describe the nonlinear kinetics of microbial inactivation by HPP, including the Weibull model (Peleg and Cole 1998; Chen and Hoover 2003a, b), log-logistic model (Chen and Hoover 2003a, b), and modified Gompertz model (Patterson and Kilpatrick 1998; Chen and Hoover 2003a, b) (Table 14.6).

Kinetic models can also be used in developing HACCP plans and process validation studies. More research is needed to develop databases of kinetic model parameters for various target pathogenic and spoilage microbes. Such databases that use defined process conditions would be useful for the evaluation of various critical process parameters. Further, it is important to investigate the influence of lethal doses (e.g., pressure) on reducing microbial populations using the proper experimental design. This includes a statistically valid collection of data taken at different process conditions using realistic foods, so that kinetic parameters are quantified. Researchers must properly document the equipment, process conditions, and microbial techniques (Balasubramaniam et al. 2004). Chapter 17 provides discussion on microbial safety kinetic models based on transition state theory.

Table 14.6 Selected models describing the kinetics of microbial inactivation by high-pressure processing

Model	Equation[a]	Description	Reference
First-order kinetics	$\log \frac{N_t}{N_0} = -\frac{t}{D}$	t = treatment time D = decimal reduction time (time required to reduce microbial population by 1.0 log)	Chen and Hoover (2003a, b)
Weibull model	$\log \frac{N_t}{N_0} = -bt^n$	t = treatment time b = scale factor n = shape factor	Peleg and Cole (1998); Chen and Hoover (2003a, b)
Log-logistic model	$\log \frac{N_t}{N_0} = \frac{A}{1+e^{4\sigma(\tau-\log t)/A}} - \frac{A}{1+e^{4\sigma(\tau+6)/A}}$	t = treatment time A = lower asymptote − upper asymptote σ = maximum rate of inactivation $\tau = \log_{10}$ time to the maximum rate of inactivation	Chen and Hoover (2003a, b)
Modified Gompertz model	$\log \frac{N_t}{N_0} = Ce^{-e^{BM}} - Ce^{-e^{-B(t-M)}}$	M = time at which the absolute death rate is maximum B = relative death rate at M C = lower asymptote–upper asymptote	Patterson and Kilpatrick (1998); Chen and Hoover (2003a, b)

[a] N_0 and N_t are the numbers of viable cells in the medium (CFU/ml or CFU/g) before and after being exposed to a lethal treatment for a specific time of t

14.5 Mechanisms of Microbial Inactivation by High Pressure

Understanding the mechanisms of inactivation of microorganisms under pressure is critical in order to design and develop efficient HPP equipment as well as identify safe process conditions for effective inactivation of microorganisms in food products. The pressure-induced inactivation of vegetative cells is generally believed to be due to damage to the subcellular structures and morphological, biochemical, and genetic alterations that not only inhibit microbial growth but can also cause cell death (Landau 1967; Hoover et al. 1989; Osumi et al. 1992; Cheftel 1995; Kobori et al. 1995). Many microbial subcellular structures including the cell membrane, nucleoid, ribosomes, and enzymes are affected by high pressure (Gross et al. 1993; Cheftel 1995; Smelt 1998; Balci and Wilbey 1999). The mechanisms of pressure-induced microbial inactivation are yet to be fully understood. Generally, damage to the cell membranes and membrane-bound enzymes (e.g. ATPases), responsible for controlling transport phenomena involved in nutrient uptake and waste disposal is proposed to be the primary mechanism of inactivation (Morita 1975; Earnshaw

1992; Wouters et al. 1998; Ulmer et al. 2000; Gänzle and Vogel 2001; Chilton et al. 2001; McClements et al. 2001; Casadei et al. 2002; Torres and Velazquez 2005). However, other mechanisms, such as denaturation of various proteins, might be involved (Hoover et al. 1989; Linton and Patterson 2000).

A complex mechanism is assumed for spore inactivation by combined pressure-heat treatment. Depending upon the process conditions, spores may exhibit different inactivation mechanisms:

- *Two-stage strategy*: An initial low pressure (60–100 MPa) and mild temperature (<30 °C) with an extended holding time are used to induce spore germination by activating the nutrient germinant receptors (Wuytack et al. 2000; Paidhungat et al. 2002). The germinated spores are subsequently inactivated by applying a shorter treatment at moderate pressures (>300 MPa) and temperatures (<30 °C).
- *High-pressure treatment at moderate temperatures*: A high-pressure treatment (500–700 MPa) is applied at moderate temperatures (<60 °C) to germinate and inactivate the spores (Mills et al. 1998). The treatment initially triggers stage I germination in which ions, in particular Ca-DPA, are released from the spore core through specific channels in the inner membrane or on the membrane itself. In addition, the core is partially hydrated during stage I germination due to the net water movement into the core, presumably into a space created by the release of the large amount of Ca-DPA. These events subsequently trigger stage II germination in which the cortex is hydrolyzed and core is fully hydrated (Paidhungat et al. 2002; Black et al. 2007b, c). However, if the process conditions cause damage to the spore germination systems such as cortex lytic enzymes (CLEs), the spores will only slowly go through stage II (Black et al. 2007b, c). The stage II-germinated spores are more sensitive than the stage I-germinated spores to agents such as moist heat (Black et al. 2007b).
- *High-pressure treatment at elevated temperatures*: When a high-pressure treatment (500–700 MPa) at elevated temperatures (>90 °C) is applied, a direct spore inactivation mechanism that bypasses germination is more likely expected since the spore germination systems are damaged (Ananta et al. 2001). Ahn and Balasubramaniam (2007b) and Ratphitagsanti et al. (2010) reported insignificant numbers of germinated spores of *B. amyloliquefaciens* TMW 2.479 Fad 82 after PATP treatment (700 MPa, 105 °C). These were in agreement with earlier findings by Ananta et al. (2001) for the inactivation of *G. stearothermophilus* ATCC 7953 spores in mashed broccoli by high-pressure treatment at temperatures above 95 °C.
- *Hit-and-wait strategy*: A short pressure pulse (600–800 MPa) at high temperature (>60 °C) is applied which releases a significant amount of the spore DPA content, and the spores are subsequently inactivated upon pressure release (Margosch et al. 2004a, b). Generally, the more DPA is released, the higher level of spore inactivation is achieved. Margosch et al. (2004b) reported the release of DPA from *B. subtilis* TMW2.485 and *B. amyloliquefaciens* TMW 2.479 Fad 82 spores by 96 % and 58 %, respectively, after a high-pressure treatment at 800 MPa and 80 °C for 2 min.

14.6 Documenting HPP-Induced Injury and Subsequent Recovery of Microorganisms

High-pressure processing may cause injury of a proportion of the microbial population. The injured cells may recover after the treatment if they are placed in a suitable substrate and stored under optimal conditions (Mackey 2000; McClements et al. 2001; Bozoglu et al. 2004). The occurrence of sublethal injury may lead to an overestimation of the process lethality, since the injured cells might not be detected using selective conditions for the enumeration of survivors (Mañas and Pagán 2005). It is important to consider the extent of bacterial injury by various treatments (Balasubramaniam and Farkas 2008). Use of nonselective microbiological media is recommended to allow detection of all viable organisms of concern posttreatment at various incubation temperatures. Recovery of injured cells is usually inhibited at acidic pH levels or in the presence of sodium chloride and antimicrobial compounds added to the growth media (Patterson et al. 1995). The following are examples of the pressure-induced injury and recovery of vegetative microorganisms and spores.

Bozoglu et al. (2004) detected two types of pressure-induced injury (symbolized as I1 and I2) in four relatively pressure-resistant strains of foodborne pathogens (*L. monocytogenes* CA, *S. aureus* 485, *E. coli* O157:H7 933, and *Salmonella* Enteritidis FDA) in milk (pH 6.65) when treated at 350, 450, and 550 MPa and stored at 4, 22, and 30 °C. The I1-type injury (most likely a structural damage such as cell wall and/or cell membrane injury) prevented the growth of colonies on selective but not on nonselective agar. The I2 type injury (most likely a metabolic injury involving the damage of the functional components of the cells) was a major injury, and after its repair (recovery to an I1 state), the cells could form colonies on nonselective but not on selective agar. The colony formation on both selective and nonselective media occurred only after full recovery of injury (I1 to active cells). The mechanism of injury repair varies depending on the type and degree of the damage and involves specific metabolic processes for the synthesis of ATP, RNA, DNA, proteins, and lipids (Bozoglu et al. 2004; Wesche et al. 2009).

Daryaei et al. (2010) reported the recovery of pressure-injured *Candida lipolytica* (spoilage yeasts) in a fermented milk test system (initial count: 10^5 CFU/ml) over a pH range of 4.3–6.5 during storage of treated milk (300 MPa, 5 min) at 4 °C for 8 weeks. The enumeration of yeasts on dichloran rose bengal chloramphenicol agar immediately after treatment showed a 5.0 log reduction in the viable count. However, the yeast count increased, during refrigerated storage, from week 3 onwards and reached 10^6–10^7 CFU/ml within 8 weeks.

Ratphitagsanti et al. (2010) reported significant recovery and growth of *B. amyloliquefaciens* TMW 2.479 Fad 82 spores in carrot purée treated at 700 MPa and 105 °C for 5 min. The treatment initially reduced the spore viable count, as enumerated on trypticase soy agar with yeast extract, to below the detection limit ($<10^2$ CFU/ml); however, the spore count increased during subsequent storage of the treated product at 32 °C and exceeded 10^7 CFU/g. The spore recovery was inhibited by increasing the treatment holding time from 5 to 15 min or adding organic acids

to the inoculated carrot purée prior to treatment. Daryaei et al. (2013) also found that the recovery and growth of *B. cereus* ATCC 9818 spores in cooked rice treated at 600 MPa and 60–85 °C was influenced by the extent of pressure holding time and process temperature.

More research is needed to fully understand the mechanisms of inactivation or injury of vegetative microorganisms and spores by HPP and combined pressure-heat treatment. It is also necessary to determine kinetic model parameters for target microorganisms in various food matrices for each technology in order to validate and optimize process conditions (Balasubramaniam and Farkas 2008).

14.7 Conclusions

High-pressure processing is a commercially viable and practical alternative technology to preserve foods with reduced thermal requirement. Pressure treatment can extend product shelf life without the use of preservatives or additives, and the process has a minimal or no impact on the sensory attributes of the food material. Depending upon the treatment intensity (with or without combination of heat), this technology can result in both pasteurization and sterilization effects. Due to the emerging nature of the technology, it is vital to understand the mechanisms of microbial inactivation under pressure. It is critical to continue research to identify surrogate organisms for various pressure pasteurization and sterilization processes. Furthermore, developments of suitable kinetic models for bacterial destruction and food quality degradation are needed. Intelligent combination of HPP with traditional heat processing or other emerging food preservation techniques may further advance the commercial application of these technologies in the food industry.

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Chapter 15
High-Pressure Effects on Viruses

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Abstract This chapter starts with brief reviews of high-pressure processing with regard to its history, advantages for use as a food processing technology, and basic description of the processing parameters. It also surveys viral food safety along with the biochemistry and pathology of foodborne infectious viruses, most especially norovirus, hepatitis A, and Aichi virus. The chapter further delves into pressure inactivation kinetics of viruses and describes the effects of treatment temperatures and food composition found in the literature. Another highlighted subject is the potential use of viral vaccines produced by pressure treatment that first arose in 1956 with investigation of pressure inactivation of poliomyelitis virus with subsequent measurement of immunogenic potential.

Keywords High pressure • Virus • Food safety • Inactivation kinetics • Vaccine

15.1 Introduction

Research published by Bert Hite and researchers (Giddings et al. 1929) was among the first reported literature on pressure sensitivity of viruses. Tobacco mosaic virus (TMV) was selected for examination and found to be extremely resistant to pressure. In fact, these workers found a pressure of 920 MPa was necessary to demonstrate any measurable inactivation of the virus. Later investigations in the twentieth century showed that not all viruses possessed the same level of pressure resistance as TMV. After this work in 1929 until the 1980s, the effects of hydrostatic pressure on viruses were occasionally studied, but there was little follow-up work by contributing laboratories. However, once high-pressure processing (HPP) emerged as a promising commercial food processing method and potential application to biomedical products, the number of investigations involving responses of viruses and other microorganisms to HPP increased many-fold.

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The primary use of HPP and other nonthermal food processing technologies is for the treatment of foods for purposes of preservation and safety while maintaining sensory and nutrient qualities of a food product similar or identical to the raw or fresh form. In other words, HPP is intended to kill microorganisms associated with the food while causing minimal changes to the food. While viruses have no effect on food spoilage (as they cannot replicate in foods), the presence of infective human viruses in foods does carry obvious safety concerns.

Shellfish, particularly oysters, clams, and mussels, are susceptible from viral contamination. These filter-feeding sedentary animals are harvested in estuaries and are commonly consumed raw or lightly cooked. Foodborne illnesses from consumption of raw shellfish have been caused by noroviruses, hepatitis A virus, and Aichi virus. These three groups of human viruses are also the viruses of primary concern in other foods.

Gastroenteritis caused by viruses is generally ranked as the primary cause of foodborne illness in the USA by a very wide margin (Mead et al. 1999). The last decade has seen a growing awareness of the significant role viruses play in foodborne disease. In the USA, the noroviruses are by far the most common cause of foodborne disease; noroviruses are estimated to be responsible for approximately 9.2 million cases per year, which corresponds to more than 67 % of all cases, 33 % of hospitalizations, and 7 % of deaths annually (Mead et al. 1999). Evidence exists that noroviruses are becoming increasingly virulent (FSN 2005). Regarding hepatitis A virus, the US Centers for Disease Control and Prevention (CDC) had ranked it as the fourth most frequently identified cause of foodborne disease in the USA (Jaykus 2000). On the list of important foodborne viruses, hepatitis A is usually ranked second behind noroviruses. Hepatitis A virus causes a more modest number of cases, projected as approximately 12,600 foodborne cases per year, but its symptoms are usually more life-threatening as a key symptom of hepatitis A is infection of the liver. Confirmed cases of hepatitis A caused by contaminated foods number nearly 4200 per year. Outbreaks continue to occur throughout the world despite an increase in sanitation and hygiene standards (Grohmann and Lee 2003).

This review focuses on the current body of knowledge related to viral responses to applications of high pressure treatment and the mechanisms involved. There is specific emphasis on high-pressure applications on viruses of foodborne concern using HPP as an intervention strategy to inactivate contaminating viruses, and also there is attention on use of pressure as a means to inactivate viruses in the manufacture of attenuated vaccines.

15.2 High-Pressure Processing

Exposure to high pressure does cause a range of effects and changes on molecular interactions in the treated foods. Ionic bonds and at least a proportion of hydrophobic interactions are broken or distorted by high pressure (Hoover et al. 1989). Covalent bonds are unaffected (Ledward 1995). The pressure-induced changes to

ionic bonds and hydrophobic interactions starting at pressures of 100–200 MPa at room temperature cause proteins to denature. Oligomeric structures dissociate into their subunits, monomeric structures partially unfold and denature, and protein aggregate and gel. The conformation of proteins is further altered by increases in pressure, due to irreversible changes to the secondary, tertiary, quaternary, and supramolecular structures (Palou et al. 1999). Denaturation may result when proteins are exposed to pressure beyond that of the individual protein-specific pressure threshold (Cheftel 1995). Additionally, the structure and function of lipids, polysaccharides, and starch are altered by HPP (Ledward 1995), while smaller molecules such as vitamin C and ß-carotene remain unaffected (Bull et al. 2004). Oxidative reactions in foods and enzymatic browning in some fruits are reportedly enhanced by HPP, while partial discoloration has been noted in treated red meats (Ledward 1995).

Compared to HPP inactivation of bacteria, the mechanism of viral inactivation by pressure is less well understood. Kingsley et al. (2002) determined that HPP does not damage nucleic acids as shown by comparative RNase treatment of pressure-treated HAV and thermally released viral RNA. Incubation of virus treated at 500 MPa with RNase before release of RNA showed that this pressure-inactivated virus did not liberate its RNA, as evidenced by a positive RT-PCR result. This suggests that the viral capsid remains intact following HPP inactivation. Given that the RNA is still infective, it was deduced that the loss of infectivity was a result of the denaturation of the capsid proteins that prevented attachment to the appropriate cellular receptor or blocked the penetration and virion-uncoating mechanisms occurring post-viral attachment (Kingsley et al. 2002).

Coxsackievirus A9 and human parechovirus-1, which are both sensitive to pressure to some degree, interact with integrin-like cellular receptors via an arginine-glycine-aspartic acid (RGD) motif on peptide loops within the VP1 capsid protein. On the other hand, coxsackievirus B5, which showed strong resistance to HPP inactivation, does not have this RGD motif and binds to cellular receptors at the base of canyon-like depressions on the surface of VP1 (Kingsley et al. 2004).

As with other preservative food processes, the required pressure treatment parameters to produce microbiologically safe and stable products are dependent on the target microorganism(s) to be inactivated. Bacterial vegetative cells, yeasts, molds, and some viruses are sensitive to pressures in the range of 200–700 MPa. Bacterial endospores have been shown to survive pressurization above 1000 MPa (Arroyo et al. 1999; Cheftel 1992; Sale et al. 1970). Spoilage of food and food safety issues due to the outgrowth of bacterial spores can be controlled by additional hurdles such as acidification and refrigerated storage. Various common factors influence the pressure resistance of microorganisms, including the type of target microorganism and its physiological state, the intrinsic properties of the food, the processing temperature, and the time and magnitude of pressure treatment (Hoover et al. 1989). Considerable variation in susceptibility to high-pressure inactivation has been observed among various microbial species, strains, and related to microorganisms in different substrates (Patterson et al. 1995). Certain foods offer pressure protection to microorganisms from inactivation or injury. For example, milk is said

to be more protective to bacteria during HPP than a buffer solution (Cheftel 1995) or meat (Patterson et al. 1995).

In the case of bacteria and fungi, the primary site of pressure damage leading to inactivation is the cytoplasmic membrane. Under pressure, cell permeability is altered and ion exchange disrupted (Yuste et al. 2001) due to crystallization of membrane phospholipids (Cheftel 1995) and protein denaturation. The majority of pressure-sensitive bacteria begin to lose viability at approximately 180 MPa (Lado and Yousef 2002). In a pressure range of approximately 200–400 MPa irreversible changes, such as cell leakage leading to cell death, have been demonstrated by the release of UV-absorbing material from *E. coli* (Farr 1990; Isaacs et al. 1995; Smelt 1998). For viruses, mechanisms for pressure inactivation differ due to the different structures and morphologies of viruses as well as their means of replication. As obligate parasites, interaction with the host plays a distinctive role.

15.3 Foodborne Viruses

Viruses differ greatly from other microorganisms in that they have no cellular structure and contain one or more molecules of either RNA or DNA enclosed in a protein coat or capsid (Madigan et al. 2000). The capsid functions as the primary protective barrier for the virion or viral particle. The capsid of some viruses is additionally enveloped in an outer lipid membrane and sometimes other layers containing carbohydrates, lipids, and additional proteins (Prescott et al. 2002). The lipid envelope does not confer extra stability to the virus. Enveloped viruses tend to be susceptible to adverse environmental conditions and are generally destroyed by the low pH and bile found in the gastrointestinal tract of humans (Adams and Moss 2000).

Viruses exist in extracellular and intracellular phases and are often referred to as obligate intracellular parasites (Prescott et al. 2002). In the extracellular phase, viruses are ametabolic or dormant and possess few if any enzymes. In the intracellular phase, viruses predominately exist as replicating nucleic acids controlling the host's replicative machinery to reproduce necessary components and then eventually release large numbers of new virions.

As a group, the viruses are far smaller than bacteria; however, the diameters of virus particles range from 10 nm (a little larger than ribosomes) to 400 nm (such as poxviruses that are equivalent in size to the smallest bacteria) (Prescott et al. 2002). Therefore, most viruses cannot be visualized with a light microscope, but rather with an electron microscope. Their shapes vary as well; the four major types of morphological structures are icosahedral, helical, envelope, and complex.

Those viruses of concern in foods are the noroviruses, picornaviruses (specifically, hepatitis A, Aichi virus, enteroviruses, and human parechovirus), hepatitis E virus, rotavirus, adenovirus, and astrovirus (Grove et al. 2006). As described earlier, the foodborne viruses of primary concern are norovirus and hepatitis A virus. These two types of human viruses represent the overwhelming majority of viral foodborne illnesses in the USA. The remaining listed viruses are seldom linked to documented

cases of contaminated foods or beverages. For example, foodborne rotavirus and astrovirus cases are far more difficult to quantify and have milder disease symptoms than hepatitis A. Therefore, rotaviruses and astroviruses are considered less of a concern in foods and are commonly ranked lower in importance with neither virus routinely reported. Additionally, the percentages of foodborne illness due to rotavirus and astrovirus are thought to be very low, probably less than 1 %. The number of foodborne cases caused by rotaviruses and astroviruses are each estimated to be <39,000 per year in the USA (Mead et al. 1999).

15.3.1 Noroviruses

Noroviruses are non-enveloped viruses containing single-stranded positive-sense RNA within capsids constructed of a single polypeptide of 59 kDa. This polypeptide is the primary reason for classification of norovirus as a calicivirus, even though the overall structure of the capsid does not resemble the typical cup-shaped morphology that is a characteristic of caliciviruses (Grohmann and Lee 2003). In the past, noroviruses were referred to as Norwalk viruses, and due to any distinctive capsid, morphology were also previously called small round-structured viruses (Grohmann and Lee 2003). Capsids of noroviruses have diameters of only 27–35 nm; this limited capacity permits each virion to house only enough RNA for three genes or three open-reading frames (Kapikian et al. 1996).

Human viral gastroenteritis caused by noroviruses is characterized as a generally mild disease featuring symptoms that include nausea, vomiting, diarrhea, malaise, abdominal pain, muscle pain, headache, anorexia, and low-grade fever (Cliver and Matsui 2002; Grohmann et al. 1981). Symptoms generally begin 1–2 days following consumption of contaminated foods or water and persist for anywhere from 1 to 8 days (Grove et al. 2006). The vomiting is often characterized as sudden-onset vomiting, which can be projectile in infected adults.

Norovirus is considered to be the leading cause of foodborne enteric illness in the USA, estimated to cause a total of over 23 million infections annually (Mead et al. 1999). Worldwide, the actual occurrence of viral gastroenteritis caused by norovirus is probably greatly underreported as well (Ang 1998). Often, with many cases associated to norovirus, medical attention is not sought due to self-limiting and mild nature of the infection (Ang 1998; McDonnell et al. 1995; Murphy et al. 1979). Only in a small number of cases, hospitalization may be required (Koopmans et al. 2002). Another factor causing underestimation of foodborne norovirus illness is the substantial strain diversity resulting from the multiple genetic and antigenic types of norovirus. Such variety in analytical types contributes to misdiagnosis of the prevalence of norovirus.

Inactivated by normal cooking, noroviruses are relatively heat sensitive (SCVM 2002). Consequently, foods typically implicated in a norovirus outbreak are those that have not undergone heat treatment prior to consumption, even though any food theoretically may be implicated in the transmission of noroviruses. Past examples of

foods contaminated with norovirus and causing illness are salads, pastry frostings, boxed lunches, and raw oysters (Cliver and Matsui 2002). Ready-to-eat foods can be contaminated by infected food handlers; however, foodborne transmission is not the only possible method for transmitting infection. Fecally contaminated water is an important vector in spread of the disease; ice has been implicated as a norovirus vector.

The primary symptom of illness caused by norovirus is vomiting, and vomiting generates aerosols and causes extensive surface contamination (Ho et al. 1989; Patterson et al. 1997). When combined with its low infectious dose (as low as 10–100 particles; Caul 1994), the norovirus attack rate (the proportion of infected individuals in relation to those who were at risk of infection during the event) is commonly quite high during outbreaks, up to 90–100 % (Lees 2000). Vomiting during norovirus infection may release an estimated 30 million viral particles from the body (Caul 1994). This accentuates transmission of infection either by the inhalation and subsequent ingestion of aerosolized virus particles or by the contamination of food or surfaces such as bench tops and sinks in food preparation areas (Marks et al. 2000; McDonnell et al. 1995).

People of all ages can be infectedby norovirus. Outbreaks are very difficult to prevent and control as demonstrated by the consistent occurrence of viral gastroenteritis on cruise ships in spite of extensive preventative actions (Patterson et al. 1997; FSN 2004). The low infective dose and high attack rate ensure infection is quickly spread through small communities and establishments, such as kindergartens and schools (Perrett and Kudesia 1995), restaurants (Patterson et al. 1997), hotels (Marks et al. 2000; McDonnell et al. 1995), nursing homes (Kaplan et al. 1982), and, as noted, cruise ships (Ho et al. 1989; McEvoy et al. 1996). A classic norovirus outbreak described by Marks et al. (2000) exemplifies the ease in which norovirus particles are transmitted by aerosolized vomit.

There is also a lack of long-term immunity. During infection, antibody to the virus is produced, but immunity does not usually last longer than a year (Cliver and Matsui 2002). The disease can occur with re-infection and thus it is difficult to develop a vaccine (CDC 2004). The highly contagious nature of the virus and the short-lived immunity following infection delivers the very high attack rates during outbreaks as described above (Marks et al. 2000).

Since norovirus particles from the outbreak in Norwalk, OH, in 1968 were first detected using immune electron microscopy, study of the virus has been hampered by the inability to culture the virus; there also has been no animal model identified (Jiang et al. 1993). Historically, diagnosis of norovirus infection has been achieved by clinical presentation (Grohmann and Lee 2003) or by visualization with electron microscopy which is a relatively insensitive technique (Atmar and Estes 2001).

Noroviruses have not yet been routinely cultured in the laboratory, although methods for the detection of the virus from fecal specimens have improved. Sequencing of the norovirus genome has enabled use of RT-PCR to detect noroviruses in environmental and clinical samples (Gassilloud et al. 2003; Grohmann and Lee 2003). Real-time quantitative PCR is a method for enumerating nonculturable and difficult-to-culture viruses. It is a popular tool for enumerating, within hours,

viruses purified from environmental or food samples; however, it and other PCR assays are at a disadvantage because these methods cannot discriminate between inactivated viruses and whole infectious viral particles, potentially delivering false-positive results from foods that are actually safe (Slomka and Appleton 1998).

Due to the culturing difficulties with human norovirus, feline calicivirus has been one of the more popular surrogates used by many laboratories as a replacement organism for study of norovirus. Feline calicivirus causes a variety of diseases in cats, such as conjunctivitis, ulcers in the oral cavity, and limping syndrome (Thiel and Konig 1999). As with other caliciviruses, feline calicivirus is very similar in primary sequence and genomic organization to human noroviruses (Jiang et al. 1993); however, feline calicivirus can be easily grown in cell culture (Doultree et al. 1999). Historically, the only source of norovirus particles has been from stools of infected individuals which characteristically contain low concentrations of the virus (Atmar and Estes 2001).

As an alternative to feline calicivirus, the mouse norovirus (murine norovirus-1, MNV-1) is more closely related to human norovirus and is the first norovirus to be propagated in cell culture (Wobus et al. 2006). The murine norovirus shares many genetic features with human noroviruses including the size and shape of the virus particles (Fig. 15.1). Murine norovirus is included in the genogroup GV of norovirus, which are closer in sequence comparison to the GII human noroviruses (Hutson et al. 2004).

MNV-1 causes a lethal infection in mice resulting in hepatitis, pneumonia, or inflammation of the nervous system (Karst et al. 2003). These symptoms are different from those of human noroviruses, but since the mouse norovirus is transmitted via the fecal-oral route, it is able to withstand lower pH levels and is a better surrogate for human noroviruses. Cannon et al. (2006) demonstrated the ability of mouse norovirus to survive at a pH range from 2 to 10, whereas FCV was only able to withstand a pH range from 3 to 9. Acid resistance is crucial for successful human infection since enteric viruses must survive acidity in the stomach in order to reach the target cells of the small intestine. The mouse norovirus is fairly sensitive to HPP and disinfectants; however, there is some speculation that human noroviruses may not be as sensitive as the mouse norovirus (Gary Richards personal communication 2010).

15.3.2 Hepatitis A Virus

The positive-sense single-stranded RNA of hepatitis A virus (Cuthbert 2001) is surrounded by a protein capsid of approximately 28–30 nm in diameter without distinctive surface features (Cliver and Matsui 2002). This nonenveloped virus is a member of the family *Picornaviridae* and possesses extreme stability to environmental conditions, especially to heat and drying (Cliver and Matsui 2002). Hepatitis A virus is more resistant to low pH (pH 2.0), gamma rays, UV light, and low levels of chlorine and ozone than other picornaviruses (Grohmann and Lee 2003).

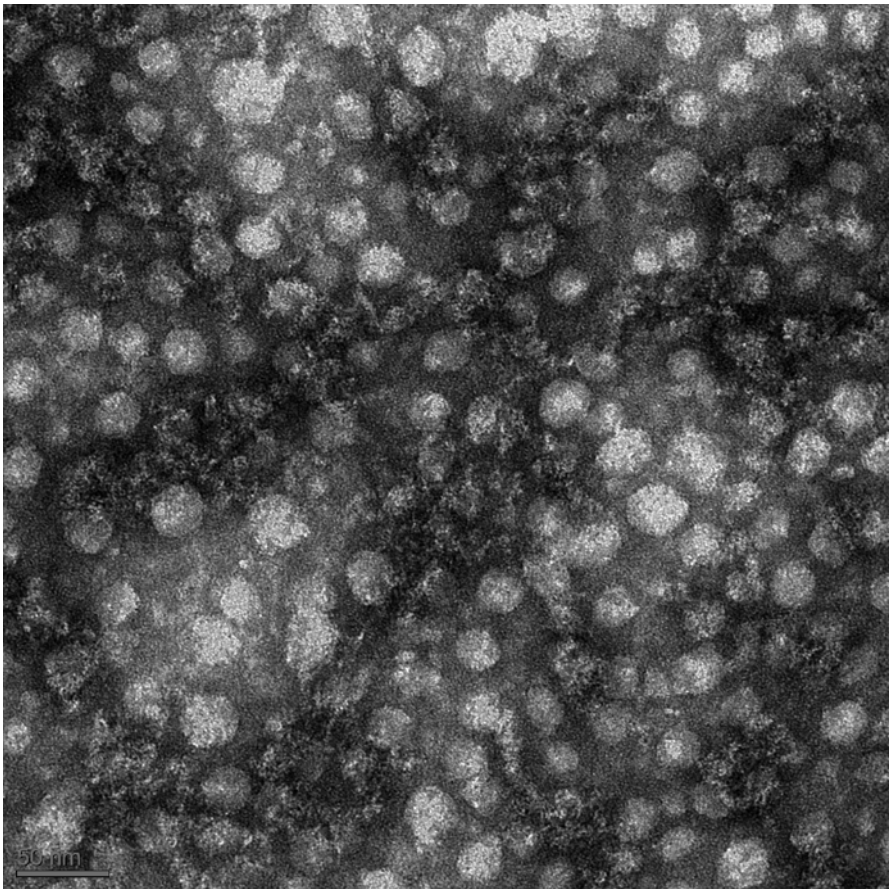


Fig. 15.1 Electron micrograph of mouse norovirus MNV-1. Magnification is 50,000×

Infection by hepatitis A starts with ingestion of fecally contaminated food or water (Cliver and Matsui 2002). After penetrating and replicating in the intestinal epithelial cells, the virus infects the liver where replication may take place inside hepatocytes (Cuthbert 2001). When an immune response is evoked, cytotoxic T cells destroy infected liver cells, severely disrupting regular body functions controlled by the liver. Virions of hepatitis A are excreted from the body in feces following secretion of the virus from the liver in bile.

The incubation period of hepatitis A virus averages 28–30 days. The disease is rarely fatal. In infants and children under 5 years of age, infection is often mild or asymptomatic; immunity to hepatitis A is usually life-long (Grohmann and Lee 2003). There is an effective vaccine; nonetheless, there are approximately 80,000 infections annually in the USA (Grohmann and Lee 2003).

Tissue culture is an effective method for the growth and quantification of a variety of viruses including human pathogens, but growth of hepatitis A virus in tissue

culture can be challenging and only inconsistently successful; this is particularly true for environmental isolates or wild-type strains (Koopmans et al. 2002). Tissue culture assays develop plaques and cytopathic effects in infected cells enabling enumeration of viable viruses. Although virus particles are released from infected cells into the surrounding liquid medium where they may be recovered for further analysis, only a relatively small proportion of hepatitis A virus is released from infected tissue culture cells. Instead, most of the infectious virus particles remain in the cell cytoplasm (Bishop et al. 1994). Tissue culture methods for analyzing and enumerating hepatitis A virus remain lengthy and labor-intensive, thereby encouraging the use of PCR techniques.

The largest documented outbreak of viral foodborne illness occurred in 1988 from the consumption of contaminated raw clams in China that killed 32 people and resulted in nearly 300,000 cases of illness (Halliday et al. 1991). The clams were contaminated by release of untreated sewage from a nearby residential area which had reported an epidemic of hepatitis A in the preceding months and from boats dumping human waste overboard in the vicinity of shellfish-harvesting areas.

In 2003 in the Pittsburgh, PA area, one of the more notable US hepatitis A outbreaks occurred (Marler-Clark 2004). Fecally contaminated scallions or green onions from Mexico were confirmed as the cause of four deaths with over 650 people demonstrating symptoms of hepatitis A infection. Apparently the scallions were grown under field conditions that exposed them to human feces, and the virus particles were taken up by the plant so that sautéing the onions prior to consumption did not completely inactivate the contaminating virus.

15.3.3 Aichi Virus

Aichi virus is related to hepatitis A virus and belongs to the family *Picornaviridae* (genus *Kobuvirus*). It is a nonenveloped positive-sense, single-stranded RNA virus. Aichi virus is one of the newer members of human viruses in which foodborne transmission has been established. The disease presents itself as a self-limiting gastroenteritis (Yamashita et al. 1991). Through phylogenetic analysis, two genotypes of AiV (A and B) have been identified (Yamashita et al. 2000). AiV was first isolated from a stool specimen of a gastroenteritis patient in Aichi, Japan in 1989, that was associated with an outbreak involving shellfish. Aichi virus is now common across Asia (Yamashita et al. 1995). Symptoms do not go beyond gastroenteritis and the disease appears to primarily infect 15- to 34-year-old people. There has been a high seroprevalence in teens and young adults with limited seroprevalence in younger children in Japan (Yamashita et al. 1993). Recently it was noted that Aichi virus (AiV) has been isolated from clinical specimens in Germany and Brazil (Oh et al. 2006), so it appears this virus is spreading among human populations of the world and likely causes illness within the USA. In Asia, oysters are the most common vehicle of AiV transmission; however, it has been suggested that there are other vehicles for AiV transmission, but they have yet to be identified

(Yamashita et al. 2000). It is likely that the aquatic environment was initially contaminated with virus in these cases, which questions the role of water in the contamination of other foods and ultimately in the transmission of AiV.

15.4 Pressure Inactivation of Viruses

As noted at the beginning of this chapter, the work of Giddings et al. (1929) was the first attempt to estimate the pressure sensitivity of viruses by studying pressure effects on tobacco mosaic virus (TMV). TMV was found to be extremely resistant to pressure; pressurization at 920 MPa was necessary to show any measurable inactivation. Fortunately, the pressure resistance of most human and animal viruses is lower than that of TMV. Most of these viruses can be inactivated to a significant extent at pressures <450 MPa. For example, Nakagami et al. (1992) found 10-min exposures above 300 MPa at 25 °C to eliminate infectivity of pathogenic herpes simplex type 1 virus and human cytomegalovirus. Hepatitis A virus in initial concentrations of 10^7 infectious units in tissue culture medium can be reduced to nondetectable levels after 5 min at 450 MPa (Kingsley et al. 2002). Among the human and animal viruses studied to date, poliovirus appears to be one of the most resistant to pressure surviving 60 min at 600 MPa with only modest reductions in infectivity (Wilkinson et al. 2001). Other viruses that are extremely pressure-resistant and resistant to inactivation at 600 MPa are Aichi virus and coxsackievirus B5 (coxsackieviruses can be spread in foods) (Table 15.1).

A 5-$\log_{10}$ $TCID_{50}$/mL reduction in rotavirus was found after a 70-s exposure to 300 MPa at 25 °C; however, after a 10-min treatment 1-$\log_{10}$ $TCID_{50}$/mL of rotavirus still remained (Khadre and Yousef 2002). Herpes simplex virus and human cytomegalovirus could not be recovered following 10 min of treatment at the same pressure. It appeared that these enveloped viruses were prevented from binding to host cells and initiating infection due to damage to their envelopes.

For nonenveloped hepatitis A virus, Kingsley et al. (2002) suggested that the capsid can remain intact following inactivation by HPP. HPP may denature the capsid proteins essential for host cell attachment to initiate infection (Khadre and

Table 15.1 Pressure response of human foodborne and potentially food-related viruses

Virus	log reduction at *P*/*T*/time	References
Hepatitis A	7 $\log_{10}$; 450 MPa for 5 min at 22 °C	Kingsley et al. (2002)
Aichi virus	*nr*; 600 MPa for 5 min at 21 °C	Kingsley et al. (2004)
Rotavirus	8 $\log_{10;}$ 300 MPa for 2 min at 25 °C	Khadre and Yousef (2002)
Coxsackievirus A9	7 $\log_{10}$; 500 MPa for 5 min at 21 °C	Kingsley et al. (2004)
Coxsackievirus B5	*nr*; 600 MPa for 5 min at 21 °C	Kingsley et al. (2004)
Norovirus (mouse Surrogate MNV-1)	6.9 $\log_{10}$; 450 MPa for 5 min at 20 °C	Kingsley et al. (2007)

nr no reduction

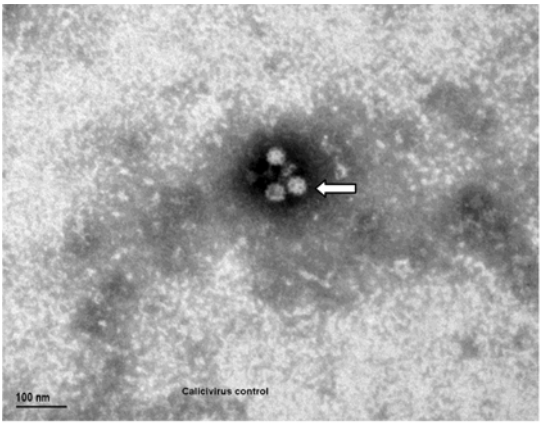
Yousef 2002), but not release RNA from virus particles (Kingsley et al. 2002). RT-PCR analysis of the RNA from noninfectious virus particles still yielded a positive result, indicating the fundamental unreliability of RT-PCR to determine the viability of pressure-treated virus. Kingsley et al. (2002) also showed that increased salinity protected hepatitis A virus when pressure-treated when compared to the virus suspended in an isotonic tissue culture medium. It is unclear whether the increased ionic strength stabilized viral proteins against denaturation from pressure, but nonetheless there are potential implications of this observation regarding the applications of HPP to marine products. Electron micrographs of feline calicivirus (Fig. 15.2) show that exposure to increasing pressure magnitudes causes loss of internalized RNA (along with infectivity) for this virus as evidenced by the hollow capsid shell.

15.4.1 Pressure Inactivation Kinetics

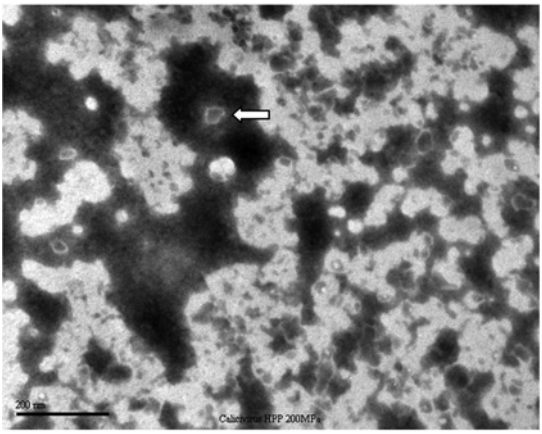
The inactivation of microorganisms by heat and other processing methods has been traditionally assumed to follow first-order kinetics, which results in a linear relationship between the logarithm of the number of survivors and treatment time. Deviations from linearity for virus inactivation have been reported frequently in the literature (Chen et al. 2004, 2005; Kingsley et al. 2006, 2007; Buckow et al. 2008). Typically, pressure inactivation curves are characterized by rapid reduction of titers at the beginning of pressurization followed by a diminishing increase in inactivation, or tailing effect, as treatment time increases. For example, a treatment of 200 MPa for 20 min at room temperature reduced the titer of feline calicivirus by 2.8 logs; however, extending the treatment time to 76 min only increased the level of reduction by an additional 0.9 log (Chen et al. 2005). The tailing effect suggests that to reach a target level of virus inactivation, it might be more desirable to increase treatment pressure so that treatment time can be substantially reduced.

15.4.2 Temperature Effects

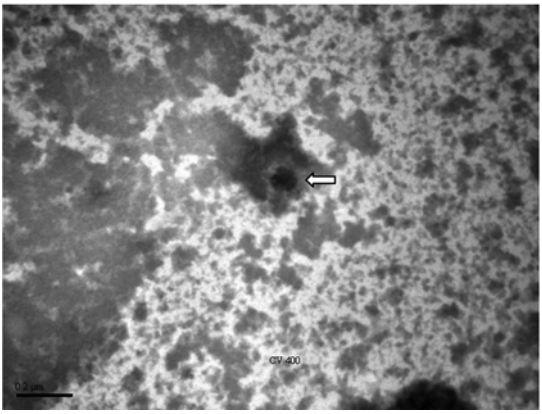
A number of reports have indicated that the dissociation and denaturation of proteins and viruses by pressure is promoted by low temperature (Weber 1993; Foguel et al. 1995; Gaspar et al. 1997; Bonafe et al. 1998; Tian et al. 2000; Kunugi and Tanaka 2002; Chen et al. 2005; Buckow et al. 2008). The explanation for this phenomenon is that low temperatures promotes the exposure of nonpolar side chains to water, and the nonpolar interactions are more affected by pressure because they are more compressible. Figure 15.3 is a schematic representing the different capsid proteins and their amino acid side chains exposed to low temperature and pressure with configuration back to a noninfectious particle after return to original environmental conditions. Besides virion inactivation, this phenomenon carries important



Control, FCV



200 MPa, FCV



400 MPa, FCV

Fig. 15.2 Electron micrograph of the norovirus surrogate, feline calicivirus (FCV; *arrows*), exposed to increasing levels of pressure, showing intact virion to ghost (courtesy of C.M. Stewart, Silliker Laboratories)

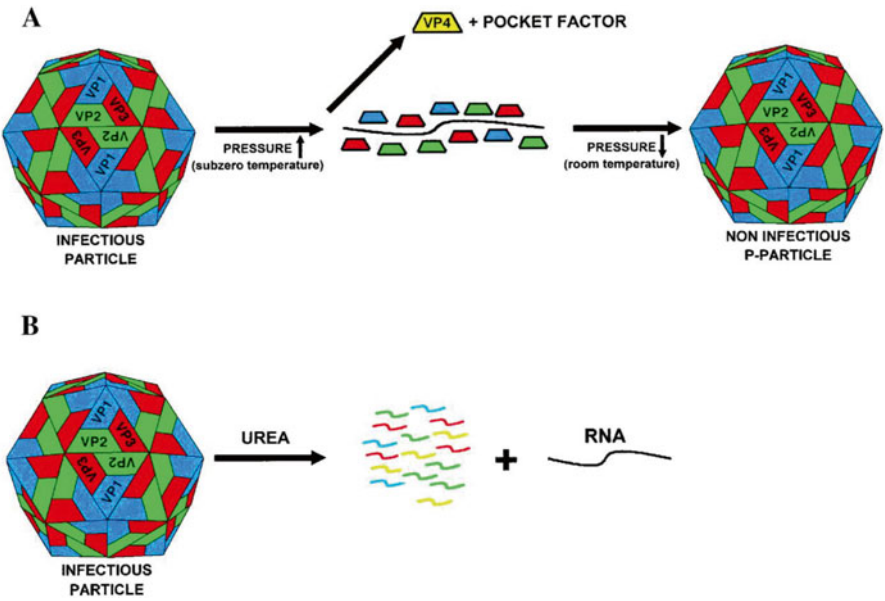


Fig. 15.3 Proposed model for disassembly of picornaviruses by pressure and low temperature (Oliveira et al., 1999). *Panel* **a** depicts disassembly by high pressure and low temperature; *panel* **b** depicts complete disassembly by high concentrations of urea. Pressure and low temperature disrupt the structure of the virus, but the capsid proteins (VP1, VP2, and VP3) remain bound to RNA. Return to atmospheric pressure and ambient temperature results in a noninfective virion that appears intact

implications for pressure-induced immunogens as discussed later in the chapter concerning pressure-treated vaccines. In Fig. 15.3 it is assumed capsid components (i.e., "pocket factor") are lost during exposure to pressure and low temperature.

Oliveira et al. (1999) examined the combined effect of low temperature and pressure on the stability of foot-and-mouth disease virus (FMDV), an animal virus that causes great losses in the production of meat. FMDV was sensitive to pressure and pressurization; a treatment at 240 MPa for 2 h caused a loss of infectivity of 4 log units at room temperature while a 240 MPa treatment at 4 h caused a reduction of 6 log units at −15 °C. The effect of treatment temperature on the inactivation of λ phage under high pressure was more thoroughly studied by Bradley et al. (2000). The phage was suspended in human plasma and pressurized at 275 MPa for 7.5 min at temperatures ranging from −62 °C to 44 °C. Only a slight decrease in titer (<1 log reduction) was observed when λ phage was pressurized at temperatures below −40 °C or above 28 °C; however, a 3- to 4-log decrease in phage titer was found for samples treated at 4 °C.

Pressure inactivation increased by several log units when HPP was performed at refrigeration temperatures as compared to room temperature (Chen et al. 2005; Kingsley et al. 2007). Two studies of hepatitis A virus (HAV) by Kingsley et al. (2006) and Kingsley and Chen (2009) suggested that the low-temperature effect on

pressure inactivation of viruses might require evaluation on a case-by-case basis since pressure inactivation of HAV was actually reduced by colder treatment temperatures. Isbarn et al. (2007) observed that an increase in temperature from 15 to 30 °C significantly reduced the pressure level needed to achieve similar inactivation rates of avian influenza A virus.

15.4.3 Effects of Food Composition

For coliphage suspended in fluid substrates, Chen et al. (2004) found that λ bacteriophage had a much higher resistance to pressure in 2 % fat milk than in a suspension medium (SM) buffer. Pressurization of λ phage in SM buffer at 300 MPa for 300 min, at 350 MPa for 36 min, and 400 MPa for 6 min reduced the titer of λ phage by >6.7 log PFU/ml, while the same processing parameters reduced phage counts in milk by <6.0 log PFU/ml. The results demonstrated that some components of the milk are baroprotective for λ phage.

HAV suspended in different substrates were found to deliver varying levels of pressure inactivation. Dulbecco's modified Eagle medium with 10 % fetal bovine serum, strawberry puree, green onions, and oyster meats have been examined using identical or similar HPP parameters which resulted in different inactivation results (Calci et al. 2005; Kingsley et al. 2002; Kingsley et al. 2005). Murchie et al. (2007) demonstrated that both bovine enterovirus and FCV were more pressure resistant when treated in mussels and oysters than when treated in seawater and culture medium. To elucidate the baroprotective effect of individual food components, Kingsley and Chen (2008) conducted a pressure inactivation study of FCV exposed to various concentrations of NaCl and sucrose. A baroprotective effect was observed for NaCl and sucrose at concentrations up to 12 % and 40 %, respectively. The baroprotective nature of NaCl was also demonstrated for HAV (Kingsley and Chen 2009). In addition to the composition of the substrate, pH also affects the pressure inactivation of viruses. Kingsley and Chen (2008) found that FCV was more resistant to pressure at ≤pH 5.2 over the pH range of 3–8. On the other hand, HAV was found to be more sensitive to pressure at lower pH over the pH range of 3–7 (Kingsley and Chen 2009). In light of these results, it is advisable that substrate composition be taken into account when pressure resistances of viruses are compared.

As discussed above, for inactivation of viruses by HPP, the pressure level, the treatment time, the temperature at which pressure is applied, and the nature of the substrate surrounding the viruses can substantially influence the efficacy of pressure inactivation. Understanding the influence of these factors on pressure inactivation of viruses would be beneficial for formulating HPP products and identifying appropriate processing parameters. For example, the observation that HAV inactivation is enhanced in acidic matrices is information that may be useful for designing product formulations and processing parameters for high-pressure processing of products such as low-pH fruit juices and salsa (Kingsley and Chen 2009).

15.5 Viral Vaccines Produced by Pressure Treatment

High-pressure inactivation of viruses for the enhanced safety of foods has been the focus of considerable research since the 1980s. Strategically inactivated viruses are also important for medical applications, and the potential value of pressure-treated viruses for vaccine preparation has been recognized for some time. Prior to the resurgence in HPP research for inactivation of foodborne microorganisms, there was at least one report that explored the inactivation of a human virus and its subsequent vaccinating potential. Basset et al. (1956) reported pressure inactivation of poliomyelitis virus with retention of some immunogenic potential. Notably, this work was pursued without benefit of the current understanding of mechanistic effects of pressure on viruses that provide deeper insight for the suitability of pressure in the preparation of viral immunogens. Ultimately, a polio vaccine was not developed in 1956 utilizing high pressure but rather by more conventional methods of the time, and the worldwide campaign to eradicate this debilitating disease ensued.

The immunogenicity of pressure-treated viruses was evaluated by their ability to elicit neutralizing antibodies or by protection of animals, either model or natural host, against challenge after vaccination with pressure-treated virus. Bovine rotavirus (Pontes et al. 1997), FMDV (Ishimaru et al. 2004), Mayaro virus (Freitas et al. 2006), and simian rotavirus (Pontes et al. 1997, 2001) were reported to elicit neutralizing antibodies as well as their native viral particles. Mice immunized with pressure-treated coxsackievirus B (Chen et al. 2001) or YFV (Gaspar et al. 2008) survived challenge with native virus, and chickens vaccinated with pressure-treated IBDV had no disease symptoms upon challenge (Tian et al. 1999, 2000).

As previously noted, it is generally assumed that pressure affects noncovalent interactions within macromolecules to such a degree that bacterial cell membranes and enzymes are disrupted. Several studies have examined the changes in viruses as a result of pressure treatment, which renders them nonpathogenic, but with sufficient integrity to elicit an immune response. As determined by microscopy, pressure appears to affect integrity of some viral surfaces including altered morphology of pressure-treated IBDV (Tian et al. 1999, 2000), envelope deterioration of Rift Valley fever virus (Perche et al. 1997), simian rotavirus outer capsid discontinuity (Pontes et al. 1997, 2001), and shell irregularity of VSV (Da Poian et al. 1996; Silva et al. 1992). Pressure affects the capsid of murine norovirus (MNV) such that it was more susceptible to protease degradation (Tang et al. 2010); however, capsid integrity of FMDV was retained after pressure release (Ishimaru et al. 2004). Resistance to RNase treatment after pressure exposure suggests capsid integrity is sufficiently maintained for protection of genomic material of rotavirus (Pontes et al. 1997), HAV (Kingsley et al. 2002), and MNV (Tang et al. 2010), though immunogenicity was not evaluated in the latter two studies.

Surface alterations may disrupt virus attachment or internalization of virus by host cells. Tang et al. (2010) reported that pressure-treated MNV has reduced binding capacity with cellular receptors. Pressure treatment has also been reported to perturb the hemagglutinin protein of simian rotavirus (Pontes et al. 2001).

VSV capsid proteins have been shown to be altered by pressure, and pressure-treated VSV was found capable of attachment to, but not internalization by, host cells (Da Poian et al. 1996; Silva et al. 1992). It has been proposed that pressure may induce changes to some viruses similar to that which would occur during fusion with a cell (Gaspar et al. 2002; Oliveira et al. 1999; Silva et al. 2002), but genomic replication does not occur. While surface changes may be severe enough to disrupt viral attachment, internalization, or subsequent replication, pressure treatment may not be severe enough to disrupt epitopes and their recognition. It has been further proposed antigen accessibility could possibly be enhanced by rearrangement of other molecules (Silva 1993). The impact of pressure-altered tertiary or quaternary structures of surface macromolecules on viral host or tissue tropism is unknown.

A viral vaccine produced by pressure treatment could theoretically have the safety of an inactivated virus with respect to reversion to virulence; however, the inactivated virus may have less potency in eliciting a full and robust immune response as compared to a live attenuated virus. The addition of adjuvants and use of booster injections may be necessary. For viruses propagated outside of a system of attenuation and subsequently inactivated by pressure, the technology may also provide an alternative to approaches that pose allergen risk such as passage through eggs to achieve attenuation.

As a stand-alone technology, pressurization requires energy, appropriate packaging, and a pressure medium, usually water. If pressure can serve as an acceptable alternative to chemical treatment (e.g., formalin or formaldehyde use), the technology would potentially address safety and environmental issues for vaccine preparation; however, high pressure is not always sufficient alone, especially when multiple agents must be inactivated.

Considerable research is needed to advance the understanding of high-pressure processing for vaccine preparation. Among the viruses tested, studies are preliminary and require evaluation in model and natural host systems for efficacy, longevity of response, side effects, and stability of the preparations. An enhanced understanding of the mechanism of pressure inactivation of viruses with retention of immunogenicity would help in the anticipation of possible pitfalls with use of the technology and develop methods to circumvent problems, improve efficiency in preparation, and lead to acceptance of successful commercial vaccines.

15.6 Conclusions and Future Needs

In food manufacturing, viruses are an important factor in food safety if for no other reason the sheer numbers of cases of viral foodborne illness; their importance in food safety cannot be overemphasized. HPP has demonstrated effectiveness as a process to reduce numbers of infective virus particles in treated foods with minimal changes in sensory quality. Some the technological challenges include throughput restrictions of HPP as a batch operation hampering treatment of high-volume commodities and cost considerations. Nonetheless, as HPP continues to mature as a

food processing technology, one can expect HPP to be applied to specialty or niche products where effectiveness for inactivation of specific viruses warrants commercial application. The pressure sensitivities of hepatitis A and norovirus suggest HPP will contribute to their elimination as a food safety threat in pressure-treated products. As knowledge accumulates related to the mechanisms of viral pressure inactivation and pressure process optimization, additional product applications will evolve.

While results have been generally favorable regarding the effectiveness of pressure treatment to produce vaccines, to our knowledge, there has yet to be any commercial production of a pressure-treated vaccine for humans or animals. As previously mentioned, use of pressure would seem superior to use of chemicals, such as formalin, especially for safety and environmental reasons, but apparently enough reluctance exists in the industry to prevent acceptance. Further development to solidify the proof of concept for this technology is required to improve consistency and wider applications of pressure treatment in vaccine production.

Future Needs. Research and development on HPP continues. Sterilization of biomedical products will also maintain interest in HPP for the foreseeable future as a possible thermal alternative for certain products. Although use of HPP as a replacement or enhancer for thermal processing is well-established, HPP can lack the power to ensure complete elimination of microorganisms. Information is needed to predict and optimize viral inactivation by HPP as well as identify additives and co-treatments for judicious enhancement of the effects of pressure.

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Chapter 16
High-Pressure Inactivation of Transmissible Spongiform Encephalopathy Agents (Prions) in Processed Meats

Paul Brown, Franco Cardone, Richard Meyer, and Maurizio Pocchiari

Abstract The epidemic of bovine spongiform encephalopathy (BSE) that began in the late 1980s and has so far been responsible for over 200 human fatalities caused by the ingestion of contaminated beef products stimulated research into disinfection methods appropriate for both animal feed and human food. As it turned out, the risk of infection was almost entirely eliminated by government and industry bans, first on the use of recycled bovine tissues in cattle feed (the source of the epidemic in bovines) and, second, the exclusion of potentially contaminated bovine tissues from beef products consumed by humans. A third approach, had the first two not been successful, was a disinfection method compatible with the aesthetic and nutritional quality of beef products. In this chapter, we review the results of a series of experiments that were undertaken to explore optimal conditions for the commercial use of a combined high-temperature and high-pressure inactivation of BSE prions in processed meats. In repeated experiments, we were unable to detect any significant effect on infectivity from a two-hour exposure to 60 °C at pressures between 400 and 800 MPa. In a further large series of experiments conducted with a variety of machines at different facilities, we found that a 5-min exposure to temperatures and pressures in the range of 120–130 °C and 600–700 MPa yielded, on average, a 100-fold (2 log) reduction in infectivity. Depending on assumptions about the potential infectious load in a given beef product, this level of disinfection, although useful, cannot be guaranteed to eliminate the risk of disease transmission to humans.

Keywords High pressure • Prion • Bovine spongiform encephalopathy • Food safety

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16.1 Introduction

Transmissible spongiform encephalopathy (TSE) parades under a variety of names in humans and animals, but is in reality a single disease caused by a misfolded host protein, also known as a prion, that differs slightly in its molecular structure in the different affected species. It is notoriously resistant to standard methods of disinfection, resulting in recent outbreaks of disease due to unsuspected contamination of human tissues or tissue extracts used for therapeutic purposes, and to the ingestion of contaminated meat products from diseased cattle with bovine spongiform encephalopathy (BSE, or "mad cow" disease).

TSE in humans may take various forms (sporadic, familial, or iatrogenic), but its most common sporadic occurrence is called Creutzfeldt-Jakob disease (CJD), in recognition of the two German neurologists who originally described it nearly a century ago. The form of disease acquired from cattle has been named "variant" Creutzfeldt-Jakob disease (vCJD) because it differs somewhat from other forms of the disease in its clinical, pathological, and molecular biological features.

The names and interrelationships of TSE in humans and animal species are shown in Fig. 16.1. Although in this chapter we will limit discussion to foodstuffs derived from bovines, it should be apparent that any affected species used for food could be cause for concern and stimulate consideration of measures by which contaminated tissues or fluids might be disinfected.

16.2 The Human Diseases

The great majority of cases of Creutzfeldt-Jakob disease—approximately 90 %—occur sporadically, a word that conjures up spontaneity and randomness, but which also (correctly) implies that we do not know why it occurs in 1–2 individuals per year per million population observed in every country under active surveillance. Its age distribution is not unlike that of Alzheimer's disease, having a peak occurrence in the 55–75 years age group, with progressively smaller number of cases found in progressively younger and older individuals. Comparatively few cases of sporadic CJD have been identified in people under the age of 30 and almost none in adolescents, a fact that marks a sharp distinction between the sporadic and variant forms of CJD, and was the first clue to the discovery of vCJD.

Typically, sporadic CJD begins with memory loss, confusion, incoordination, and visual disturbances, alone or in combination. In contrast, the earliest signs of vCJD are usually behavioral abnormalities and sensory symptoms (often, pain in the lower extremities). However, as the illness progresses, the clinical picture in both forms of disease becomes indistinguishable, terminating after a period of several months in a state of full-blown dementia, incoordination, involuntary movements (especially myoclonus), and one or more of a wide assortment of other neurological deficits. Death typically follows from infection or aspiration due to a

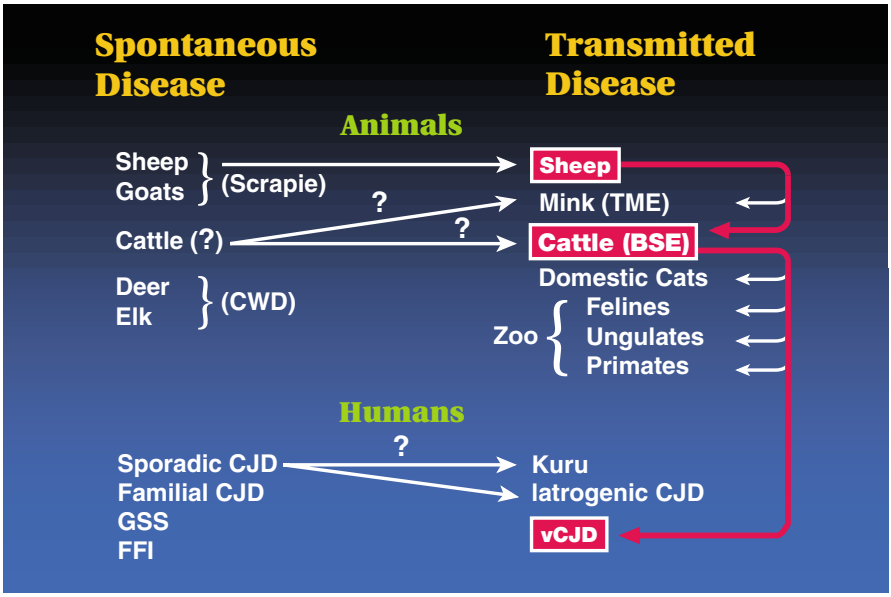

Fig. 16.1 Known or suspected interrelationships between animal and human forms of transmissible spongiform encephalopathy (TSE). Scrapie, first recognized in the mid-eighteenth century in Europe and now global in distribution, may have been responsible for the twentieth century outbreaks of transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle. Sporadic Creutzfeldt-Jakob disease (CJD) is by far the most common form of human disease, but about 10 % of cases are familial, as are most cases of fatal familial insomnia (FFI) and all cases of Gerstmann-Sträussler-Scheinker disease (GSS). Several outbreaks of iatrogenic CJD have occurred in recent decades, together with the only recognized zoonotic form of TSE, variant CJD (vCJD) due to oral exposure to bovine meat products contaminated by BSE

comatose bedridden condition. The mean duration of illness is about 8 months in sporadic CJD and about 14 months in vCJD.

When the brain is examined at autopsy, the most obvious abnormality is a myriad of small vacuoles producing a "spongy" appearance. Neuropathologists also recognize a widespread loss of nerve cells accompanied by an increase in astrocytes, a type of structural and nutritional "support" cell. Like the clinical triad of dementia, incoordination, and myoclonus that is the cornerstone of pre-mortem diagnosis, the triad of spongiosis, neuronal loss, and gliosis forms the basis of neuropathological confirmation. The presence of misfolded protein aggregates, demonstrable by immunohistochemistry or by a distinctive protein banding pattern in immunoblots of brain homogenates, secures the diagnosis. When viewed microscopically, the misfolded protein may appear in aggregates large enough to qualify as amyloid plaques. The morphology of such plaques is an important diagnostic feature of vCJD, in which the plaques are not only far more numerous than in other forms of CJD, but are also surrounded by vacuolar haloes—the so-called "florid" or "daisy" plaque (Fig. 16.2).

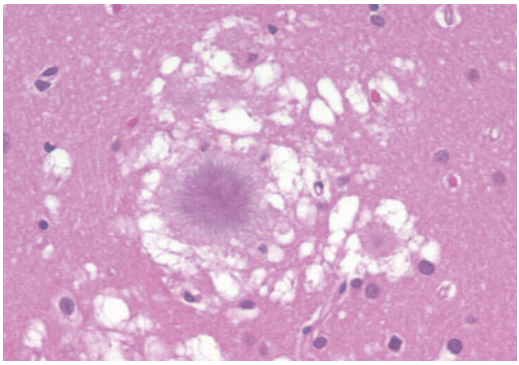

Fig. 16.2 Light microscopic view of the distinctive form of amyloid plaque surrounded by a vacuolar "florette" that is diagnostic of the variant form of CJD (vCJD) due to BSE infection. The plaque consists mainly of misfolded "prion" protein. Magnification 400×. Courtesy of Dr. James Ironside, National CJD Research & Surveillance Unit, University of Edinburgh, Edinburgh, Scotland

16.3 Bovine Spongiform Encephalopathy as the Cause of Variant CJD

In 1986, a new disease of cattle was identified in the United Kingdom as a spongiform encephalopathy that within a few years attained epidemic proportions and also began to spread to other countries, mostly but not entirely limited to Europe (Wells et al. 1987).

The origin of BSE remains arguable, but the means by which it spread is certain: recycling of contaminated carcass meat and bone meal (MBM) as nutritional supplements in cattle feed (Wilesmith et al. 1988). Fortunately, this mechanism was quickly identified and various feed bans and other preventive measures truncated to a few years what might have become a massive and continuing global epidemic.

The possibility of human infection from the ingestion of bovine milk and meat products was also considered at an early stage of the epidemic, but was thought unlikely because of the innate resistance to disease susceptibility across species as different as bovines and humans. Some precautions were nevertheless put in place to minimize human exposure, notably a ban on the use of mechanically recovered meat, which could include residual spinal cord tissue. In the event, humans did turn out to be susceptible to BSE infection, but because human exposure to BSE would have been maximal before its cause was recognized and because of the long (10–15 year) incubation period, the first cases of human disease did not appear until 1994 (Will et al. 1996).

It is estimated that several million cattle were infected with BSE, and as of June 2015, 229 individuals, mostly in the UK, have died of vCJD. The epidemic occurrence of these two diseases renewed interest in the fundamental biology of

TSEs and in the exploration of new methods to reduce or eliminate infectivity in contaminated tissues, including feed and foodstuffs. Although the outbreaks of BSE and vCJD are both winding down and will soon pass into history (Brown et al. 2012), we cannot predict whether some as yet unforeseen conditions may create other episodes of zoonotic TSE, whatever the origin, and the remainder of this chapter will summarize the evolution of attempts at disinfection using a combination of heat and pressure.

16.4 The Original Disinfection Study

The conjunction of our interest in high-pressure inactivation of conventional pathogens, even highly resistant examples like endobacterial spores, and the ongoing epidemics of BSE and vCJD caused by a highly resistant "unconventional" pathogen led us to explore the possibility that high pressure, either with or without accompanying high temperature, might be an effective and practical method to disinfect potentially contaminated human foodstuffs.

Our original study was conducted in a commercial high-pressure processing facility in Sweden (Avure) and yielded some encouraging results (Table 16.1) (Brown et al. 2003). Using a moderately resistant strain of TSE (the 263 K strain of hamster-adapted sheep scrapie), we determined that a single 5-min exposure or multiple 1-min exposures to pressures ≥1000 MPa at temperatures ≥135 °C reduced the level of infectivity by 5–6 log LD_{50} (mean lethal dose) per gram of tissue and,

Table 16.1 Proteinase-resistant protein (PrP^{TSE}) and infectivity reductions in a hot dog paste spiked with 263 K hamster-adapted scrapie brain tissue under various pressure, temperature, and exposure time conditions

Material	Pressure (MPa)[a]	Temperature (°C)	Exposure time (min)[b]	Log_{10} PrP^{TSE} reduction/g	Log_{10} LD_{50} reduction/g
Brain[c]	1200	135	3	3.0	3.5
Brain in hot dog[d]	690	124	3	2.0	2.8
	1000	135	3	3.5	3.8
	1200	135	3	4.5	5.8
	690	121	10	2.0	3.0
	1000	137	10	4.5	5.7
	1200	135	10	4.5	5.6
	1200	142	5	3.5	6.3
	0.2–0.3	121–134	5 (autoclave)	≥3.5	≥6.7

[a]690 MPa = 100,000 psi

[b]Multiple 1-min pulses except for the two 5-min test runs (one high pressure and one autoclave), which were continuous

[c]Input infectivity = 8.8 log_{10} LD_{50}/g

[d]Input infectivity = 9.6 log_{10} LD_{50}/g

not surprisingly, was significantly more effective than exposures to lower pressures and temperatures. None of these conditions, however, produced as great a reduction of infectivity as a 5-min exposure to 132 °C at 0.3 MPa in a steam autoclave. Results were comparable for brain homogenates diluted in saline or hot dog macerates (hot dogs were chosen for ease of blending with brain homogenates). Overall, there was a reasonably good correlation between reductions in infectivity determined by bioassay in hamsters and the level of misfolded proteinase-resistant protein (PrP^{TSE}) determined by Western blot titrations.

We therefore initiated a series of further studies designed to explore the consistency of results in different high-pressure machines and facilities, using a variety of meat products and TSE strains (including a mouse-adapted strain of BSE) under a wide range of pressure and temperature conditions.

16.5 Disinfection Studies

Our follow-up experiments were conducted in a different commercial facility (Avure in Parma, Italy) and in two different academic research settings (University of Cork in Ireland and the Robert Koch Institute in Berlin) (Cardone et al. 2006a, b, 2012; and unpublished data). We first set about exploring the relative contributions of pressure, temperature, and time in reducing the concentration of PrP^{TSE}, a surrogate index of infectivity (Table 16.2). It should be noted that measurements of PrP^{TSE} are useful to avoid the time and expense of performing bioassays that require up to 2 years of surveillance and eventual neurohistochemical examinations of hundreds of animals. It would be wrong, however, to assume that the results always parallel the bioassays, and therefore for critical experiments, it is necessary to verify PrP^{TSE} results with infectivity bioassays, preferably using serial dilution end-point titrations rather than estimates based on dose-response incubation period curves of positive controls.

From our initial PrP^{TSE} results, it was evident that a progressive increase of either pressure at a constant high temperature or temperature at a constant high pressure resulted in a progressive reduction of PrP^{TSE}. At a lower pressure and temperature, an exposure time $\geq$3 min produced maximum effect. Despite the best results from pressures in the 1000 MPa range and temperatures $\geq$135 °C, these conditions are not commercially practical, and we therefore conducted most of our subsequent experiments at 600–690 MPa and temperatures of 120–134 °C, using exposure times of 5 min to allow for any variability in the 3-min threshold for maximum activity.

Three obvious questions needed answers: Do different meat substrates influence the results? Are results reproducible in replicate tests on aliquots of the same sample? And do different strains of TSE show similar results? To answer the first question, we tested beef hot dogs, hamburger meat, tinned beef paté, baby food meat puree, corned beef, and cat food, all spiked with 263 K hamster scrapie and run in the same test at 600 MPa and 130 °C for 5 min. Western blots consistently showed

Table 16.2 Proteinase-resistant protein (PrP^{TSE}) reductions in a hot dog paste spiked with 263 K hamster-adapted scrapie brain tissue when two of three variables were kept constant while one variable was examined

Test conditions			Weight equivalent brain (μg) in sample								
MPa	(°C)	Min	1000	330	100	33	10	3.3	1	0.3	0.1
Untreated control sample					+	+	+	+	+	+	−
Varying pressure series											
600	134	5			+	+	−	−			
800				+	±	−	−				
1000			+	±	−						
1200			−	−	−						
Varying temperature series											
1200	115	5	+	+	+	−					
	118		+	+	+	−					
	125		+	+	−						
	130		+	±	−						
	134		−	−	−						
Varying time series											
600	127	1			+	+	+	+	−		
		2			+	+	+	+	−		
		3			+	+	±	−			
		4			+	+	±	−			
		5			+	+	±	−			

The ± symbol represents a trace positive staining reaction

PrP^{TSE} reductions of 3.5–4.0 logs. To test reproducibility, we ran eight separate tests on aliquots of hot dog paste spiked with 263 K hamster-adapted scrapie under nearly identical conditions (690 MPa and 123–128 °C for 5 min). Western blots showed an identical 2 log reduction of PrP^{TSE} in all eight samples, with a narrow range of 1.5–2.5 log LD_{50}/g in hamster infectivity bioassays.

To test the comparability of different TSE strains, we conducted parallel tests using the same conditions on hot dog paste spiked with the 263 K hamster-adapted scrapie strain, a strain of mouse-adapted vCJD, a strain of mouse-adapted BSE, and a homogenized sample of BSE-affected cattle brain. Western blots showed a 1.5 log reduction of both the hamster scrapie and mouse vCJD strains, a 1.0 log reduction of the mouse BSE strain, and only a 0.6 log reduction of the cattle BSE brain. This was not a surprising result in view of earlier studies showing a greater resistance of BSE than either vCJD or scrapie to autoclaving (Taylor et al. 2002), but was nevertheless disappointing because, for practical reasons, we could not conduct bioassay experiments on the most appropriate strain (cattle BSE) due to the need for cattle as assay animals. We therefore continued to use hot dog pastes spiked with either hamster-adapted scrapie or mouse-adapted vCJD as our "base" conditions, with the understanding that the reduction of PrP^{TSE} (and presumably infectivity) of BSE strains would probably be less than if we were using the native strain of BSE.

Table 16.3 Proteinase-resistant protein (PrP^{TSE}) and infectivity reductions in SRM spiked with mouse-adapted BSE brain under various pressure/ temperature conditions

Material	Pressure[a] (MPa)	Temperature (°C)	Log_{10} PrP^{TSE} reduction	Titer	Log_{10} LD_{50}/g reduction
SRM[b]	Ambient	Ambient	–	7.7	–
	690	121	0.5	5.3	2.4
	690	121	1.0	5.0	2.7
	690	134	0.5	5.4	2.3
	690	134	0.5	5.1	2.6
	690	134	≥2.0	4.6	3.1
	1000	121	1.0	5.5	2.2
	1000	134	≥2.0	4.8	2.9

[a]Continuous 5-min exposures
[b]*SRM* specified risk materials

A substrate not directly consumed by humans particularly interested us–tissues that had been experimentally identified by research laboratories as infectious in cattle with BSE–the so-called specified risk materials (SRM) that were consequently prohibited from being included in processed meat products for human consumption. These materials consist of the entire skull and vertebral column, the small and large intestines, spleen, and tonsil. For our study, bones were removed, tissues were homogenized and spiked with mouse-adapted BSE, and then exposed to various pressure and temperature conditions (Table 16.3). Infectivity was reduced by 2–3 log LD_{50}/g of tissue in all tested samples, with no major differences whether exposed to 690 or 1000 MPa, or to 121 or 134 °C.

One final critical issue needed study: the assumption that is often made in disinfection studies that a multi-log reduction of a high infectivity sample–for example, a 4 log reduction of a 7 log input infectivity sample—will be more than enough to totally inactivate a duplicate sample diluted to a much lower input infectivity of say, 2 logs. However, experience has shown that the total population of infectious particles is heterogeneous and may contain a proportion of particles that survive a given decontamination process, whatever the level of input infectivity. Thus, it is essential to show that a multi-log reduction of a high infectivity sample will in fact fully sterilize a low infectivity sample, and we therefore performed two experiments, one using the 263 K hamster scrapie strain and the other using the mouse BSE strain. Spiked brain samples were diluted with the aim of lowering the infectivity to around 2–3 log LD_{50}/g, but we only succeeded in lowering the input titers to around 5 log LD_{50}/g. In each experiment, bioassays yielded the usual reductions of about 2 log LD_{50}/g.

We then tried a two-stage approach of serially diluting a 263 K hamster scrapie brain spike into the hot dog paste, treating the samples, and then titering serial dilutions of each sample, as shown in Table 16.4. The untreated 10^{-1} brain dilution had a titer of 9.8 log LD_{50}/g and the 10^{-6} brain dilution titered at ≤4.5 log LD_{50}/g (probably about 3.5 logs). Even at this low level of infectivity in a sample that had been subjected to 690 MPa and 124 °C for 5 min, 1 of 9 mice died. Thus, sterility was still not quite achieved.

Table 16.4 Mortality rates in mice inoculated with hot dog paste spiked with different dilutions of 263 K hamster adapted scrapie brain

Log dilution of brain spiked into hot dog	Pressure/temp/time	Log dilution of inoculated sample						Inoculum titer (log LD^{50}/g)[a]
		−5	−6	−7	−8	−9	−10	
−1	Ambient			4/4	2/4	2/4	0/4	9.8
−4	690 MPa/124 °C/5 min	9/9	6/10	2/10	0/10	0/10		7.6
−5	690 MPa/124*C/5 min	5/9	0/10	0/10	0/10			7.4
−6	690 MPa/124 °C/5 min	1/9	0/10	0/10				≤4.5

[a]Titer per ml = observed mortality rate from 50 µl inoculum + log of 20 (1.3)

16.6 Some Conflicting Results

The research group at the Federal Research Centre for Nutrition and Food in Karlsruhe, Germany, has reported large reductions in both PrP^{TSE} and infectivity in 263 K brain homogenate following a 2-h exposure to pressures ≥500 MPa at 60 °C (Garcia et al. 2004; Heindl et al. 2008). In view of our failure to observe any reduction in PrP^{TSE} after exposing 263 K hamster brain homogenate to similar conditions conducted in different laboratories using different machines, we obtained permission to visit the Karlsruhe laboratory to duplicate their experimental conditions in a strictly comparative test.

In a preliminary experiment, three different samples were subjected to 400, 600 or 800 MPa at 60 °C for 2 h. As before, we found no significant reduction of PrP^{TSE} in our samples. We therefore undertook a second experiment, in which one of our samples and one sample from the Karlsruhe laboratory were placed in the same pressure vessel and exposed to 600 or 800 MPa at 60 °C for 2 h. The machine (U101) was a laboratory scale prototype made of an insulated pressurization cylinder with a water thermostat temperature control. The starting pressure and temperature settings were held until the end of the interval of treatment with virtually no variation. The results of this rigorous comparison including the Karlsruhe sample, shown in Table 16.5, were similar to the preliminary experiment and to what we had observed in both of our previous experiments.

In view of the earlier Karlsruhe observations of a parallel reduction in PrP^{TSE} and infectivity, the absence of a PrP^{TSE} reduction in this study dampened enthusiasm for conducting bioassays on the tested specimens. We would also point out that the Western blot method used in Karlsruhe was less sensitive than ours and that their infectivity values were estimates based on an incubation curve published by Prusiner in 1982, rather than their own “in-house” curve or, even better, end-point dilution infectivity measurements. Although we remain without a proven explanation for the disparity between the Karlsruhe results and those observed in this comparative study, a total of four failures to confirm the Karlsruhe results (including parallel tests on a Karlsruhe sample) convince us that our own results are more likely to be correct.

Table 16.5 Proteinase-resistant protein (PrP^{TSE}) reductions in 263 K hamster-adapted scrapie brain homogenate subjected to high pressure at pasteurizing temperatures for 2 h

	Test conditions			Weight equivalent brain (μg) in sample					Log reduction
Sample[a]	MPa	(°C)	Time (hrs)	33	10	3.3	1	0.33	PrP^{TSE}
ISS	Ambient	60	2		+	+	±	–	
ISS	600	60	2	+	+	±	–		0.5
FRCN	600	60	2	+	+	±	–		0.5
ISS	800	60	2	+	±	–			1.0
FRCN	800	60	2	+	–	–			1.5

The ± symbol represents a trace positive staining reaction
[a]*ISS* Istituto Superiore di Sanitå, *FRCN* Federal Research Center on Nutrition

16.7 Sensory, Nutritional, and Cost Considerations

No method of disinfection is of any practical use if it results in an unappetizing product, and our own (non-blinded) taste tests of autoclaved meat products confirmed our expectation that autoclaved meat is indeed unappetizing. For this reason we undertook a series of sensory tests of several meat products that were cooked in standard fashion and then either subjected to three 1-min pulse of 690 MPa at 130 °C or left untreated. Each test was conducted in blinded fashion on 47–52 subjects, who were asked to evaluate appearance, aroma, texture, and flavor. Scoring ranged from 1 (least liked) to 9 (most liked).

Two tests were conducted: in the first test, the overall scores of pressure-treated roast beef, chuck roast, and steak equaled or exceeded the scores given to the untreated samples, usually because of increased tenderness and roasted flavor. However, the untreated hamburgers and hot dogs were much preferred to the pressurized samples due to drying of the hamburger and "mushiness" of the hot dogs. These were therefore reformulated for a second test. Hamburger meat was mixed with a USDA-approved gum blend (carrageenan) and seasoned salt. Hot dogs were made with a leaner meat blend (7 % fat), using a coarse grind (1/2 in. plate) and drying to a lower moisture content by holding in the smokehouse until the internal temperature reached 160 °F (71 °C). In addition, 5 % dry potato buds were added to soak up free moisture and simulate fat texture. The sensory results of this repeat test are summarized in Table 16.6. For every product, the appreciation of pressure-treated meat either equaled or exceeded that of the untreated control.

A number of articles have been published in the last decade about nutritional changes in foods subjected to high-pressure processing, including several recent reviews (Bajovic et al. 2012; Considine et al. 2008; Gupta and Balasubramaniam 2012; Simonin et al. 2012), but no information exists for the pressure/temperature conditions used in our experiments and, in particular, high pressure at comparatively high temperatures. The principal nutritional value of meat resides in its protein component, and because pressure acts chiefly on comparatively weak chemical

Table 16.6 Sensory testing of various meat products exposed to high pressure and temperature

	Preference		Mean score		
Meat product	Pressure	Control	Pressure	Control	Statistical significance
Sausage	22	25	5.28	5.68	No difference
Hamburger patties	38	12	6.38	5.80	0.01
Sliced roast beef	26	23	6.39	6.69	No difference
Chuck roast	40	12	6.94	5.81	0.01
Ribeye steak	34	17	6.82	6.35	0.05

Notes: tests were conducted in blinded fashion. Rating scale from 1 to 9 (dislike extremely to like extremely). All statisically significant differences favored the pressure treated product

bonds (van der Waals forces, electrostatic interactions, and hydrogen bridges) without breaking covalent bonds, the overall effect on the energy potential of proteins and amino acids is minimal. However, the effect of even short exposures to heat in the range of 120–132 °C would, in contrast, probably result in irreversible protein denaturation, but this would not destroy the caloric value of the residual amino acids and is in any case lower than the temperature at which normal cooking occurs.

The current imperfect state of knowledge about high pressure and nutrition was summed up in a recent review that concluded with a plea for more systematic experimental designs, a more rigorous approach in making comparisons between studies, and a continuing need to examine the changes in nutrient bioavailability that occur in high-pressure processing (Gupta and Balasubramaniam 2012).

Industrial-size high-pressure vessels are available from several manufacturers: Avure Technologies, Elmhurst Research, Inc., NC Hyperbaric, and Uhde GmbH, and over 300 industrial units are presently in use worldwide (personal communication from Avure and NC Hyperbaric, the two globally dominant manufacturers). The added cost of high-pressure processing depends on a number of factors, including vessel price and depreciation, magnitude of use, processing batch size, level and holding time of pressure exposure, and plant operating conditions. The most up-to-date cost estimates for a variety of processing conditions have recently been published by Mùjica-Paz et al. (2011); their estimates for moderate to intensive use under the pressure, time, and temperature conditions most closely resembling our own experimental studies are shown in Table 16.7 and range from about 10 cents per pound for intensive production to 30 cents per pound for moderate production.

16.8 The All-Important Question of Risk

Is high-pressure reduction of prion infectivity adequate to eliminate the risk of becoming infected by BSE contamination of processed meat products? The answer depends on the amount of infectivity consumed, which in turn depends on a number of assumptions that may or may not accurately reflect reality. Mechanically recovered meat (MRM), the likely vehicle of BSE transmission to humans in processed

Table 16.7 High-pressure processing cost estimates for a range of yearly product outputs

	Moderate use		Intensive use
Yearly production (tons)	528	2880	8640
Vessel volume (liters)	55	300	300
Cost estimates			
Depreciation (5-year)	0.21	0.11	0.04
Wear of parts	0.04	0.02	0.03
Labor	0.06	0.03	0.03
Total cost (US$ per lb)	0.31	0.16	0.10

Processing conditions: 600 MPa, 3 min, 7.5 min total cycle time, 8 cycles/h, 60 % vessel filling ratio. Modified from Table 1 in (Mùjica-Paz et al. 2011)

meat products, is presently prohibited from human consumption, but for purposes of risk calculation, we will assume that MRM can still be used in such products. The arithmetic was set forth in our original paper (Brown et al. 2003), in which the calculation for infectivity in a contaminated hot dog was based on (1) the herd incidence of BSE, (2) the estimated amount of infectivity in the MRM constituent tissues, (3) the proportion of MRM in a processed meat product, and (4) the amount of product typically consumed in a single meal. The resulting estimated range of infectivity was 0.5–5 intracerebral LD_{50} per g, or 30–300 LD_{50} in an entire 60-g hot dog. A high-pressure processing reduction of infectivity of between 2 and 3 logs (100–1000 LD_{50}) would consequently not provide an adequate margin of safety.

It may be asked why, with a potential ingested dose as high as 300 LD_{50}, there have been only 174 cases of disease in a widely exposed UK population (estimated to run into the millions). The answer probably lies in the likelihood of a bovine to human species barrier to infection and of a highly heterogeneous distribution of infectivity in MRM so that only the occasional batch of hot dogs or other processed meat products was actually contaminated. Moreover, the oral route of infection in humans is less efficient than direct intracerebral inoculation. In cattle, the transmission of BSE requires about 300,000 times more brain when given orally than when inoculated intracerebrally (Wells et al. 2007). If human exposure risk followed suit, as little as a 1 log LD_{50}/g infectivity reduction, if consistently reproducible, would eliminate risk altogether.

In point of fact, this discussion has been rendered superfluous by elimination of the contaminating vehicle (MRM) from the human diet, and the near disappearance of BSE from the world bovine population, but it does underscore the need for a more effective level of infectivity reduction than we were able to achieve. In particular, a great deal of more work is needed to ensure the reproducibility of results using various meats and meat products in a variety of commercially available machines. Should a new source of contamination appear in the future—for example, from the expanding prevalence of chronic wasting disease in deer and elk, or some other yet unrecognized form of animal TSE—the method as presently practiced could still accomplish a substantial reduction in the risk of food-borne prion disease, a not inconsiderable benefit for products that defy all other processing methods to make them both safe and palatable for human consumption.

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Chapter 17
Mathematical Models Based on Transition State Theory for the Microbial Safety of Foods by High Pressure

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Abstract Prior to the development of transition state theory, the Arrhenius equation was the principal relationship used in describing the temperature dependence of chemical reaction rates. Research into determining the theoretical basis for the Arrhenius parameters A (pre-exponential factor) and E_a (activation energy) led to the development of transition state theory and the Eyring equation, whose central postulate is a hypothetical *transient state* called the *activated complex* that forms through interactions between reactants before they can become products during the process of a chemical reaction. It is from the perspective of transition state theory that we develop two secondary models to reflect the effects of temperature and of high pressure on microbial inactivation by the emerging nonthermal technology of high pressure processing (HPP), and we designate these as transition state (TS) models TST and TSP, respectively. These secondary models are applied to data obtained with two primary models, the enhanced quasi-chemical kinetics (EQCK) differential equation model and the Weibull distribution empirical model, that were used to evaluate nonlinear inactivation kinetics for baro-resistant *Listeria monocytogenes* in a surrogate protein food system by HPP for various combinations of pressure (207–414 MPa) and temperature (20–50 °C). The mathematical relationships of TST and TSP involve primarily the unique model parameter called "processing time parameter" (t_p), which was developed to evaluate inactivation kinetics data showing tailing. These detailed secondary models, as applied to the parameters of the EQCK and Weibull primary models, have important ramifications for ensuring food safety and the shelf life of food products and support the growing uses of HPP for the safe preservation of foodstuffs.

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Keywords Transition state theory • Kinetics • Enhanced quasi-chemical kinetics model • Food safety

17.1 Introduction

Predictive microbiology commonly employs kinetics models in the form of mathematical equations to describe and predict conditions that support the growth of microorganisms in foods to ensure their safe shelf life. The logistic function, Weibull distribution model, and Gompertz equation are empirical functions that have been used successfully to model microbial growth kinetics in actual foods or representative nutritive media, such as surrogate foods or laboratory broth. Some of these models can be modified and inverted to describe nonlinear microbial inactivation kinetics occurring when a kill-step, such as thermal treatment, is applied to the food product of interest. The log-linear equation is often invoked in circumstances when linear inactivation kinetics are observed. As part of research on decontamination technologies, we reported (Doona et al. 2012) the application of the enhanced quasi-chemical kinetics (EQCK) model to characterize nonlinear inactivation kinetics showing tailing obtained with the emerging nonthermal technology of high pressure processing (HPP- see Doona and Feeherry 2007). In this chapter, we apply transition state theory to develop novel temperature-dependent and pressure-dependent secondary models that are tested with results from the EQCK and Weibull primary models for the inactivation kinetics of *Listeria monocytogenes* inactivation by HPP.

HPP is an alternative food processing technology that ensures the safety of foods from microbial pathogens without causing the extent of chemical or physical changes to foods associated with conventional thermal methods, and thereby preventing the unwanted deterioration of food qualities such as flavor, color, and texture (Lau and Turek 2007). In appropriate conditions of high pressure and temperature, HPP safely eliminates *L. monocytogenes* from foods without compromising food quality. Given the low infective dose of *L. monocytogenes* (<100 total organism in food-see FDA-DHHS 2011) and the seriousness of listeriosis infections (500 estimated fatalities yearly), suitable interventions to control *L. monocytogenes* from contaminating a broad range of foods are essential. The commercial uses of HPP to eliminate *L. monocytogenes* from ready-to-eat meats and other foods provide tremendous benefits in ensuring consumer safety while retaining food quality.

The original quasi-chemical model is based on differential equations and fits growth-death dynamics of microbial populations in foods controlled by "hurdle technology" (Taub et al. 2003; Ross et al. 2005). The quasi-chemical kinetics model consists of four (4) quasi-chemical reaction steps with associated chemical rate equations. The model emulates microbiological dynamics that include the exponential growth phase of a living culture as modeled by an autocatalytic reaction that displays the same concentration-time profile as a dividing cell population, and cell death that is accelerated by an antagonistic metabolite as a mimic to extracellular lethality factors involved in quorum sensing. The quasi-chemical model fits a variety of observed kinetics patterns, and it has been used to evaluate the nonlinear inactivation kinetics of

Escherichia coli by HPP (Doona et al. 2005, 2007) that showed protracted lag times but no observable tailing (defined as the occurrence of a persistent subpopulation of survivors). A novel secondary model called the "equivalence chart" defined equivalent sets of HPP conditions that effectuated a 6-log reduction of *E. coli*. Heat-sensitive matrices could be treated at lower temperatures and higher pressures, and pressure-sensitive materials could be treated at lower pressures and higher temperatures and still ensure microbiological safety in all cases.

The enhanced quasi-chemical kinetics (EQCK) model we recently reported (Doona et al. 2012) was developed to evaluate nonlinear inactivation kinetics obtained with HPP showing tailing, particularly as it pertained to the inactivation the inactivation of a baro-resistant strain of *L. monocytogenes* (Feeherry et al. 2005; Tay et al. 2003). The EQCK model derives from a set of six (6) chemical reaction steps and their associated mathematical rate equations, with the additional steps included to provide the capability of modeling tailing in the observed dynamics. We also introduced the model parameter called the "processing time parameter" (designated t_p) to estimate the time required to reduce a microorganism population by 6-logs for a given set of HPP conditions, particularly in instances when tailing occurs. The temperature and pressure dependences of the model parameter t_p were evaluated using the Arrhenius activation energy model and the partial molar activation volume model, respectively. Overall, these results provided a basis for modeling the inactivation kinetics of resistant bacterial spores such as *Clostridium botulinum* and *Bacillus anthracis*, the causative agent of anthrax (Cléry-Barraud et al. 2004). Bacterial spore populations are heterogeneous, comprising in some cases identifiable "superdormant" subpopulations (Ghosh and Setlow 2009, 2010; Ghosh et al. 2009; Wei et al. 2010) that can appear as tailing in inactivation kinetics.

Herein we examine in detail novel secondary models for microbial inactivation in foods by HPP from a fundamental kinetics perspective developed for chemical reactions, that is, from the view of "transition state theory," also known as "activated complex theory." In studies of chemical reaction rates, it had been noted by the early or mid-nineteenth century that increasing temperature increased a reaction's rate constant exponentially. This influence of temperature was explained by Arrhenius, who postulated that only molecules with energy above a certain threshold were able to react. In the early twentieth century, the Arrhenius approach was formulated more rigorously by postulating a critical molecular configuration of reactants with a high probability that it transforms into products. This critical configuration was treated as a molecule called the "activated complex," which could then be described with molecular statistical and quantum mechanical tools (Glasstone et al. 1941). This more accurate depiction of the temperature dependence of rate constants thus developed led to the formation of the renowned Eyring equation. As an example, consider the bimolecular, elementary, single-step reaction of hydrogen atom abstraction in the gas-phase reaction of hydrogen and deuterium ($H + HD = H_2 + D$) that would involve forming a tri-atomic activated complex in the transition state with two possible outcomes: a return to the reactants or a rearrangement of bonds to form the products. Today, with the advent of powerful and rapid-acting computers, the theory has been successfully applied to a large variety of multi-atom reactions,

including enzyme-catalyzed reactions. Applying this theory to reactions of complex stoichiometry in solution depends on knowing the slowest or rate-determining step in a sequence of steps or complex reaction network, with modifications of the activated complex to include interactions with solvent molecules.

Microorganisms present more complexity than reaction networks, because of the myriad chemical and biochemical reactions governing the physiological processes of metabolizing cells and the need for retaining morphology and structure to function. The successful application of an activated complex model like the Arrhenius equation to nonchemical, nonmolecular systems such as bacteria, spores, and fungi (Braverman and Berk 1976) may have some limitations but may also imply that the effects of food processing methods that destroy microorganisms might exert their influence predominantly on a single, rate-determining step, such as an enzymatically catalyzed reaction. Moreover, intensive variables other than temperature, such as pressure, can also profoundly affect the microbiological safety (and other intrinsic properties) of foods when using the nonthermal technology of HPP. We therefore prefer to designate two secondary models that treat the variables of HPP (temperature and pressure) as separable in the context of transition state theory: these transition state models are TST and TSP, respectively.

17.2 Transition State Theory: Temperature Dependence

Transition state theory posits equilibrium between the activated complex and reactants leading to the fundamental equation:

$$k = \frac{k_B T}{h} e^{-\Delta G^* / RT}$$

where k is a rate constant (units determined by the order of the reaction it is applied to), k_B is Boltzmann's constant, T is absolute temperature, h is Planck's constant, R is the universal gas constant, and ΔG^* is the free energy of formation of the activated complex. Inserting the classical thermodynamic definition of the free energy of formation of the activated complex in terms of enthalpy of activation, ΔH^*, and entropy of activation, ΔS^*, and taking the natural logarithm of k leads to the TS expression

$$\ln(k) = \ln\left(\frac{k_B}{h}\right) + \ln(T) + \frac{\Delta S^*}{R} - \frac{\Delta H^*}{RT}$$

The Arrhenius equation is an empirical (experimental) relationship with its traditional form written as

$$k = A e^{-E_a / RT}$$

where E_a is the activation energy and the pre-exponential A-term is constant. The relationship between $\ln(k)$ and $1/T$ has the form

$$\ln(k) = \text{constant} - \frac{E_a}{RT}$$

By differentiating the TS and Arrhenius equations for $\ln(k)$ with respect to T and equating, we obtain

$$E_a = RT + \Delta H^*$$

In determining activation energies for thermally processed foods, a range of temperatures is employed. As a midpoint of such a temperature range, consider the temperature 70 °C, which is close to the traditional thermal pasteurization temperature of milk (71.7 °C). At the corresponding absolute temperature (343.15 K), the RT term has the value 2.85 kJ mol^{-1}, whereas E_a values for thermal inactivation of microbes are typically >50 kJ mol^{-1} (Ross 1993, exemplified later). Thus, with the approximation that $\Delta H^* >> RT$, the value E_a is primarily due to ΔH^*, with the RT contribution being too small to detect in thermal food processing experiments. Therefore, the experimental Arrhenius equation is an approximation of the TS equation when

$$\ln\left(\frac{k_B}{h}\right) + \ln(T) + \frac{\Delta S^*}{R} \cong \text{constant} = \ln(A)$$

17.2.1 TST: The Temperature-Dependent Model

The Arrhenius equation in this form, where the pre-exponential A-term is constant, is an approximation of the more complete TS pre-exponential term. In TS theory, the pre-exponential term is often referred to as a "collision number" or "frequency factor." These designations have little or no meaning in the context of food processing to inactivate microorganisms, and we prefer to rearrange the Arrhenius equation into a form more suitable for meaningful analysis of microorganism inactivation dynamics. Specifically, we express the Arrhenius rate constant expression in terms of a reference rate constant, k_r, where the subscript "r" refers to a reference temperature (e.g., T_r=273.15 K), and a dimensionless activation energy term, U_a (Ross 1993). To accomplish this transformation, we define the dimensionless temperature function x as $x \equiv T_r/T - 1$, which can be rewritten as $T_r/T = 1 + x$. Multiplying the exponential term in the Arrhenius equation by T_r/T_r and rearranging yields

$$k = k_r e^{-U_a x}$$

where

$$k_r = Ae^{-U_a} \text{ and } U_a = \frac{E_a}{RT_r}$$

This expression for k, as written above, is designated TST model for thermal inactivation of microorganisms. This expression can be used in conjunction with the parameter called processing time (t_p), which is the time required for certain HPP hold-time conditions to effectuate a 6-log reduction of target organisms in a foodstuff or representative nutritive media. Since t_p (units of *min*) = $1/k$ (in units of min^{-1}) and $t_{pr} = 1/k_r$, the values of t_p can be used to determine the corresponding dimensionless activation energy (U_a) through the TST model as a graph of $\ln(t_p)$ against x according to the following equation:

$$\ln(tp) = \ln(tp_r) + U_a x$$

17.3 Transition State Theory: Pressure Dependence

The starting point for development of the temperature dependence of the observed rate constant was the TST model with a temperature-independent pre-exponential term. To develop the pressure-dependent model designated TSP, we begin by differentiating the TS rate constant expression with respect to pressure (P) at constant temperature (T). This process yields

$$\frac{d\left[\ln(k)\right]}{dP} = -\frac{1}{RT}\left(\frac{dG^*}{dP}\right) = -\frac{1}{RT}\Delta V^*$$

where R and T have their previously assigned meanings and ΔV^* is the difference in partial molar volume between the activated complex and reactants. Integrating this equation between the upper limit $P = P$, $k = k$ and the lower limit $P = P_0 = 0$, $k = k_0$ gives (Jordan 1998)

$$k = k_0 e^{-\left(\frac{\Delta V^*}{RT}\right)P}$$

This TSP model rate constant equation predicts a linearly dependent graph of $\ln(k)$ against P. When $\Delta V^* > 0$, that is, when the partial molar volume of the activated complex is greater than that of the reactants, the rate constant should decrease linearly as pressure increases. Conversely, when $\Delta V^* < 0$, that is, when the partial molar volume of the activated complex is less than that of the reactants, the rate constant should increase linearly as pressure increases. However, in our experience with *L. monocytogenes* inactivation by HPP, and that of many investigators working with molecular systems, plots of $\ln(k)$ against P are often curved, indicating that pressure

exerts more than a thermodynamic, Le Chatelier-type perturbation of ΔG^*. When dealing with the pressure dependence of chemical reaction rate constants, this curvature is explained by postulating a compressible activation complex (Van Eldik et al. 1989). Accordingly, the more complete TSP model expression for the partial molar volume of activation contains a compressibility term, as follows:

$$\Delta V^* = \Delta V_c^* - \Delta \beta_c^* P$$

In this equation ΔV_c^* is the partial molar volume of activation in the presence of compressibility and $\Delta \beta_c^*$ is the partial molar compressibility of activation.

Insertion of the pressure-dependent partial molar volume of activation into the TS rate constant expression and integrating over the same k and P limits as before yields an expression for the TSP pressure-dependent rate constant model when $\Delta \beta_c^* \neq 0$.

$$k = k_0 e^{-\left[\left(\frac{\Delta V_c^*}{RT}\right)P - \left(\frac{\Delta \beta_c^*}{2RT}\right)P^2\right]}$$

17.3.1 TSP: The Pressure-Dependent Model

In a treatment analogous to that for the TST model, the TSP model can be expressed in terms of a reference rate constant and dimensionless activation parameters. Inserting the definition $y \equiv P/P_r - 1$ and $P/P_r = 1 + y$, where $P_r = 6.984757$ MPa, into the TSP pressure-dependent rate constant model when $\Delta \beta_c^* \neq 0$ yields

$$k = Ce^{-C_1 y} e^{C_2 y^2}$$

Taking the natural logarithm of this expression and utilizing the relation $\ln(t_p) = -\ln(k)$ yields

$$\ln(tp) = C_0 + C_1 y - C_2 y^2$$

From the rate constant equation above and using P_r and dimensionless coefficients, let $V_r = \Delta V_c^* P_r/RT$ and $V_{cr} = \Delta \beta_c^* P_r^2/2RT$, so that $C = k_0 exp[-(V_r - V_{cr})] = k_r$, $C_1 = V_r - 2V_{cr}$, and $C_2 = -V_{cr}$. And from the natural logarithm expression, we obtain $C_0 = \ln(1/C) = \ln(t_{pr})$.

17.4 Materials and Methods

The baro-resistant strain *L. monocytogenes* OSY-8578 was obtained courtesy of the Ohio State University. Doona et al. (2012) previously reported the details of the methods for maintaining the culture, preparing the inoculum using a $Klett_{54}$ counter

(Klett-Summerson Colorimeter, Thomas Scientific, Swedesboro, NJ, USA), and inoculating whey protein samples (Whey Protein Concentrate 7504, Calpro Ingredients, Corona, CA, USA) with about 10^8 CFU/ml inside sterile Stomacher bags (Model 400, Seward, Ltd, London, England, UK), and then sealing the Stomacher bags (Röschermatic Vacuum Packaging Machine, Reiser & Co., Canton, MA, USA) for HPP experiments using a 2-l high-pressure unit (Engineered Pressure Systems, Inc., Haverhill, MA, USA). Samples were treated with HPP under approximately isobaric and isothermal conditions over the ranges of 207 – 414 MPa and 20–50 °C at appropriate time intervals to obtain detailed kinetics. Survivors were diluted in Butterfield's phosphate buffer (pH 7), plated on Tryptose Agar, and enumerated after 48–96 h incubation at 35 °C using a New Brunswick Colony Counter (Bio-technologies, Salem, NJ USA). Kinetics data analysis was carried out with the model written in MATLAB software with built-in differential equation problem solvers and a nonlinear regression analysis toolbox.

The present set of HPP conditions was determined to be the range most suitable for capturing detailed inactivation kinetics. One reason was that the inactivation of several types of microorganisms (*L. monocytogenes*, *E. coli*, and *Bacillus stearothermophilus* and *Bacillus amyloliquefaciens*) tended to occur more slowly in whey protein compared to other surrogate food matrices (e.g., wheat and potato starches). For example, in HPP conditions of 621 MPa and 25 °C and aqueous wheat starch suspensions as the surrogate food matrix, there were no surviving *L. monocytogenes* within the equipment come-up time (ca. 1.5–2.0 min). Table 17.1 shows the %survivors of *L. monocytogenes* OSY-8578 in the whey protein surrogate food system after the come-up time of the high-pressure equipment at lower values of high pressure (276–414 MPa). The general trend observed in Table 17.1 is that increased inactivation occurs during the equipment come-up with increasing severity of the HPP conditions (i.e., increasing T and P). Additionally, the microbial inactivation dynamics were not dependent on the gelation of the whey protein matrix. Table 17.2 shows the effects of HPP treatments on the whey protein matrix, with the effects of increasing the HPP conditions (T and/or P) tending to increase the rate of protein denaturation commensurately.

The effects of temperature at 50 °C with ambient pressure on *L. monocytogenes* and *E. coli* are shown in Fig. 17.1 (plotted as $\log(N/N_o)$ vs. time, t, in min). Exposure

Table 17.1 %Survivors at HPP "come-up" for *L. monocytogenes* OSY-8578 with T in units of °C and P in units of MPa

	T			
P	*20*	*30*	*40*	*50*
207	122.1	90.3	107.4	79.2
276	91.7	82.9	92	21.8
345	87.3	104.1	49.7	19.9
414	141.1	84.9	44.3	1.1

Table 17.2 Denaturation (gelation) of 50 % aqueous whey protein (a_w=0.988, pH 6.13) at 50 °C (time *t* in units of min, *P* in units of MPa)

	t			
P	*0*	*1.5*	*7.5*	*50*
207	No gel	No gel	No gel	Soft gel
241	No gel	No gel	Soft gel	Gel
276	No gel	Soft gel	Gel	Gel
310	Soft gel	Gel	Gel	Gel
345	Gel	Gel	Gel	Gel
414	Gel	Gel	Gel	Gel

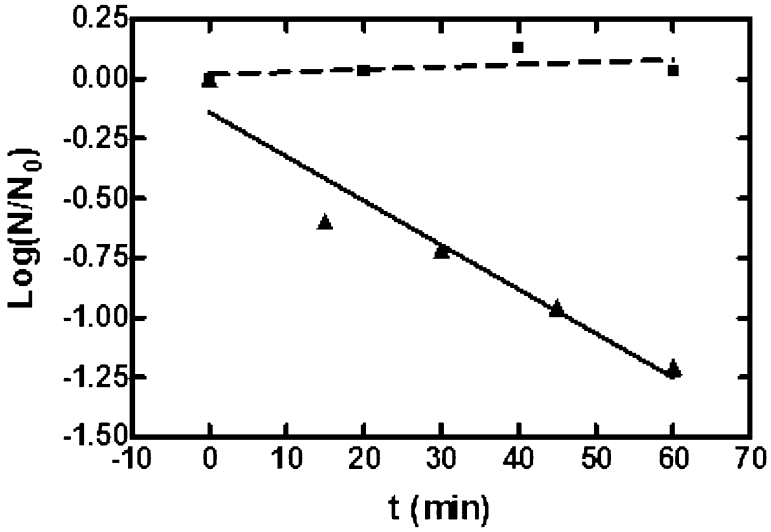

Fig. 17.1 Survival of *E. coli* and *L. monocytogenes* at 50 °C without high pressure, and plotted as Log(survivors) vs. *t* (time): (*filled square*) *E. coli* experimental; (*dashed line*) *E. coli* calculated; (*filled triangle*) *L. monocytogenes* experimental; (*solid line*) *L. monocytogenes* calculated

to these conditions for 60 min produced a 1.21-log reduction of *L. monocytogenes* and no inactivation of *E. coli*. Interestingly, the HPP inactivation kinetics of *E. coli* often show protracted lag times without showing tailing (Doona et al. 2007), and the HPP inactivation kinetics of *L. monocytogenes* show no discernible lag and sometimes tailing (Doona et al. 2012). For *L. monocytogenes* with HPP conditions of 207 MPa and 50 °C, the Weibull model estimated t_p=65 min, whereas the thermal inactivation rate estimated a value of $t_p \approx 324$ min. Thus, both temperature and high pressure exert lethal effects individually and in concert with respect to these microorganisms.

17.5 Primary Models

Doona et al. (2012) have reported previously that the EQCK model and the Weibull models are effective primary models for evaluating the HPP inactivation kinetics of *L. monocytogenes*, that in some cases show tailing, and for estimating suitable values of t_p. The estimates of t_p from these fittings are compiled in Table 17.3. The qualities of fits obtained separately with the EQCK and Weibull primary models have to some extent already been compared, showing that the Weibull model, though statistically adequate, cannot account for all of the nonlinearities observed in the microbial dynamics as does the EQCK model. These differences in modeling behaviors result from basic differences in the mathematical properties of the two models.

17.5.1 The Enhanced Quasi-Chemical Kinetics (EQCK) Model

The EQCK model is a six-step differential equation model that is semi-mechanistic (Doona et al. 2012). The six (6) quasi-chemical steps and corresponding set of ordinary differential equations comprising the EQCK model are listed in Table 17.4. The symbols M, M*, E, BR, and D represent metabolizing cells, multiplying cells, antagonistic metabolite (analog of the extracellular lethality factor secreted by stressed *E. coli* cells—see Kolodkin-Gal et al. 2007), the baro-resistant subpopulation of cells, and dead cells, respectively. This full version of the EQCK model contains the

Table 17.3 Estimated values t_p obtained with the EQCK or Weibull models as functions of P and T for the HPP inactivation of *L. monocytogenes* (Doona et al. 2012)

P (MPa)	T (°C)	t_p (EQCK)	t_p (Weibull)
414	50	4	5
345		12	12
276		31	32
207		65	67
414	40	11	14
345		24	25
276		64	64
207		354	279
414	30	42	26
345		48	46
276		125	117
207		543	446
414	20	32	31
345		74	68
276		285	446
207		718	584

Table 17.4 The rate equations and differential equations for EQCK and model A (Doona et al. 2012)

EQCK	*Rate equations*	Model A
$M \rightarrow M^*$, k_1		$M \rightarrow M^*$, k_1
$M^* \rightarrow 2\,M^* + E$, k_2		$M \rightarrow BR$, k_2
$M^* + E \rightarrow D$, k_3		$M^* \rightarrow D$, k_3
$M^* \rightarrow D$, k_4		$BR \rightarrow D$, k_4
$M \rightarrow BR$, k_5		
$BR \rightarrow D$, k_6		
	Differential equations	
$dM/dt = -(k_1 + k_5)M$		$dM/dt = -(k_1 + k_2)M$
$dM^*/dt = k_1M + [k_2 - (k_3hE + k_4)]M^*$		$dM^*/dt = k_1M - k_3M^*$
$dE/dt = (k_2 - k_3hE)M^*$		$dBR/dt = k_2M - k_4BR$
$dD/dt = (k_3hE + k_4)M^* + k_6BR$		
$dBR/dt = k_5M - k_6BR$		

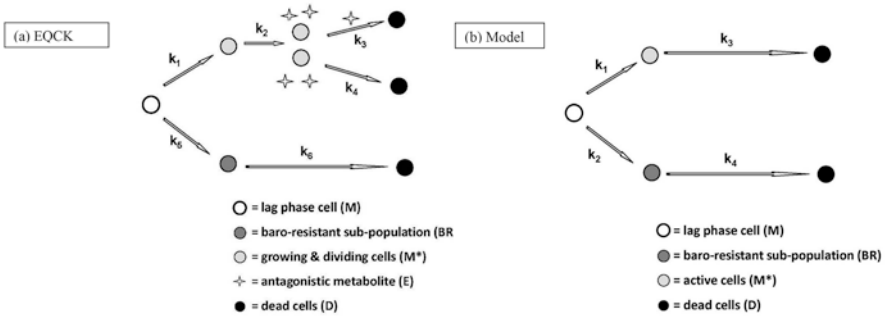

Fig. 17.2 (**a**, **b**) Reaction scheme of the EQCK model and its parsimonious subset model A (see Table 17.4) to account for inactivation kinetics showing tailing (Doona et al. 2012)

growth-death steps of the original quasi-chemical model and additional steps that account for the presence of a baro-resistant subpopulation and its gradual disappearance that are observed as tailing in the inactivation kinetics. The growth parameters are superfluous for the present purposes of describing the HPP inactivation kinetics of *L. monocytogenes* showing tailing, where no discernible growth is observed. Accordingly, we devised a more suitable, four-step subset version of the EQCK model called Model A, which omits steps relating to growth and accurately fits the inactivation kinetics data. The reaction steps and differential equations comprising Model A are also listed in Table 17.4. Reaction schema for the EQCK model and Model A are shown in Fig. 17.2a, b (Doona et al. 2012; Peleg and Corradini 2011).

The relationships of t_p values estimated with the EQCK model to temperature at constant pressure and to pressure at constant temperature are shown in Fig.17.3a, b, respectively.

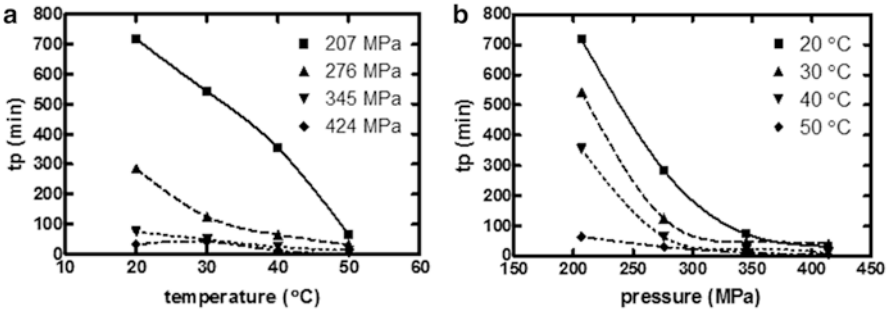

Fig. 17.3 (**a**, **b**) Relationships of t_p estimated from the EQCK model to temperature and pressure, respectively

17.5.2 The Weibull Model

The Weibull distribution function is a popular empirical function used in predictive microbiology (Peleg and Corradini 2011; Corradini et al. 2007; Peleg 2006). The conventional expression of the two-parameter Weibull model is as follows:

$$\mathrm{Log}_{10}\left(S_t\right) = \mathrm{Log}_{10}\left(S_0\right) - bt^n$$

with S_t=microbial counts at time $t=t$, S_0=counts at $t=0$, b=rate parameter, and n=shape parameter. The coefficients b and n are pressure and temperature dependent, and *ad hoc* empirical expressions have been used to describe the pressure and temperature dependence of the Weibullian-power law model's parameters (Doona et al. 2007).

One characteristic of the Weibull model is that the parameter n can, depending on the data being fit, have a range of possible values that significantly influence the shape of the fitted curve. For values of $n>1$, the curve shows downward concavity; for values of $n<1$, the curve shows upward concavity ("tailing); and for values of $n=1$, the curve is essentially linear. In the case of the inactivation of *E. coli* by HPP (Doona et al. 2007), the values of n ranged from 3.4 to 0.4 for conditions of 276–512 MPa and 30–50 °C. In the present instance for the HPP inactivation of *L. monocytogenes* with conditions of 207–414 MPa and 20–50 °C, the estimates of values of n occur over a narrower range ($1.54<n<0.62$). For the eight (8) cases in Table 17.3 with values of $n\approx 1$ [$0.9\le n\le 1.13$], the corresponding plot of ln(tp) vs. ln(6/b) produces a straight line with slope≈ 1 (Fig. 17.4), which confirms the following expression of tp, the time to effect a 6-log kill, with the Weibull model:

$$tp = \left(6/b\right)^{1/n}$$

The relationship of tp to T at constant P from the Weibull model's estimates of tp is shown in Fig. 17.5a. Similarly, in Fig. 17.5b, the relationship of tp to P at constant T is shown.

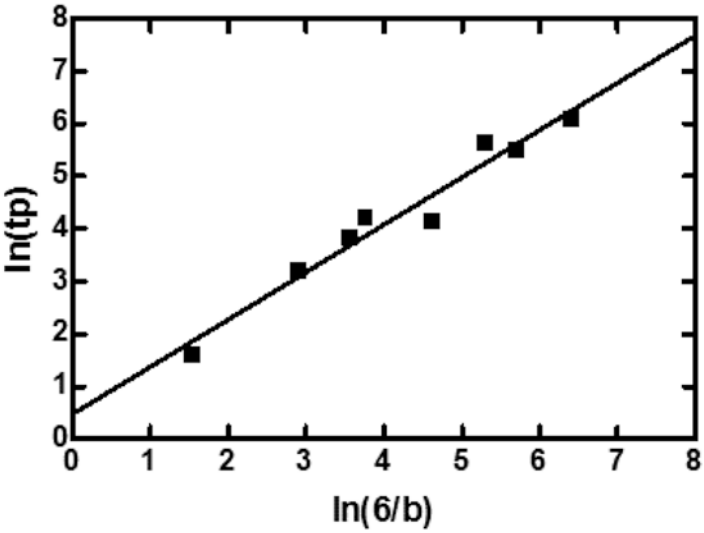

Fig. 17.4 Plot of ln(*tp*) vs. ln(6/*b*) for *L. monocytogenes* HPP inactivation data

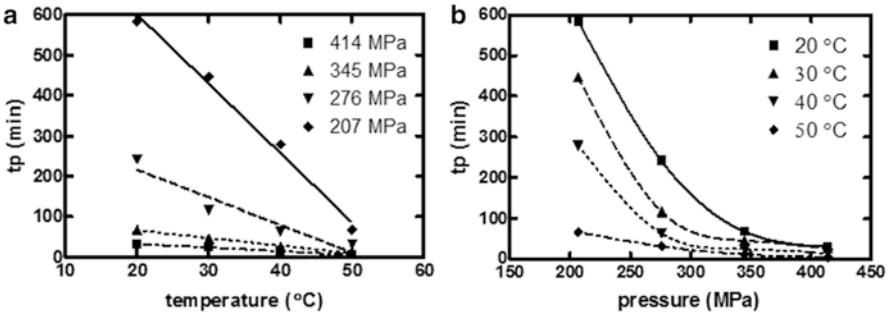

Fig. 17.5 (**a**, **b**) Relationships of *tp* estimated from the Weibull model to temperature and pressure, respectively

17.6 TST and TSP Analysis and Comparison of Primary Models

17.6.1 TST Secondary Model for the EQCK and Weibull Models

For the sake of comparison, we evaluate the TST model from estimates of t_p obtained from the EQCK model and from the Weibull model. Using the estimated values of t_p obtained for all HPP treatment conditions with either the EQCK or Weibull primary models, the estimated value of k ($t_p = 1/k$) can be used to

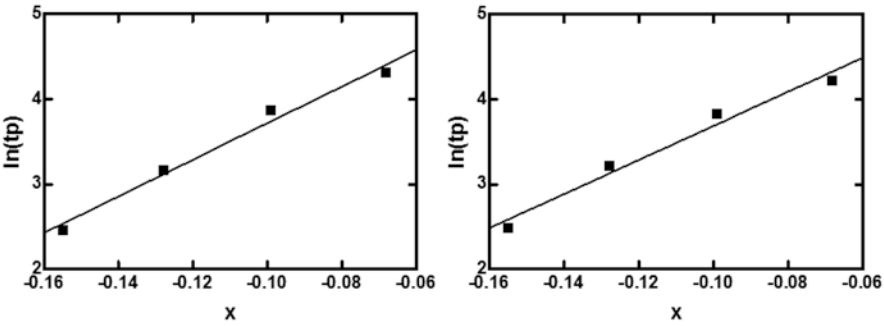

Fig. 17.6 (**a**, **b**) TST (Arrhenius) plots of $\ln(t_p)$ against x at 345 MPa: (**a**) EQCK analysis, (*closed squares*) data, (*solid line*) calculated $\ln(t_p) = 5.867 + 21.506\times$ (Doona et al. 2012). (**b**) Weibull analysis, (*filled squares*) data, (*solid line*) calculated $\ln(t_p) = 5.694 + 20.07\times$

Table 17.5 TST secondary models-determination of dimensionless activation energy, U_a, and E_a from EQCK and Weibull estimates of t_p at 345 MPa and 50–20 °C ($R = 0.008313$ kJ mol^{-1} K^{-1})

	EQCK model	Weibull model
T (°C)	t_p (min)	t_p (min)
50	12	12
40	24	25
30	48	46
20	72	68
U_a	21.51	20.07
E_a (kJ/mol)	48.8	45.6

determine the corresponding dimensionless activation energy. Typical secondary model fittings with the TST model (Arrhenius plots) as $-\ln(k) = \ln(t_p)$ against x for tp values estimated from the EQCK or Weibull primary models, respectively, are shown in Fig. 17.6a, b. The TST secondary model produces good fits with the sets of t_p values obtained with both primary models and yields comparable values for activation energy ($U_a = 21.5$ and 20.1, respectively) and reference rate constants (k_r-see Table 17.5).

For most chemical reactions, the activation energy is independent of temperature. By extension, it is reasonable to assume that the activation energy remains constant regardless of the pressure on the system over the range of high pressures considered in these experiments. The corresponding plots of $\ln(t_p)$ vs. x at $P = 414$, 276, and 207 MPa should also yield a constant value for U_a and E_a. The results of these plots are summarized and averaged in Table 17.6. Over the experimental pressure range, the estimated average value of U_a and E_a using the t_p values determined separately from the EQCK and Weibull models is reasonably constant and equal to one another over the experimental pressure range examined ($U_a = 24.53$ and 22.01 and $E_a = 55.70 \pm 4.69$ and 49.96 ± 4.08, respectively).

This relation should be likely to hold at any value for the reference temperature, T_r. In this instance, the arbitrarily chosen value was $T_r = 273.15$ K. The pressure-dependent processing time at T_r and designated tp_r is calculable from the inverse of

Table 17.6 Secondary models (activation energy, E_a, and reference processing time, tp_r) at four experimentally investigated pressures: $R = 0.008313$ kJ mol^{-1} K^{-1}, 1 kcal = 0.239006 kJ

P (MPa)	U_a	E_a (kJ mol^{-1})		t_{pr} (min)
	EQCK model			
207	26.16	59.41		5827.25
276	25.32	57.50		1579.24
345	21.51	48.83		353.22
414	25.12	57.04		267.12
			E_a (kcal mol^{-1})	
	Average:	55.70	13.31	
	StdDev:	4.69		
	%Error:	8.42		
	Weibull model			
P (MPa)	U_a	E_a (kJ mol^{-1})		t_{pr} (min)
207	23.88	54.22		3869.96
276	23.14	52.53		1175.80
345	20.07	45.58		297.17
414	20.93	47.51		161.95
			E_a (kcal mol^{-1})	
	Average:	49.96	11.94	
	StdDev:	4.08		
	%Error:	8.17		

k_r, which is the intercept of the TST secondary model. The estimates of the quantity t_{pr} are also summarized in Table 17.6.

For the t_p values estimated from both primary models using the experimental data, the calculated t_{pr} values are large in comparison, which reflects the low temperature (0 °C) selected as the reference temperature. The t_{pr} values decrease with increasing pressure for estimates from both primary models, as expected. For both models, the values of the activation energies lie within the range of values observed for thermal inactivation of microorganisms. Within experimental error, the E_a values are constant and have similar standard deviations and percent errors. However, the Weibull model estimates a lower E_a value, compared with the EQCK model, which is compensated by shorter reference processing times. In contrast, the EQCK model produces a higher, more realistic E_a value and longer reference processing times.

17.6.2 TSP Secondary Model for the EQCK and Weibull Models

The polynomial expression above for $\ln(t_p)$ comprises the TSP model for the HPP inactivation of microorganisms. Similar to the TST analysis, the qualities of the primary model fits using either the EQCK or Weibull models previously (Doona

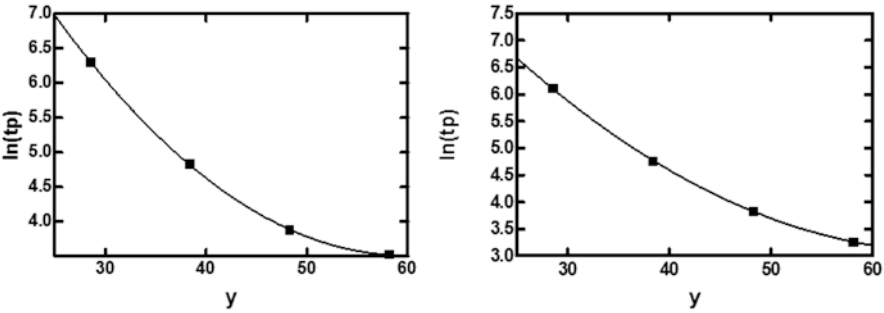

Fig. 17.7 (**a**, **b**) TSP plots of $\ln(t_p)$ against y at 30 °C (**a**) EQCK analysis, (*filled squares*) data, (*solid line*) calculated $\ln(t_p) = 13.793\text{-}0.345y + 0.0029y^2$ (Doona et al. 2012). (**b**) Weibull analysis, (*filled squares*) data, (*solid line*) calculated $\ln(t_p) = 12.121 - 0.267y + 0.0020y^2$

Table 17.7 Comparisons of activation and reference parameters

T (K)	tp_r (h)	ΔV_c^* cm^3 mol^{-1}	$\Delta\beta_c^*$ cm^3 mol^{-1}MPa^{-1}
EQCK			
293.15	400	−44.4	0.020
303.15	16296	−136.7	0.351
313.15	8330	−118.7	0.256
323.15	5	−13.4	−0.077
		Avg = −78.3	Avg = 0.138
		StdDev = 51.0	StdDev = 0.173
		%Err = −65.1	%Err = 126
Weibull			
293.15	284	−43.0	0.020
303.15	3061	−95.0	0.207
313.15	3706	−110.2	0.213
323.15	8	−22.8	−0.044
		Avg = −67.7	Avg = 0.099
		StdDev = 36.0	StdDev = 0.131
		%Err = −53.1	132

et al. 2012) have been evaluated as a function of P for the HPP inactivation of *L. monocytogenes*. Both primary models yielded comparable results. Using the values of t_p estimated from those results (Table 17.4), regression analysis was used for plots of $\ln(t_p)$ vs. y. The quality of the fits obtained for TSP plots of $\ln(t_p)$ vs. y is illustrated for a temperature of 30 °C in the secondary fittings shown in Fig. 17.7 using the t_p values estimated from the EQCK and Weibull models.

The reference processing time, activation volume, and activation compressibility are collected in Table 17.7. The data analysis used units of minutes for time and liters for volume. For convenience, these units have been changed in Table 17.7 to hours and cubic centimeters. A small temperature dependence of the activation volume can arise as a consequence of the temperature dependence of the chemical

potential, but this effect is too small for us to detect. A second, possibly large, temperature dependence may be observed if there is a change in the type or number of reactions under observation, or if there are two or more competing processes, one of which is favored as temperature changes. We believe that neither explanation is relevant to high pressure processing and ascribe the observed differences in measured values to experimental error. Accordingly, we average the values for the activation parameters and discuss these parameters and t_{pr} below.

The trends exhibited by the three parameters gathered in Table 17.7 are similar for both the EQCK and Weibull models. The values of each parameter are measured at different temperatures. Yet, it is obvious that the values display broad maxima and would not be successfully modeled by linear TST graphs, e.g., of $\ln(t_{pr})$ against $1/T$. Both the EQCK and Weibull models yield relatively large errors, with the Weibull model performing slightly better than the EQCK model in this regard. Compressibility is defined to be positive; therefore, the negative value produced by both models signifies that, within experimental error, the compressibility is very small.

17.7 Conclusions

The EQCK model evaluates all stages of the microbial lifecycle continuously, as does its progenitor, the original quasi-chemical model. In dealing with growth, division, and mortality simultaneously to describe the overall dynamics of a microbial population, the EQCK model is accordingly a population dynamics model (Peleg 2006), whose model parameters have mechanistic implications, which distinguishes the EQCK from empirical models.

Using transition state theory, we have developed two secondary models, TST and TSP, to reflect the effects of temperature and of high pressure on microbial inactivation by HPP. These secondary models are applied to data obtained with the EQCK and the Weibull distribution model for the nonlinear inactivation kinetics of baroresistant *L. monocytogenes* in a surrogate protein food system by various HPP combinations (207–414 MPa, 20–50 °C) that in some instances show tailing. The mathematical relationships of TST and TSP involve the unique parameter called "processing time" (t_p), which was developed particularly to evaluate inactivation kinetics data showing tailing. These results are significant for ensuring the safety of foods with respect to *L. monocytogenes* and also have implications for bacterial spore inactivation by HPP in the production of commercially sterile foodstuffs and in the bio-decontamination of *B. anthracis*.

Activation volume values calculated with the TSP for both models were relatively large compared with typical activation volumes for organic and inorganic reactions. Large volumes of activation are typical of proteins, and values in the range 100–300 $cm^3\ mol^{-1}$ are not uncommon. Interestingly, for food technology where the goal of HPP is microbial inactivation, these large activation volumes are ascribed to structural misalignments within or between protein molecules caused by the application of high pressure. Moreover, in a recent study of the structural changes occurring in

L. monocytogenes in response to treatments with gamma radiation, pulsed electric field, and high pressure (Mohamed et al. 2012), HPP treatments designed to effectuate a 3-log inactivation of *L. monocytogenes* Scott A (400 MPa, 24 °C, 6 min) caused aggregation of the cytoplasm (indicating extensive protein denaturing) observable with transmission electron microscopy without causing DNA lesions (in contrast to the effects of γ-irradiation). While those studies helped distinguish the loci targeted by these technologies, they also helped in understanding the mechanism of bacterial inactivation by these same technologies. These results are consistent with the TSP results regarding the occurrence of protein denaturation.

In defining t_p, we arbitrarily chose a 6-log magnitude of reduction, but other values also could have been chosen without altering the fundamental mathematics and methods involved in the process of this derivation. A minimum 5-log reduction of *L. monocytogenes* or other pathogens is required for products like juices (FDA-DHHS 2002), and t_p could be estimated for a 5-log reduction. However, commercial food processors are required to validate microbial inactivation using their actual process and product of interest. The present results with *L. monocytogenes* inactivation in a surrogate food system (Doona et al. 2012) are not intended to be directly transposable to any specific product of interest for purposes of commercial validation, and we retain t_p as an indication of a 6-log reduction for consistency with published literature and assume it imparts an extra margin of safety. Nonetheless, this methodology is modifiable and extensible; the present results provide a guide that can be customized to address practical validation studies of actual food products with other organisms and other nonthermal processing technologies, as required.

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Part V
Food Chemistry and Quality

Chapter 18
Effects of High Pressure on Food Proteins

Jian Yang and Joseph R. Powers

Abstract High-pressure modification of proteins involves changes in protein secondary, tertiary, and quaternary structures from the native state through intermediate states to the fully denatured state. High pressure changes protein structure primarily by rupturing or forming non-covalent bond-electronic interactions, hydrophobic interactions, and hydrogen bonds. High pressure also induces formation of new disulfide bonds stabilizing the denatured proteins or producing protein aggregation. Generally, high pressure decreases protein volume by compressing internal cavities and changing the solvation volume. However, the conditions for high pressure to denature or modify food proteins depend on the structure of individual proteins. Large numbers of disulfide bonds in a native protein helps that protein to withstand high-pressure denaturation. Temperature is a variable that along with pressure can be manipulated to either promote or retard protein denaturation. In addition to pressure intensity and holding time, the pH, ionic strength, and solvent conditions can greatly affect high-pressure modification of food proteins. Pressure-modified protein functional properties—solubility, gelation, emulsification, foaming, binding, coagulation, and water-holding capacity—can positively or negatively affect the organoleptic and nutritional quality of ingredients or food products. To improve food quality, modification of food proteins for desirable functional properties requires careful selection of pressure-treatment conditions or parameters (pressure intensity and holding time, temperature, pH, ionic strength, etc.). High pressure is a unique and approved tool to modify protein functional properties yet needs further exploration in areas of bioactive proteins and peptides and protein allergens.

Keywords High pressure • Protein functionality • Denaturation • Gelation • Solubility • Digestion

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18.1 Introduction

Proteins are macromolecules made up of amino acid polypeptide chains. The structures and behaviors of proteins in a food system render functional properties, influencing the quality of food products (Messens et al. 1997). Protein denaturation or conformational changes during food preparation, processing, storage, or consumption greatly alter protein functional properties and result in changes in food quality and organoleptic properties (Messens et al. 1997). As two independent physicochemical parameters, pressure and temperature cooperatively induce protein denaturation or conformational changes, affecting protein functional properties in foods.

The mechanism of protein denaturation induced by high pressure is different from that due to heat. Bridgman (1914) first observed that high-pressure treatment at 500–700 MPa for 30–60 min induced coagulation of egg albumen. More recently, physicists, chemists, and biologists have used high pressure as a tool to study protein stability, folding/unfolding, and biological properties (Suzuki et al. 1963; Zipp and Kauzmann 1973; Heremans 1982; Weber and Drickamer 1983). Beginning in the 1980s, food scientists and technologists have had increased interest in high-pressure technology because high pressure can improve quality and safety of food products (Elgasim and Kennick 1980; Hayashi et al. 1989; Knorr 1993).

High pressure is a unique tool to modify food proteins and produce texture and functional products different from thermal-processed food products. Although intensive studies of high pressure on proteins' stability and denaturation have been well documented during the last several decades (Gross and Jaenicke 1994; Heremans and Smeller 1998; Lullien-Pellerin and Balny 2002; Scharnagl et al. 2005; Knorr et al. 2006), this chapter will provide the basic and current knowledge of protein stability and denaturation, functional properties, and nutritional aspects as affected by high hydrostatic pressure.

18.2 Protein Functionality

According to conformational structures and physicochemical characteristics of proteins, protein functional properties in food science are classified into hydration, protein-protein interactions, and surface properties (Nakai and Li-Chan 1993; Damodaran 1994; Messens et al. 1997). The hydration properties include water absorption and retention, wettability, swelling, adhesion, dispersibility, solubility and viscosity. The protein-protein interactions include gelation and precipitation. The surface properties include surface tension, emulsification, and foaming characteristics (Messens et al. 1997; Galazka et al. 2000b).

The hydrophilicity or hydrophobicity of proteins plays a significant role in protein functionality. Polar or nonpolar amino acids build up the environment of hydrophilicity or hydrophobicity of protein molecules. For example, polar and charged amino acids at the surface of globular proteins provide hydrophilicity, which contributes to

protein solubility and water-binding properties (Damodaran 1988). Nonpolar and uncharged amino acids on the surface of globular proteins provide hydrophobicity, which favors protein aggregation and affinity for nonpolar ligands (Damodaran 1988). Nonpolar amino acid residues in the interior of the protein structure provide hydrophobic pockets contributing to protein ligand binding (Wu et al. 1999). The amphiphilicity of a protein contributes functional properties of emulsion and foaming through protein migration to the oil–water or air-water interfaces, forming viscoelastic films and stabilizing emulsions and foams (Damodaran 1996).

18.3 Protein Stability

The primary structure of protein—polypeptide chains—is constructed by the sequence of amino acids bonded covalently through peptide bonds. The polypeptide chain forms the secondary structures—α-helices or β-sheets—by intra- or intermolecular hydrogen bonds. The secondary structure domains fold into a three-dimensional configuration by non-covalent interactions between amino acid side chains. Therefore, the quaternary structure is formed by the spatial arrangement of subunits by non-covalent bonds between the polypeptide subunits (multimeric proteins). The non-covalent interactions to stabilize the secondary, tertiary, and quaternary structures include: (1) electrostatic interactions, (2) hydrogen bonds, (3) hydrophobic interactions, and (4) Van der Waals force. Semi-covalent bonds—disulfide bonds—also stabilize tertiary structure of proteins.

Proteins may exist in three structurally distinct, thermodynamically stable states: the native state, the molten globule state, and the fully unfolded (or denatured) state (Ptitisyn 1995). A native protein with biological activity has a delicate balanced conformational structure through interactions within the polypeptide chains and with a surrounding solvent (Lullien-Pellerin and Balny 2002). In the presence of solvent, such as water, native proteins are stable in a narrow physicochemical zone. Changes of the environmental conditions, such as temperature, pressure, solvent, and pH, can perturb the subtle balance of intramolecular and solvent-protein interaction and unfold the polypeptide chain, resulting in protein denaturation (Lullien-Pellerin and Balny 2002).

18.4 Protein Denaturation

Protein denaturation induced by pressure involves conformational changes in quaternary, tertiary, and/or secondary structures, leading to partially or completed unfolded polypeptide. Generally, protein denaturation can be described as a three-stage model: native state (N) $\Leftrightarrow$ intermediate states (I) $\Leftrightarrow$ denatured state (D) or unfolded state (U). The intermediate state may include many different sub-intermediate states during protein unfolding, providing a unique structure and various

functional properties (Fink et al. 1994; Uversky 1997). For example, protein in the molten globule state—a structural intermediate of protein between the native and denatured states—retains the secondary structure and has a loosely compact tertiary structure with increased mobility of the protein chain (Ptitisyn 1995). Although many reversible or irreversible intermediates exist during protein denaturation, the three-stage model simplifies the process of protein from native state to various intermediate states and then to the denatured state. The fully denatured state is a random coil of protein polypeptide chain, in which polypeptides can interact with each other to form a protein network.

18.5 Effect of High Pressure on Conformational Changes of Food Proteins

18.5.1 Protein Volume Changes by High Pressure

The protein partial volume (V_i) consists of V_{atom}, $V_{cavities}$, and $\Delta V_{hydration}$, where V_{atom} is volume of atoms of protein primary structure, $V_{cavities}$ is volume of cavities due to imperfect folding, and $\Delta V_{hydration}$ is volume of solvation due to the interactions of the protein molecule with the solvent (Frye and Royer 1998; Heremans and Smeller 1998).

Protein conformational changes or denaturation under pressure and/or temperature follows the principle of Le Chatelier: changing external factors (such as pressure or temperature) imposed on a system at equilibrium will shift the system to a new equilibrium counteracting the effects of the changes in external factors. Pressure negatively changes protein volume ($-\Delta V$), corresponding to small changes less than 1 % of the specific volume of the proteins (Royer 2002). Volume changes of unfolding of proteins by pressure are reported from −4.5 to 188 ml/mol, which are contributed by electrostriction, elimination of cavities, and exposure of hydrophobic and polar moieties (Royer 2002).

High pressure generally does not result in formation or breakage of covalent bonds of a protein, instead changing bond angles and exchanging disulfide bonds. The changes in bond angle and semi-covalent bonds do not change protein volume (ΔV) (Mozhaev et al. 1996). The volume changes of protein by high pressure are mainly ascribed to the changes in $V_{cavities}$ and $\Delta V_{hydration}$. The protein volume changes in cavities ($V_{cavities}$) include compressing the internal cavities or losing cavities upon protein unfolding (Frye and Royer 1998). Local or global unfolding of a protein induced by high pressure allows water access into the cavities, resulting in a loss of partial molar volume. The protein volume changes in solvation ($\Delta V_{hydration}$) involve rupturing and rearranging non-covalent bonds with solvent molecules, contributing to the largest volume changes of a protein induced by pressure (Frye and Royer 1998).

Non-covalent bonding between protein molecules and solvent includes electrostatic interactions, hydrogen bonds, and hydrophobic interactions. High pressure ruptures electrostatic interactions and decreases the solvation volume by electrostriction, in which induced ions in the ion's coulombic field compress the nearby

water dipoles in solution. The volume decrease (ΔV) of solvation for singly charged ions in water is −10 ml mol^{-1} and for a neutral molecule dissociated into two ions is −20 ml mol^{-1} (Mozhaev et al. 1996). Generally, high pressure stabilizes hydrogen bonds. At extreme high pressure, ruptured hydrogen bonds result in negligible changes of the solvation volume (ΔV) of proteins (Mozhaev et al. 1996). High pressure stabilizes interactions through stacking among aromatic rings of proteins, causing negative volume changes (Mozhaev et al. 1996). However, the effects of high pressure on the actual behavior of hydrophobic residues within proteins are still unclear (Boonyaratanakornkit et al. 2002). Generally, high pressure disfavors hydrophobic interactions due to positive volume changes (ΔV). Nevertheless, elevated pressure may also stabilize hydrophobic interactions because of increasing an unfavorable volume resulted from the solvation of apolar surfaces during protein unfolding (Boonyaratanakornkit et al. 2002). The exposure of hydrophobic groups to water may lead to a volume increase or decrease, depending on the types and concentrations of adjacent hydrophobic groups (Low and Somero 1975; Hendrickx et al. 1998). More recently, Sarma and Paul (2012) using molecular dynamics simulation investigated the effects of pressure on water-mediated interaction of neopentane as a model for hydrophobic residues of proteins and concluded that high pressure causes water molecules to penetrate into the protein interior.

18.5.2 Protein Stability and Denaturation by High Pressure

High pressure has little effect on the protein primary structure formed by the sequence of amino acids through peptide bonds. High pressure, however, induces exchange reactions of semi-covalent bonds—disulfide bonds— with free sulfhydryl groups (Visschers and de Jongh 2005). High pressure shortens hydrogen bonds and stabilizes secondary structures, such as α-helices (Mozhaev et al. 1996; Boonyaratanakornkit et al. 2002), but extreme high pressure (1000 MPa) can rupture hydrogen bonds of proteins leading to irreversible loss of the secondary structure (α-helices) (Hayakawa et al. 1996). High pressure also favors disruption of electrostatic and hydrophobic interactions (Mozhaev et al. 1996). Therefore, the effect of high pressure on protein denaturation mainly influences protein tertiary and quaternary structure (Mozhaev et al. 1996). Besides, high pressure also strengthens interactions of Van der Waals forces because Van der Waals forces can maximize packing density and reduce the volume of proteins under pressure (Boonyaratanakornkit et al. 2002).

Mozhaev et al. (1996) described that high pressure first destabilizes the protein tertiary structure but the secondary structures remains; the pressure-denatured proteins resemble molten globule-like state with increased hydrodynamic radii and exposed hydrophobic residues. A moderate pressure (50–200 MPa) can destabilize protein quaternary structure by dissociating the subunits of oligomeric proteins to form individual non-denatured subunits, which are subsequently subjected to conformational changes (Mozhaev et al. 1996). Normally pressure less than

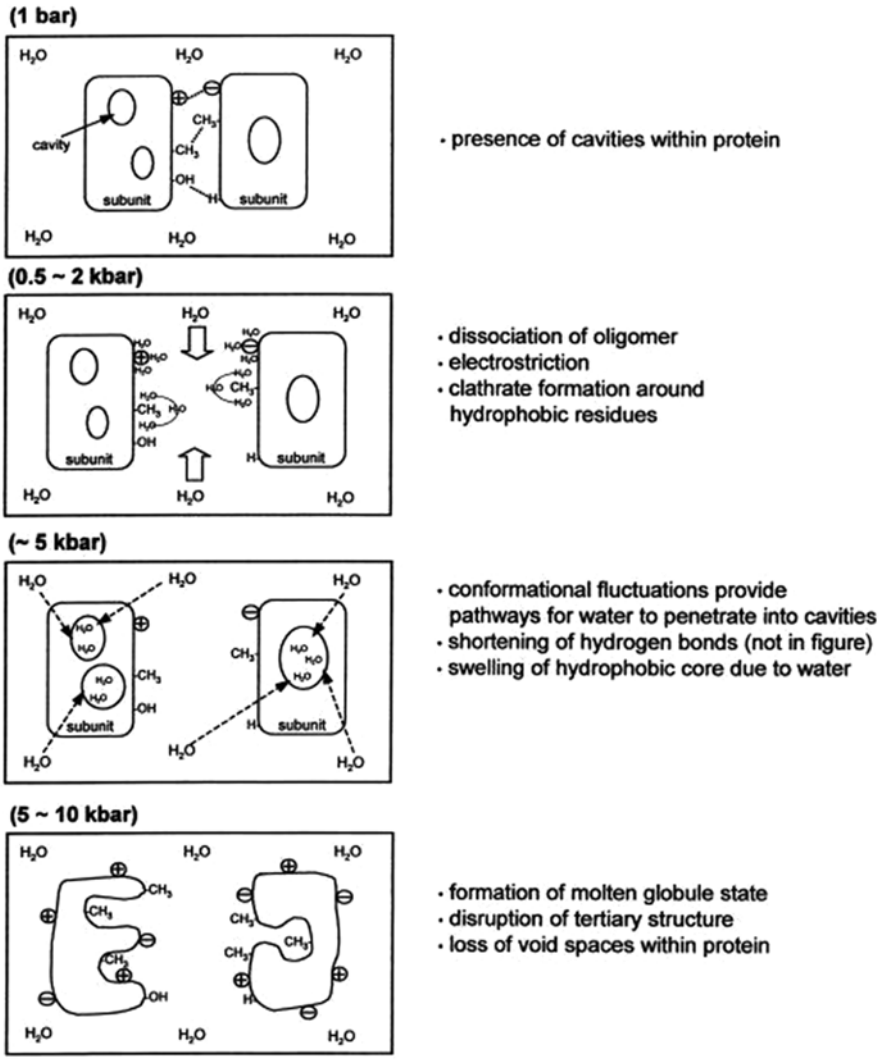

Fig. 18.1 Effects of pressure on molecular interactions during protein denaturation. Adapted from Boonyaratanakornkit et al. (2002) with permission

100–200 MPa reversibly denatures proteins, but at pressures greater than 300 MPa, proteins are irreversibly denatured (Balny and Masson 1993). Smeller et al. (1999) described a scheme of pressure-induced protein denaturation in which high pressure first unfolds native proteins (nN) into intermediates (nI); then the intermediate (nI) may either be denatured to the unfolded state (nU) or forms aggregates (I_n), which can be dissociated by high pressure or converted into a pressure-insensitive network (Ag). Boonyaratanakornkit et al. (2002) proposed a diagram about the effects of pressure intensity on protein denaturation (Fig. 18.1).

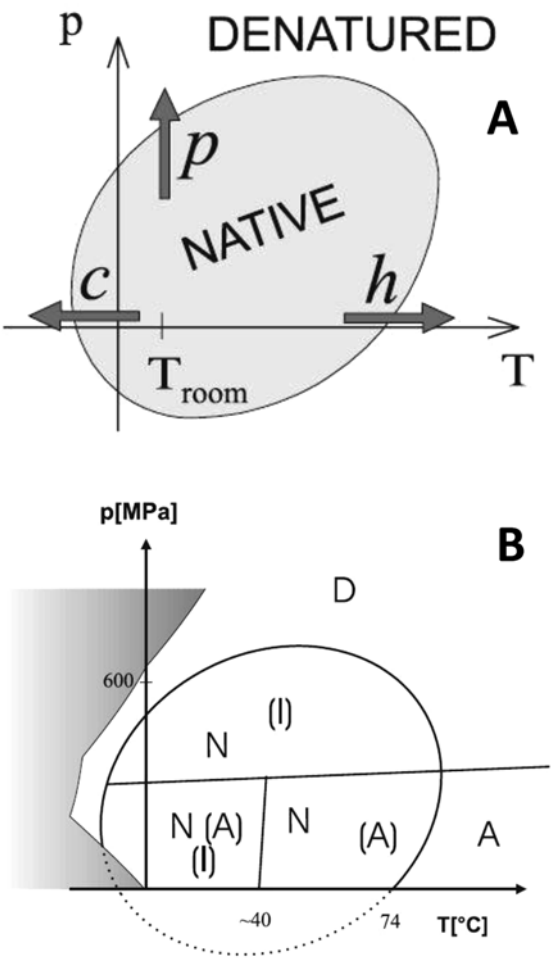

Fig. 18.2 (**a**) Schematic representation of the elliptic phase diagram of proteins. (**b**) Extended phase diagram. Adapted from Smeller (2002) with permission

18.5.3 Elliptic Phase Diagram for Protein Denaturation

Temperature and pressure are two physicochemical parameters in protein denaturation. Hawley (1971) first proposed the pressure-temperature (*p*–*T*) plane, an elliptic phase diagram, to describe the reversible denaturation of chymotrypsinogen by pressure and temperature. The *p*–*T* elliptic phase diagram shows that the state of a native protein exists, restricted to a certain range of conditions in terms of pressure, heat, or cold, and exceeding the restriction in pressure, heat, or cold can denature a protein (Fig. 18.2a) (Smeller 2002). A combination of pressure with heat or cold exhibits either promoting or counteracting effects on protein denaturation. For example, when temperature increases, the effect of temperature on protein can be minimized by high pressure establishing a new equilibrium system (Balny et al. 1997).

The phase diagram can be an aid to selection of proper pressure and temperature parameters to modulate protein structure and modify protein functional properties more broadly than using only one parameter—pressure or heat (Mozhaev et al. 1996; Lullien-Pellerin and Balny 2002). A limitation of this phase diagram is that protein denaturation is simplified to a two-stage model from a native state (N) to a denatured state (D) (Smeller 2002). The simplified two-stage model of food denaturation excludes conditions that induce the protein into intermediate state, such as the molten state, which may possess unique functional properties different from native and fully denatured proteins. Also, the phase diagram cannot differentiate the reversible denaturation from irreversible denaturation, such as protein aggregates (Smeller 2002). Smeller (2002) suggested an extended phase diagram for myoglobin, which includes protein denaturation and aggregation phenomena and describes the native and denatured states as well as aggregated and intermediate states (Fig. 18.2b). Another limitation is that the phase diagram cannot describe the effects of other environmental factors, such as pH and salt, on protein denaturation.

18.5.4 Denaturation of β-Lactoglobulin by High Pressure

β-Lactoglobulin (β-Lg) is a globular protein found in bovine milk as the most abundant whey protein consisting of 162 amino acids with a molecular weight of 18,300, containing two disulfide bridges and one free cysteine. Under physiological conditions β-Lg is a dimer with predominant β-sheet. The monomer of β-Lg has nine antiparallel β-strands and one short α-helix, forming a hydrophobic calyx site within the β-barrel and a surface hydrophobic site between the α-helix and the β-barrel (Papiz et al. 1986; Sawyer and Kontopidis 2000).

β-Lg is very susceptible to high pressure due to the structure of the calyx. Stapelfeldt and Skibsted (1999) using in situ fluorescence measurements of β-Lg noted that at pressure up to 50 MPa the protein transitions into a melted state with partial collapse of the inner calyx. The β-Lg also exhibits an increase in aromatic hydrophobicity, a decrease in aliphatic hydrophobicity, and an exposure of the free thiol group of cysteine (Stapelfeldt and Skibsted 1999). Pressure at 123 MPa leads to partial denaturation of β-Lg by exposure of hydrophobic regions with a volume change (ΔV) of −73 ml mol^{-1} (Stapelfeldt and Skibsted 1999). Pressure treatment at ≤200 MPa reversibly unfolds β-Lg, but treatment at >200 MPa irreversibly denatures β-Lg after pressure release, leading to aggregation and gel formation (Stapelfeldt and Skibsted 1999). However, Belloque et al. (2000), using NMR to examine β-Lg after pressure release and adjustment to pH 2, observed that pressure applied at 100 MPa does not alter the core of β-Lg. Treatments at 200 and 400 MPa increase the flexibility of the β-Lg core; the structure of pressure-treated β-Lg reverts to the native structure after equilibrium at atmospheric pressure (Belloque et al. 2000). Iametti et al. (1997) reported that pressure treatment of β-Lg at 600 and 900 MPa at neutral pH produces formation of irreversible soluble aggregates of protein with a loss of only 10 % of tertiary structure.

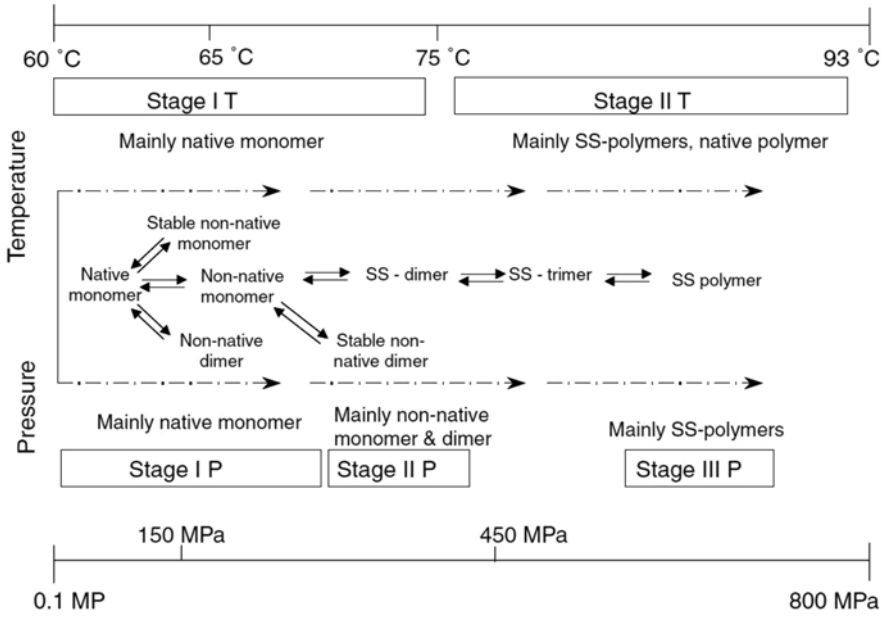

Fig. 18.3 Proposed three-stage model of the heat and pressure denaturation of β-Lg. *T* temperature, *P* pressure. Adapted from Considine et al. (2007) with permission

Multistage models have been proposed for β-Lg denaturation by high pressure. Considine et al. (2005, 2007) proposed a three-stage model for β-Lg denaturation in neutral solution (Fig. 18.3). At stage I at pressures of 0.1–150 MPa, β-Lg remains a stable native structure. Stage II occurs at pressure of 200–450 MPa, and the native β-Lg monomer interchanges reversibly with nonnative monomer, with formation of disulfide-bonded dimer. At stage III at pressure of 450–800 MPa, β-Lg is unfolded into a stable form and aggregates through disulfide bonds (Considine et al. 2005). Adding hydrophobic ligands can stabilize the native β-Lg and shift the transitions from stage I to stage II and from stage II to stage III (Considine et al. 2005). Kuwata et al. (2001) collected NMR data of β-Lg at pressures up to 200 MPa at pH 2 where β-Lg is a monomer, and they proposed a high-pressure denaturation model for β-Lg. Before being fully unfolded by high pressure, β-Lg forms either intermediate conformers I_1, which has disordered "non core region" (β B-E), or intermediate conformers I_2, which has disordered "core region" (β F-H).

Temperature of pressurization can result in changes of the tertiary and secondary structure of β-Lg. Tedford and Schaschke (2000) observed that β-Lg at pH 7.0 treated at 55 and 100 MPa undergoes changes of 44 % and 63 % in tertiary structure at 75 °C but only 8 % and 11 % at 35 °C, respectively. However, these treatments do not change the secondary structure—α-helix content—as compared to the native β-Lg. Yang et al. (2001) reported that a combination treatment at 600 MPa and 50 °C induces β-Lg at pH 7.0 into the molten globule state, in which tertiary structure is lost

with formation of nonnative secondary structure (α-helix). Aouzelleg et al. (2004) also reported pressure treatment at 280 MPa and 40° or 60 °C at pH 7.0 induced a molten globule-like state of β-Lg while losing the well-defined tertiary structure with increased α-helix content. In addition, at cold temperature, Walker et al. (2004) observed that pressure treatment of 510 MPa at 8 °C does not change α-helix of β-Lg as measured by circular dichroism but results in a loss of α-helix at 24 °C; the changes of tertiary structure of β-Lg by pressurization are greater at 24 °C than at 8 °C.

β-Lg is more stable to pressure treatment at acidic pH than at neutral pH. Belloque et al. (2007) reported that pressure unfolding of β-Lg at pH 2.5 requires greater intensity (tested at 100–400 MPa) than that at pH 6.8. The partially denatured β-Lg can return to the native structure after treatment at 400 MPa at acidic pH but not at neutral pH. Tedford and Schaschke (2000) observed that β-Lg lost greater tertiary structure due to high-pressure treatments at pH 7.0 than at pH 5.6.

During high-pressure treatment, some ligands exhibit a baroprotective effect on β-Lg. In the presence of sodium dodecyl sulfate (SDS), myristic acid (MA), and linoleic acid (CLA), β-Lg is stable against pressure at 200 MPa in the stage I of denaturation model described by Considine et al. (2005, 2007). The presence of ANS (1-anilino-naphthalene-8-sulfonate), retinol, and CLA inhibits the transition of β-Lg at 600 MPa from stage II to stage III (Considine et al. 2005, 2007).

18.6 Effects of High Pressure on Functional Properties of Food Proteins

As described above, high pressure induces changes of protein conformational structure, formation, or rearrangement of hydrogen bonds, electrostatic interactions, hydrophobic interactions, and semi-covalent bonds (disulfide bonds). Proteins modified by high pressure may exhibit positive or negative functional properties to food quality and organoleptic properties. The modification of protein functional properties by high pressure not only depends on pressure intensity and holding time but also on temperature, pH, ionic strength, solvents, and interactions with other food components.

18.6.1 Solubility

Protein solubility is critical to many protein functional properties such as emulsification, foaming, and gelation. Protein solubility depends on the structures of native, intermediate, and denatured proteins. Hydrophilic groups from charged amino acid residues on polypeptide chains promote hydration and repulsive forces between protein molecules, leading to protein solubility. Conversely, hydrophobic groups from nonpolar amino acid residues on the protein surface can promote protein-protein interactions, leading to aggregation. To better understand modification of

protein functionality due to application of pressure, it is essential to know how high-pressure treatment coupled with environmental factors, such as temperature, pH, and salt, affects the solubility of proteins.

High-pressure treatment can have limited effects on protein solubility. Funtenberger et al. (1995) observed that the solubility of 2.5 % and 5 % solutions of β-Lg at pH 7.0 was not affected by 15-min pressurization at 450 MPa at 25 °C. Lim et al. (2008a) also found that the solubility of whey protein concentrates (8.23 % solids) at pH 4.6 and 7.0 does not change after treatment at 300–600 MPa. Lee et al. (2006) reported that pressurization at 690 MPa for 30 min decreases the solubility of 1 % whey protein concentrates (WPC) about 15 % at pH 7.0. The decrease in solubility of WPC is significantly correlated with an increase of surface hydrophobicity of total treated protein during pressurization for 5–30 min. This result suggests that high pressure causes the exposure of hydrophobic groups to the protein surface promoting protein-protein hydrophobic interactions and decreasing the solubility of WPC (Lee et al. 2006). Wang et al. (2008) observed that pressurization at 200–600 MPa insignificantly decreased solubility of soy protein isolates (SPI) (1 % and 3 %) at pH 6.8. Although the surface hydrophobicity of the SPI significantly increases after pressurization, no correlation between the surface hydrophobicity and the solubility of SPI is observed. Molina et al. (2001) reported that pressurization of soybean 7S and 11S globulins and SPI at 200–600 MPa at pHs 7.5 and 6.5 resulted in variable effects on the solubility of these proteins with a trend of significantly increasing surface hydrophobicity. However, the surface hydrophobicity was not significantly correlated with the solubility of SPI. Puppo et al. (2004) also observed an increase in surface hydrophobicity of SPI (1 %) after pressurization at 200–600 MPa without significant change in solubility of SPI evaluated at pH 8.0.

High pressure can effect change in soluble aggregates of proteins. For example, Funtenberger et al. (1995) observed that pressurization from 150 to 450 MPa at 25 °C results in production of soluble aggregates of β-Lg including dimers, trimers, tetramers, pentamers, and hexamers, which increased with an increase in pressure intensity. Funtenberger et al. (1997) suggested that β-Lg aggregates are formed during pressurization by intermolecular disulfide bonds through SH/SS interchange reactions rather than from oxidation of SH groups. Tang and Ma (2009) reported that pressurization from 200 to 600 MPa does not change the total aggregate content of SPI, including insoluble and soluble aggregates. However, pressurization decreased the content of insoluble aggregates while increasing the content of soluble aggregates. Tang and Ma (2009) observed that large insoluble aggregates, formed at 200 MPa, are dissociated to small soluble aggregates by pressure at 400–600 MPa. About 50 % of insoluble aggregates transfer to soluble aggregates after pressurization at 600 MPa.

Pressurization can affect protein solubility as a function of pH. Torrezan et al. (2007) reported that 2 % SPI when pressurized at 450 MPa exhibits a maximum solubility of 53 % at pH 2.66 and 65.1 % at pH 6.84, which are about 11.5 % and 37 % higher than the solubility of untreated samples, respectively. Puppo et al. (2004) observed that pressurization at 200–600 MPa insignificantly changes the solubility of SPI at pH 8 but significantly increases the solubilization of SPI by

about 25 % at pH 3. Although the pressurization increases the solubility of SPI at pH 3, the solubility of SPI at pH 8 remains about 13 % higher than that at pH 3 after high-pressure treatment. At pH 8, high pressure produces soluble aggregates of SPI stabilized by disulfide bonds, while at pH 3 high pressure induces both aggregation and dissociation of SPI. For whey proteins, however, the solubility of WPC at pH of 4.6 and 7.0 are not affected by pressurization at 400 MPa from 0 to 30 min; the solubility of WPC at pH 7.0 is about 5 % higher than that at pH 4.6 prior to or after high-pressure treatment (Lim et al. 2008a, b). Van Camp et al. (1997a) reported that pressure at 400 MPa for 30 min significantly decreases the solubility of WPC solution (2.2 %), about 60 % at pH 5, 30 % at pH 6, and 36 % at pH 7. The solubility of WPC treated at pH 6 and 7 further decreases by adding $CaCl_2$ up to 7 mM. The decrease in solubility by $CaCl_2$ is attributed to diminished repulsive forces between negatively charged carboxyl groups on protein and enhanced cross-linking negatively charged protein molecules. However, solubility of WPC treated at pH 5 increases by adding $CaCl_2$ from 0 to 7 mM (Van Camp et al. 1997a). At pH 5, near-isoelectric point, the added calcium may enhance positive repulsive forces between proteins, leading to the increase in solubility of WPC.

High pressure affects protein solubility more at a high-protein concentration than at a low protein concentration. Funtenberger et al. (1995) showed that pressure at 450 MPa (25 °C, 15 min) induces more aggregates of β-Lg in 5 % solution than 2.5 %. Solubility of β-Lg evaluated at pH 4.7 is 61 % soluble for the 5 % solution versus 71 % for the 2.5 % solution. Wang et al. (2008) observed that the solubility of 5 % SPI dispersions treated at 200 and 400 MPa is significantly lower than that of 1 % or 3 % SPI.

In addition, a combination of high pressure with temperature can promote a reduction of protein solubility. Van der Plancken et al. (2005) found that pressurization at 100–700 MPa at 10 and 25 °C did not decrease the solubility of 10 % egg white protein solution at pH 8.8. However, the pressure treatment combined with a temperature of 60 °C results in a 20 % loss of solubility at 200–500 MPa and a loss of 40 % solubility at 700 MPa as compared to pressure treatment at lower temperature. The loss of solubility is attributed to formation of large aggregates linked by disulfide bonds created through sulfhydryl-disulfide bond exchange reactions in the high-pressure treatment with temperature (Van der Plancken 2005).

18.6.2 Gelation

Gelation is a process of forming a three-dimensional network of denatured or partially denatured proteins. The structure of a gel, a well-ordered matrix, can hold significant amounts of water through protein-protein and protein-solvent interactions (Van Camp et al. 1997a). Factors influencing protein denaturation, such as high-pressure intensity and holding time, protein concentration, temperature, pH, and ionic strength, affect gelation and gel properties.

The gels induced by high pressure exhibits characteristics different from gels induced by heat. Camp and Huyghebaert (1995a) reported that the WPC gels

induced by high pressure (400 MPa, 30 min) exhibit a structure of a porous and finely stranded network with a small amount of intermolecular cross-links, while WPC gels induced by heat (80 °C, 30 min) exhibit a structure of less porous and more compact and continuous network with a high level of cross-links. The pressure-induced gels show less elasticity than that of the heat-induced gels. The G''/G' (loss modulus/storage modulus) ratio of the pressure-induced gels is greater than that of the heat-induced gels. When protein concentration increases, the G''/G' decreases for the pressure-induced gels but increases for the heat-induced gels (Camp and Huyghebaert 1995a). Although pressure-induced gels improve elastic properties at high-protein concentration, the elastic behavior of the pressure-induced gels is still less than that of the heat-induced gels. In addition, the pressure-induced WPC gels are compressed and surrounded by non-incorporated liquid (NIL), which decreases significantly at high-protein concentrations (14–18 %) (Camp and Huyghebaert 1995a). The difference in mechanisms between pressure- and heat-induced protein denaturation and network formation, which are involved in rupturing and reforming non-covalent interactions and covalent bondings, may contribute to the difference in gel characteristics produced by pressure and heat treatment.

The minimum protein concentrations and pressure intensity for gelation varies with the treatment conditions and the source of protein. For example, Camp and Huyghebaert (1995b) observed that the minimum pressure and protein concentration to form a network of WPC gel are 200 MPa and 16 %. However, Camp and Huyghebaert (1995b) observed that the start concentration for WPC to form a weak gel at 400 MPa for 30 min is 9–18 %. Kanno et al. (1998) reported that to form gels the required concentration of WPC at 400 MPa is >18 % and the required concentration of WPI at 600 MPa is 10 %. He and Ruan (2009) observed that the minimum pressure of 250 MPa (25 °C) is required to induce gels of WPI solutions (20–28 %). Alvarez et al. (2008) reported that pressure at 250 MPa induces gelation of SPI at a concentration of 15 %. Speroni et al. (2009) observed that high pressure at 600 MPa induces gelation for 10 % SPI and 10 % β-conglycinin (7S) but not for 10 % glycinin (11S).

The compositions or characteristics of a food protein also significantly influence the gel properties. Kanno et al. (1998) observed that pressure-induced WPI gel at 20 % exhibits hardness and breaking stress three times greater than the pressure-induced WPC gel at 20 %. The pressure-induced WPI gel exhibits a honeycomb microstructure, whereas the pressure-induced WPC gel exhibits a coarse microstructure with larger aggregates. The aggregation of β-Lg, α-La, and serum albumin through S-S bonding is involved in pressure-induced whey protein gelation (Kanno et al. 1998). The lower protein concentration and the higher contents of lactose, lipids, and inorganic materials in WPC may cause the coarse structure of WPC gels compared to WPI gels (Kanno et al. 1998).

Protein concentration and pressure-treatment conditions are critical to the strength of pressure-induced gels and gel properties. Camp and Huyghebaert (1995b) observed that the strength of high-pressure-induced WPC gels increases with an increase of protein concentration from 11 % to 25 %. Kanno et al. (1998) also observed that increasing the concentration of WPI from 12 % to 18 % increases the hardness and breaking stress of WPI gels induced by pressure at 600–100 MPa.

Camp and Huyghebaert (1995a) found that increasing the concentration of WPC from 11 % to 18.3 % dramatically increases the storage modulus (G') and loss modulus (G'') of gels induced at 400 MPa for 30 min. Van der Plancken et al. (2007) and Alvarez et al. (2008) reported that increasing pressure from 250 to 560 MPa increases the storage modulus (G') and the loss modulus (G'') of pressure-induced SPI gels. In addition, the strength of high-pressure-induced WPC gels also increases with an escalation of applied pressure from 200 to 400 MPa, pressure holding time from 10 to 60 min, temperature from 20 to 50 or 60 °C, and pH from 4 to 7 (Camp and Huyghebaert 1995b, 1996).

Changing pH can influence high-pressure-induced gelation by affecting negative or positive repulsive forces among protein molecules. For example, at the isoelectric point (IEP) of WPC (pH 5.0), high-pressure treatment (400 MPa, 30 min) only induces formation of a weak and white coagulum with low gel strength (Van Camp et al. 1997b). Van Camp et al. (1997b) explained that at IEP the electrostatic attraction forces promote protein aggregation through random protein-protein interaction, resulting in formation of a coagulum. At pH 3–4 below the IEP of WPC and in the absence of salt, pressure treatment does not induce the gelation of the positively charged WPC (14 %) (Van Camp et al. 1997b). However, at pH 6–9 above the IEP, the same pressure treatment induces the gelation of the negatively charged WPC. The pressure-induced gels are homogeneous, ordered, and elastic and exhibit a decrease in the NIL as pH is raised from 6 to 9 (Van Camp et al. 1997b). When pH increases, protein interactions at specific reactive sites promote the formation of the ordered gel network structure because the increased negative repulsive forces among proteins delay the aggregation step (Van Camp et al. 1997b). At alkaline environments, increased reactivity of SH groups of proteins promotes formation of strong gels through disulfide bonds during high-pressure treatment (Van Camp et al. 1997b). He et al. (2010) also reported that raising pH from 5.0 to 8.0 increases the hardness and the breaking stress of WPI gels (20 %) induced by pressure at 800 MPa and 30 °C. The degree of cross-linking by S-S bonds is higher at pH 8.0 than at pH 5.0, resulting in formation of a rigid gel (He et al. 2010).

Adding salt can also affect pressure-induced gelation by influencing negative or positive repulsive forces among protein molecules. Although high pressure does not induce WPC gels at pH 3, in the presence of sodium chloride (0.5 mol/L) pressure treatment at 400 MPa (30 min) forms gels with high strength and storage modulus (Van Camp et al. 1997b). The presence of NaCl reduces the positive repulsive forces among protein molecules; the remaining repulsive forces delay the aggregation step during gelation, leading to the formation of a stronger gel network through electrostatic and hydrogen bonds (Van Camp et al. 1997b). At a pH above IEP or in alkaline conditions, the presence of NaCl decreased the strength, the storage modulus, and the NIL of pressure-induced gels by reducing the negative repulsive forces among protein molecules during the aggregation step (Camp and Huyghebaert 1995b; Van Camp et al. 1997b). He et al. (2010) also reported that an increase of ionic strength from 0.05 to 0.2 increases the hardness and the breaking stress of pressure-induced WPI gels (20 %) at pH 5.0–8.0. He et al. (2010) observed that the geometric dimensions of the microstructure decrease with increasing pH and

decreasing ionic strength. For example, at pH 5.0 and ionic strength 0.05–0.2, the microstructures of pressure-induced WPI gels are coral-like stranded network with tiny aggregates of micrometer dimensions. At pH 6.8, the pressure-induced WPI gels show fine-stranded network at ionic strength 0.05, honeycomb-like network at ionic strength 0.1, and thick-stranded network at ionic strength 0.2. At pH 8.0, the pressure-induced WPI gels exhibit dense microstructure at ionic strength 0.05, a fine-stranded network at ionic strength 0.1, and honeycomb-like network at ionic strength 0.2 (He et al. 2010).

High-pressure-treated proteins change behaviors of gelation and properties of gels induced by heat. Wang et al. (2008) reported that the gelling ability of SPI decreases after pressure treatment at 200–600 MPa and the decrease in gelling ability of pressurized SPI is concentration dependent. For example, after pressure treatment at 400 MPa, heat cannot induce gelation of pressurized 3 % SPI but can induce gelation of pressurized SPI at 5 %. The SPI dispersions (3 %) treated at 200 MPa exhibit longer gelling onset time and lower storage modulus (G'') than the SPI dispersions (5 %) treated at 200 MPa and the untreated SPI dispersion (10 %). Speroni et al. (2009) also observed that the G' value of heat-induced gels of SPI, 7S, and 11S after treatment at 300 and 600 MPa is lower than that of untreated proteins. Formation of high-molecular-mass aggregates through hydrophobic interactions and disulfide bonds by high pressure may contribute to the decrease in the gelling ability and the gel strength of pressure-treated SPI (Speroni et al. 2009). To obtain strong gels of soy proteins, Speroni et al. (2009) suggested avoiding sequential treatments of pressure and heat.

18.6.3 Emulsion

Emulsification is a process to disperse either oil droplets in water or water droplets in oil often aided by emulsifiers such as proteins. Good protein emulsifiers require that amphiphilic proteins rapidly absorb, unfold, and reorient to/at the interface and interact with neighboring protein molecules to form a strong, cohesive, and viscoelastic film stabilizing the emulsion. Therefore, protein conformational characteristics such as flexibility and adaptability of polypeptide chain to the environment and distribution of hydrophilic and hydrophobic groups on the protein surface are of significance in protein emulsion activity, capacity, and stability. Protein denaturation by high pressure at various conditions such as temperature, pH, and ionic strength can positively or negatively influence protein emulsion properties.

The effects of high pressure on emulsion properties of proteins depend on resistance of protein structure to denaturation by high pressure. β-Lg treated at pressure 200–800 MPa exhibits decreasing emulsifying efficiency by producing large droplets of oil-in-water emulsion, which continually increases during storage for 10 days (Galazka et al. 1996a). However, α-lactalbumin (α-La) treated by high pressure at 200–600 MPa has improved emulsifying activity index (EAI) at pH 3, 7, and 9 and emulsion stability (ES) at pH 3 (Rodiles-López et al. 2008). High-pressure treatment

(<400 MPa) of ovalbumin (egg white protein) does not significantly change the oil-in-water droplet size; while treatment at >600 MPa only slightly increases the droplet size of ovalbumin-stabilized emulsion (Galazka et al. 2000a). Since protein denaturation affects the solubility or aggregation of proteins, protein structure resistant to denaturation during high pressure may improve emulsifying properties. For example, β-Lg, which possesses one disulfide bond and a free SH and is susceptible to denaturation by high pressure, exhibits a decrease in emulsifying properties after treatment above 200 MPa. α-La, which possesses four disulfide bonds, is much more stable to high-pressure treatment, exhibiting improvement or little change in emulsifying properties after treatment at 200–600 MPa. Ovalbumin has a structure of four free sulfhydryl groups and one disulfide bond. The molten globule of ovalbumin induced by pressure treatment above 400 MPa may exhibit a limited change in emulsifying properties (Smith et al. 2000). Therefore, protein structure is critical to emulsifying properties as affected by high pressure.

With protein concentrates or isolates, the structure and denaturation of predominant protein play a significant role in emulsifying properties as affected by high pressure. For example, WPC treated at pressure 200–800 has a decreased emulsifying efficiency, resulting in a broader droplet-size distribution and an increase in the average of droplet size (Galazka et al. 1995). The decrease in emulsifying properties of WPC by high pressure may be primarily associated with β-Lg rather than α-La because β-Lg and α-La account for 65 % and 25 % of whey protein concentrates, respectively. Puppo et al. (2011) reported that soybean protein β-conglycinin and glycinin pressurized at 200–400 MPa (60 °C) exhibit different emulsion properties. Treating β-conglycinin (7S) at 200–400 MPa increases the droplet size and flocculation index (FI) of emulsions, while pressurized glycinin (11S) does not significantly change the droplet size and FI value of emulsions. Soy protein fractions 11S and 7S make up about 52 % and 35 % of soy protein isolates, respectively. The 11S fraction may affect the emulsifying properties of soy protein isolates more than the 7S fraction.

Solubility of high-pressure-modified proteins is critical to protein emulsifying activity. Yin et al. (2008) reported that high pressure (200–400 MPa) treatment of red kidney bean protein isolate significantly improves emulsifying activity index (EAI) and emulsion stability index (ESI). The authors suggested that an increase of protein solubility contributes to the increase of EAI and ESI (Yin et al. 2008). Tang and Ma (2009) observed that pressure treatment from 200 to 600 MPa increases soluble aggregate and decreases insoluble aggregate content of soy protein isolates (SPI). The aggregates of SPI treated at pressure of 400 and 600 MPa exhibit smaller molecular weight (MW) compared to the treatment at 200 MPa. Although enhancing protein solubility during high-pressure modification of proteins appears significant in improving protein emulsifying activity, no significant correlation between solubility and EAI of soy protein isolate was observed (Molina et al. 2001).

Protein surface hydrophobicity contributes to protein amphiphilic properties, which is favorable for protein to migrate to the interface in emulsifying. However, an increase in hydrophobicity of β-Lg treated by pressure at 200–800 MPa at pH 7 does not improve the emulsion activity of the protein (Galazka et al. 1996a, b). The pressure-treated β-Lg produces large droplets of oil-in-water emulsion compared to

the native proteins (Galazka et al. 1996a, b). Exposing hydrophobic groups by protein unfolding by high pressure may promote protein aggregation through hydrophobic interactions, resulting in reduction in emulsion efficiency and stability of pressure-treated β-Lg (Galazka et al. 1996a, b). Galazka et al. (2000a) also observed pressurization at 600–800 MPa results in an increase of surface hydrophobicity of ovalbumin accompanying a decrease of emulsifying ability as indicated by increased average droplet diameter.

The pH of the protein system during pressurization greatly affects the emulsifying properties of proteins. For example, Puppo et al. (2005) reported that SPI at pH 8 treated by 200–600 MPa (10 min, 10 °C) exhibits a decrease in the average droplet size of emulsion but at pH 3 exhibits a slight increase in the average droplet size compared to untreated SPI. The pressure treatment significantly decreases the flocculation index (FI) and increases adsorbed proteins of emulsion at both pH 8 and 3. However, the interfacial protein concentration of emulsion slightly decreases at pH 8 and significantly increases at pH 3 after the high-pressure treatment.

The emulsifying properties of pressure-treated proteins are also influenced by the pH of the emulsion system. For example, Rodiles-López et al. (2008) observed that the emulsion prepared at pH 3 with pressure-treated α-La exhibits an increase in EAI and emulsion activity (ES) with an increase of pressure from 200 to 600 MPa. Conversely, the emulsion prepared at pH 5 with pressure-treated α-La exhibits a decrease in EAI and ES. The emulsions prepared at pH 7 and 9 with treated α-La exhibits a slight increase in the EAI value but a great decrease in the ES values with an increase in pressure. Rodiles-López et al. (2008) suggested that the intermediate molten globule state of pressure-treated α-Lac contributes to the improvement of emulsifying properties at pH 3.

In addition to pH, protein concentration and ionic strength also influence the effect of high pressure on the emulsifying of proteins. Pittia et al. (1996) observed that pressure treatment at 300–900 MPa does not change the emulsifying capacity of β-Lg (pH 7.0, phosphate buffer 10 mM, n-tetradecane as the dispersed phase) at concentrations of 1 and 1.5 mg/mL but greatly decreases the emulsifying capacity of β-Lg at concentrations of 0.3 mg/mL. Galazka et al. (2000a) observed that ionic strength (0–0.1 M) does not influence the average droplet size of emulsions prepared with native ovalbumin but dramatically increases the average droplet size of emulsion prepared with ovalbumin treated by pressure at 600 MPa and 20 min.

High-pressure treatment has little effect on emulsions prepared with native proteins and thus has potential to be used for pasteurization and sterilization of emulsions. Although high-pressure-treated β-Lg and WPC exhibit a decreased emulsifying stability, high-pressure treatment (200–800 MPa) only slightly reduces the stability of emulsion prepared with native β-Lg and WPC (Galazka et al. 1996a, b). This phenomenon is explained by the flexible native β-Lg partially unfolding in the adsorbed monolayer at the interface of droplet but is unaffected by pressurization (Galazka et al. 1996a, b). Puppo et al. (2008) also reported that a combined treatment of pressure (200–600 MPa) and heat (20–60 °C) does not increase the oil droplet size, flocculation, and coalescence of emulsions prepared with soy protein isolates (SPI). Puppo et al. (2008) suggested that formation of high-molecular-mass

aggregates through subunits (7S, 11S fractions) and monomers (β-7S) contributes to the stability of emulsions under high-pressure treatment. Anton et al. (2001) also observed that pressure treatment at 200 and 500 MPa did not change the oil droplet size at pH 3.0 and 7.0 but increased the viscosity of emulsion at pH 7.0. Therefore, high-pressure treatment could be used to reduce microbes and stabilize yolk emulsion in acidic condition (Anton et al. 2001). Van de Ven et al. (2007) also noted that pressure pasteurization (600 MPa, 5 min, 20 °C) does not decrease the average droplet size of emulsions made with WPI (pH 6.7 and 3) and SPI (pH 6.7). Pressure sterilization (800 MPa, 5 min, 80 °C initial and 115 °C maximum temperature) results in only a small increase in the average droplet size of emulsions of WPI (pH 6.7, ionic strength 0.075) and SPI (pH 6.7) compared to a tremendous increase in droplet size by heat sterilization. Thus, high-pressure sterilization has advantages in stabilizing emulsions over heat sterilization (Van de Ven et al. 2007).

18.6.4 Foaming

Foaming is the process of dispersion of air in liquid to form air bubbles stabilized through surfactants at the interface. Like emulsions, foams require proteins with solubility, flexibility, and amphiphilicity to move to the interface, form a film, and stabilize the resultant foams. Therefore, protein conformational structure associated with protein solubility, hydrophilicity, and hydrophobicity can directly affect foaming capacity and foaming stability. The foaming capacity and foaming stability of pressure-modified proteins depend on treatment conditions such as pressure intensity and holding time, temperature, protein concentration, pH, and ionic strength (salt).

Denaturation and aggregation of proteins induced by high pressure greatly influences protein-foaming properties. Pittia et al. (1996) reported that β-Lg (0.3 mg mL^{-1}) treated by high pressure at 250–900 MPa for 10 min decreases the foaming ability as analyzed by the initial conductivity. The reduction of foaming ability is attributed to pressure-induced large protein aggregates, which reduces protein diffusion rate and availability for adsorption at the air-water interface (Pittia et al. 1996). The foams prepared by pressure-treated β-Lg exhibited greater stability than untreated β-Lg. Results using a Tween 20 replacement method suggested formation of strong protein-protein interactions at the air-water interface (Pittia et al. 1996). Ibanoglu and Karatas (2001) observed that increasing pressure from 150 to 450 MPa (holding time 5–15 min, pH 7.0) increases the foam volume of the pressurized WPI solution. The foam volume of the WPI solution pressurized at 150–300 MPa (pH 7.0) also increases with the holding time from 5 to 25 min. The foam stability of the pressurized WPI solution (pH 7.0) increases with holding time from 5 to 15 min but with the pressure only up to 300 MPa (Ibanoglu and Karatas 2001). The increased foam volume of the pressure-treated WPI is attributed to partially denatured proteins, which improve molecule flexibility and enhanced rate of protein adsorption at the air-water interfaces (Ibanoglu and Karatas 2001). The increase in foam stability of the pressurized WPI solution is attributed to protein aggregation forming a thick film

at the interfaces, while the reduction of foam stability of WPI treated by pressure above 300 MPa is attributed to a decrease in film viscoelasticity by reduced number of protein interactions (Ibanoglu and Karatas 2001).

Intermediate state of proteins induced by high pressure may improve the protein-foaming properties. Rodiles-López et al. (2008) reported that α-La treated by high pressure at 200–600 MPa and 25–55 °C greatly improves the foaming capacity and foam stability compared to untreated α-La. α-La treated at 400 MPa exhibits the highest value of foaming capacity tested in the pH range from 3 to 9 among α-La treated at 200–600 MPa; α-La treated at 600 MPa and 40 °C for 5 min exhibits the highest value of foaming stability at pH 9.0 (Rodiles-López et al. 2008). Rodiles-López et al. (2008) suggested that the combination of high pressure and temperature as well as pH induces α-La into molten globules, which are partially unfolded and possess the flexibility and secondary structure to form a stable film at the air-water interface benefiting foaming properties.

High-pressure treatment improves foaming properties of WPC. Lim et al. (2008a, b) observed that WPC (3 %) pressurized at 300 MPa (15 min, 25 °C) exhibits little change in solubility and greatly improved overrun and foam stability and that the pressure-treated WPC possesses smaller molecular-weight (MW) fractions of peptides. Lim et al. (2008a, b) reported that ice cream mix containing high-pressure-treated WSU-WPC (300 MPa, 15 min) exhibits greater overrun and foam stability than those of the WPC without high-pressure treatment. Again, partially denatured and soluble proteins by high pressure can enhance protein-foaming ability.

The effect of high pressure on protein-foaming ability and stability also depends on the pH of protein solution during pressurization. Although Ibanoglu and Karatas (2001) observed that WPI solution pressurized at pH 7.0 (150–450 MPa, 5–25 min) improves foam volume and foam stability, the WPI solution pressurized at pH 5.0 had decreased foaming volume and stability. High-pressure treatment at pH 6.0 changes foaming volume and stability of WPI solutions, depending on conditions of pressure intensity and holding time. The reduction of foam volume and stability at pH 5.0 or 6.0 is suggested to be due to the loss of solubility and formation of aggregates of WPI at pH near the pIs of whey proteins, decreasing protein availability or diffusion for adsorption and forming film at the air-water interfaces (Ibanoglu and Karatas 2001). Van der Plancken et al. (2007) reported the effect of pH, pressure intensity, and temperature on the foaming ability and stability of egg white protein. At pH 8.8, the foaming ability of egg white protein solution (10 % v/v) improves after the treatment at 400–700 MPa (10–60 °C). However, at pH 7.6, the foaming ability of egg white protein only increases after the treatment at 400–500 MPa (10–40 °C) but decreases after treatment at 600–700 MPa (10–40 °C). The foam stability of egg white protein only improves slightly at pH 8.8 after treatment at 600–700 MPa (10 °C) but greatly improves at pH 7.6 after treatment at 600–700 MPa (10–40 °C) (Van der Plancken et al. 2007). The effect of pH on foaming ability and stability of egg white protein is attributed to protein solubility. At pH 8.8, the pressurized egg white proteins form voluminous foams due to unfolded proteins with retained residual solubility, while at pH 7.8, the pressurized proteins form dense and stable foams due to protein unfolding with extensive loss of protein solubility and increased protein-protein interactions (Van der Plancken et al. 2007).

In addition, the effect of high pressure on protein-foaming ability and stability also depends on the pH of the tested system for foaming. Rodiles-López et al. (2008) evaluated foaming properties of pressure-treated α-La at pH 3, 5, 7, and 9. Pressurized α-La improved the foaming stability at all the tested pH values. Pressurized α-La exhibits greater improvement of foaming stability at pH 9 than at pH 3, 5, and 7; the values of foaming stability of pressurized α-La at pH 9 is about 40–70 % greater than the values at pH 3 (Rodiles-López et al. 2008).

In addition to pressure, temperature, and pH, the foaming properties of pressurized proteins also depend on protein concentration and ionic strength. For example, Ibanoglu and Karatas (2001) observed that the treatment at 300 MPa (15 min) reduces the foam volume and foam stability for 2 % WPI (pH 7.0) but not for WPI at 1 % due to severe protein aggregation at the higher-protein concentration during pressure treatment. Ibanoglu and Karatas (2001) also observed that an increase in buffer molarity from 50 to 100 mM decreases foam volume and stability because of extensive aggregation by high-pressure treatment of proteins at high-buffer molarity.

18.6.5 Binding

Flavor or ligand-binding properties of proteins can influence the flavor perception or sensory quality of food products positively or negatively by carrying desirable flavor or transmitting undesirable off-flavors (Kühn et al. 2006). The interactions between flavors and proteins can be reversible or irreversible. Reversible interactions include hydrogen bonds, hydrophobic interactions, and electrostatic interactions; irreversible interactions involve covalent bonds (Kühn et al. 2006). In high-moisture foods, the binding of proteins with flavor compounds mainly involves hydrophobic interactions between nonpolar ligand with hydrophobic patches or cavities of the proteins (Kühn et al. 2006).

18.6.5.1 Ligand Binding

The binding properties of pressure-modified proteins to ligands depend on the ligand structure and binding sites of proteins. β-Lg possesses a primary hydrophobic site—a calyx within the β-barrel—and a surface hydrophobic site between the α-helix and the β-barrel (Papiz et al. 1986). β-Lg treated at 600 MPa at 50 °C exhibits an increased affinity to aromatic hydrophobic ANS (1-anilinonaphthalene-8-sulfonate) ligand without change in the number of binding sites (Yang et al. 2001, 2003). However, pressure-treated β-Lg exhibits an increased affinity to the aliphatic CPA (cis-parinaric acid) ligand with a decrease in the number of binding sites. Pressure-treated β-Lg also exhibits a decrease of affinity to retinol with a decrease in the number of binding sites (Yang et al. 2001, 2003). The hydrophobic aromatic ligand ANS presumably binds to multiple hydrophobic

sites of β-Lg, while the hydrophobic aliphatic ligand CPA presumably binds to external hydrophobic sites and the ligand retinol binds to the hydrophobic calyx site (Yang et al. 2002). Liu et al. (2005b) also observed that whey protein concentrates (WPC) treated at 600 MPa at 50 °C for 30 min exhibited an increased number of binding sites for the ANS ligand with no change of binding sites for the CPA ligand. The binding affinity of WPC for both ANS and CPA decreases when pressure treated.

Changes of protein structure by high pressure results in an increase or a decrease in the protein affinity to ligands. High-pressure treatment alters the calyx binding site and external surface binding site of β-Lg through conformational changes or by forming dimers or trimers of β-Lg, consequently increasing or decreasing the affinity or the number of binding sites of β-Lg to ANS, CPA, and retinol ligands (Yang et al. 2001). Yang et al. (2001) combined six measures of structure changes and ligand-binding properties of β-Lg versus high-pressure-treatment holding time in a plot (Fig. 18.4), which includes: (1) intrinsic tryptophan fluorescence, (2) extrinsic fluorescence with associated ANS, (3) extrinsic fluorescence with associated CPA, (4) far-UV CD, (5) near-UV CD, and (6) disulfide bond formation (e.g., protein aggregation). The results show that the conformational changes of β-Lg by high pressure is closely associated with an increase and a decrease in binding of β-Lg to ANS and CPA, respectively. The changes in binding of β-Lg with ligand ANS and CPA match well with the changes of tertiary structural changes of β-Lg by high-pressure treatment. Therefore, these results indicate the ligand-binding properties of β-Lg depends on the conformational changes in binding sites as well as the structural characteristic of ligands.

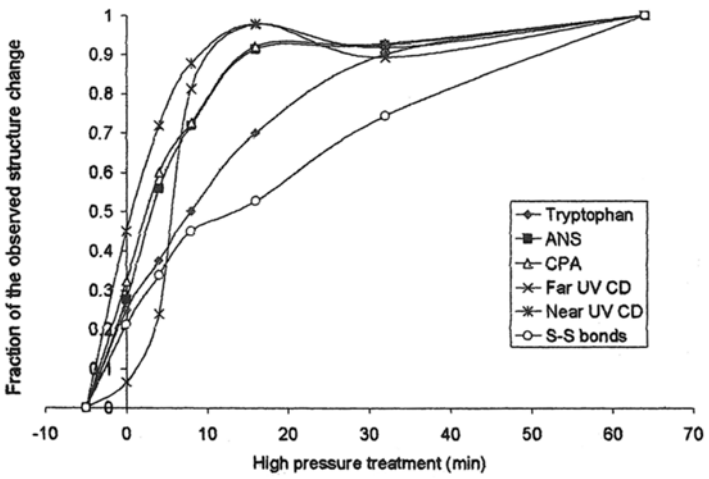

Fig. 18.4 Effects of pressure on the structure changes and binding properties of β-Lg. Adapted from Yang et al. (2001) with permission

18.6.5.2 Flavor Binding

The structure of flavor compounds greatly determines their affinity for proteins. In displacement tests with ANS, CPA, and retinol probes, Yang et al. (2003) observed that native and pressure-treated (600 MPa and 50 °C for 32 min) β-Lg exhibits specific binding to palmitic acid, capsaicin, and carvacrol and a weak and nonspecific binding to α-ionone, β-ionone, and cinnamaldehyde. Both native and pressure-treated β-Lg do not exhibit affinity for vanillin. High-pressure-treated β-Lg exhibits an increase or a decrease of binding affinity with palmitic acid, capsaicin, and carvacrol depending on the fluorescent probe (retinol, CPA, or ANS) used in the displacement test or the corresponding binding site of the probe (Yang et al. 2003). In addition, the conformational changes in primary hydrophobic site and surface hydrophobic binding sites of β-Lg after high-pressure treatment can also affect the flavor-binding properties of β-Lg because palmitic acid, capsaicin, carvacrol, cinnamaldehyde, and vanillin possess some degree of similarity in structure of aliphatic, or aromatic, or both aliphatic and aromatic characteristics of CPA, ANS, or retinol ligands.

Flavor-binding properties of pressure-treated whey proteins also depend on the structure of individual flavor compounds. Kühn et al. (2008) reported that WPI after pressurization at 250 MPa exhibits an increase in binding with trans-2-nonenal but no change with 2-nonanone and 1-nonanal. WPI treated by high pressure at 600 MPa exhibits an increase in binding with trans-2-nonenal, a decrease with 2-nonanone, and no change with 1-nonanal. Kühn et al. (2008) suggested that high-pressure denaturation weakens hydrophobic interactions and enhances covalent interactions between β-Lg and trans-2-nonenal. Liu et al. (2005a) reported that WPC treated by high pressure at 600 MPa and 50 °C increases the number of binding sites and apparent dissociation constants to benzaldehyde with an increase of pressure duration. However, the pressure-treated WPC does not exhibit a trend in increase or decrease of the binding properties to heptanone, octanone, and nonanone with pressure-treatment holding time. In addition, Kühn et al. (2008) also found that adding flavor compounds (2-nonanone, 1-nonanal, trans-2-nonenal) before or after high-pressure treatment does not significantly change the binding properties of WPI to these flavor compounds.

18.6.5.3 Binding Properties of the Molten Globule

The molten globule is an intermediate state of protein with a loosely packed hydrophobic core compared to the native state. The unique structure of the molten globule may favor the binding to ligands or flavors compared to the native state. Yang et al. (2001, 2002) reported that the β-Lg molten globule induced by high pressure at 600 MPa (50 °C, pH 7.0) increases the binding to the ligand ANS but does not with the ligands CPA and retinol. The flavor-binding properties of the pressure-induced molten globule of β-Lg depend on the structure of individual flavor compounds (Yang et al. 2003). Recently, using NMR, Tavel et al. (2010) observed that the molten globule of β-Lg produced by preheat-treatment at pH 2.6 favors binding

of flavor compounds β-ionone and guaiacol in both the calyx site and external hydrophobic site compared to the native state (Tavel et al. 2010). That the pressure-induced molten globule of β-Lg at neutral pH condition does not favor binding flavor compounds may be due to the corruption of the internal calyx binding site by high pressure.

The pH of the environment in the range from 3.0 to 11.0 can greatly change the ligand-binding properties of the pressure-induced β-Lg molten globule (Yang et al. 2002). Compared to native β-Lg, the pressure-induced β-Lg molten globule demonstrates greatly increased ANS binding from pH 6.0 to 3.0 but reduced retinol binding at pH 8.0–11.0 and significantly decreased CPA binding from pH 3.0 to 11.0. In addition, the monomers of β-Lg formed by blocking free SH group by N-ethylmaleimide (NEM) enhance retinol and CPA binding from pH 3.0 to 11.0 compared to pressure-treated β-Lg in the absence of NEM. However, the dimers of β-Lg formed through disulfide bonds by oxidation in the absence of KIO_3 decrease CPA fluorescent intensity from pH 3.0 to 11.0 (Yang et al. 2002). These results indicate that monomers of pressure-induced molten globule of β-Lg have enhanced ligand-binding properties probably due to greater accessibility to the binding sites than dimers. In the presence of NEM, the increase of retinol binding to β-Lg from the pH 11 to pH 2.5 also indicates NEM may stabilize the calyx allowing for retinol interaction under high-pressure treatment (Yang et al. 2002).

Protein flavor binding is complex involving protein structure, flavor structure, and interactions (non-covalent and covalent interactions or bonds) between proteins and flavors, as well as environmental factors affecting protein and flavor structures and their interactions. Therefore, it is challenging to elucidate the effects of high pressure on flavor-binding properties of proteins.

18.6.6 Coagulation

Casein coagulation by rennet involves a primary stage and a secondary stage. At the primary stage, κ-casein is enzymatically hydrolyzed to para-κ-casein and casein macropeptide, reducing the hydrophilicity of the casein micelle. At the secondary stage, the enzymatically modified caseins aggregate to form casein curds (López-Fandiño 2006). High-pressure treatment affects not only milk coagulation but also cheese yield and quality. In addition to high-pressure and duration, temperature, pH, and calcium are also factors influencing casein coagulation properties.

High-pressure treatment of milk has two opposite effects on rennet coagulation time (RCT) depending on intensity of pressure applied. Pressurization of whole milk at ≤200 MPa and 25 °C decreases the RCT by 30–40 %, while further pressurization to 400 MPa increased RCT to values similar to control milk (López-Fandiño et al. 1996). In similar studies with skim milk, Needs et al. (2000) and Zobrist et al. (2005) noted that pressurization for 15–30 min at 100–250 MPa resulted in a decrease in RCT, while treatment at pressures at greater than 300 increased RCT values with 400–600 MPa treatments producing coagulation times

equal to or greater than control milk. High-pressure treatment does not promote the release of casein macropeptide during the primary stage of κ-casein hydrolysis (López-Fandiño et al. 1997; Needs et al. 2000). However, pressurization at 200 MPa greatly increases the aggregation of casein micelles in the secondary stage (Needs et al. 2000). The improved aggregation results from dissociation of casein micelles by high pressure to submicelles, favoring aggregation through an increase in surface area and interparticle collision and a decrease in steric repulsion (López-Fandiño et al. 1997; Needs et al. 2000). The increase in RCT of milk pressurized above 200–250 MPa is mainly attributed to aggregates between caseins and denatured whey proteins particularly β-Lg (Needs et al. 2000; Huppertz et al. 2004). The casein whey protein interaction hinders aggregation in the secondary stage and also affects the enzymatic hydrolysis of κ-casein in the primary stage, resulting in an increase in the overall RCT (López-Fandiño et al. 1997; Needs et al. 2000).

Generally, high pressure improves coagulation properties and cheese quality. Pressurization at 100–600 MPa increases cheese yield by incorporation of additional β-Lg and moisture (López-Fandiño et al. 1996; Needs et al. 2000). The pressurization of milk at 600 MPa significantly reduces syneresis from the curds due to forming a finer gel network by incorporation of whey protein (Needs et al. 2000). An increase in pressure from 0.1 to 600 MPa enhances the complex modulus (G') by 50 % and the storage modulus by 125 % (Needs et al. 2000; Zobrist et al. 2005).

Pressurization conditions (temperature, pH, and calcium) exhibit significant effects on casein coagulation properties and cheese quality. Milk treated at 100–600 MPa at 20 °C results in a longer RCT than treatment at 5 and 10 °C (Zobrist et al. 2005). Pressurization at 40–60 °C (200–400 MPa) increases RCT of milk as compared to pressurization at 25 °C (López-Fandiño and Olano 1998). An increase of pH (6.2, 6.7, and 7.0) during treatment at 100–600 MPa greatly increases the RCT of milk. The increase in RCT of milk by pressurization temperature and pH may be attributed to the denaturation of whey protein (Zobrist et al. 2005). Adding 4 mM $CaCl_2$ decreases RCT of milk pressurized at 100 and 200 MPa compared to control but increases RCT of milk treated at 400 MPa from 0 to 60 min. The curd firmness from milk pressurized at 400 MPa is significantly improved by adding $CaCl_2$ (López-Fandiño et al. 1997).

In addition, milk first heated at 90 °C for 10 min then pressurized at 100–600 MPa for 0–30 min exhibits greatly improved rennet coagulation and cheese-making properties (Huppertz et al. 2005). The RCT of heat-treated milk decreases with an increase of pressure and treatment duration. The coagulum of the heat-treated milk pressurized at 250–600 MPa for 30 min or 400 or 600 MPa for 0 min exhibits greater strength than that of untreated milk. The yield of cheese curd from pressure-treated heated milk improved by 15 % without influencing the moisture content compared to untreated milk (Huppertz et al. 2005). A combination of heat treatment prior to high-pressure treatment appears to be an effective method to improve casein coagulation and cheese quality.

18.6.7 Water-Holding Capacity

The water-holding capacity (WHC) of muscle proteins greatly influences the yield (the cooking loss) and quality (juiciness and tenderness) of fish and meat products. The WHC reflects the ability of meat to retain its natural water content, which is mainly located within the myofibrils in the spaces between the thick and thin filaments (Offer and Trinick 1983). The changes in the interfilament spacing alter the WHC of meat (Offer and Trinick 1983). The WHC of comminuted meats and meat emulsion greatly depends on solubilization, denaturation, and gelation of myofibrillar proteins during processing (Macfarlane and McKenzie 1984; Iwasaki et al. 2006; Sikes et al. 2009). High-pressure treatment at various temperatures, salt concentrations, and pHs, which alters structures, solubilization, and denaturation of myofibril proteins, can result in both positive and negative effects on WHC and quality of meat and meat products (Cheftel and Culioli 1997).

High-pressure treatment generally decreases the WHC of fresh fish products. Lakshmanan et al. (2007) reported pressurization at 100–200 MPa for 10 and 20 min decreases the WHC of fresh salmon about 0.9–8.1 %. Pressurization at 150 MPa results in a 6.8–8.1 % loss in the WHC of fresh salmon. Lakshmanan et al. (2007) also observed that the moisture content of fresh salmon increases about 3–5 % after pressure treatment at 100–200 MPa for 10 min but decreases about 0.7 % after treatment at 200 MPa for 20 min. Angsupanich and Ledward (1998) also reported that pressurization at 200–800 MPa for 20 min decreases the moisture content of cod muscle less than 1.29 %. Wada and Ogawa (1996) noted that pressurization at 200 MPa decreases the WHC of sardine red meat 3.6 %, and the WHC of pressure-treated sardine meat further falls about 7–9 % during storage 5 °C for 5–10 days. However, the non-pressure-treated sardine meat retains the original WHC after storage for 10 days. The decrease in WHC in pressure-treated sardine red meat is closely associated with the oxidation of the meat because of denaturation of myoglobin by high pressure (Wada and Ogawa 1996). The destruction of sarcomere structure and denaturation of myofibril proteins by high pressure may contribute to the decrease in WHC and moisture content of fresh fish.

High-pressure treatment has variable effects on the WHC of smoked fish products. Gomez-Estaca et al. (2007) reported that the WHC of cold-smoked dolphinfish was unchanged after pressurization at 200 and 300 MPa for 15 min but was significantly decreased after increasing pressurization to 400 MPa. Lakshmanan et al. (2007) reported that 100–150 MPa pressurization for 10–20 min slightly increases the WHC of cold-smoked salmon about 0.7–1.9 % but greatly decreases the WHC of fresh salmon about 4.1–8.1 %. However, treatment at 200 MPa for 10–20 min decreases the WHC of cold-smoked salmon about 0.5–0.6 %. Lakshmanan et al. (2007) also noted that pressurization at 100–200 MPa for 10–20 min increases the moisture content about 2.5–7.1 % compared to non-pressurized samples. Added salt may contribute to the increases in WHC and moisture content of cold-smoked salmon by solubilizing proteins and expansion of the filament lattice by the electrostatic repulsive force from Cl^- ions (Offer and Trinick 1983; Lakshmanan et al. 2007).

Pressure treatment can reduce the cooking loss of comminuted meats and meat emulsion. Sikes et al. (2009) reported that compared to treatments at 100–400 MPa, pressurization at 200 MPa (2 min, 10 °C) results in a marked reduction in the cooking loss of beef sausage in the presence of 1 % salt. An increase in pressure from 0.1 to 200 MPa reduces the cooking loss of beef sausage from 28–32 % to 2–8 %; a further increase in pressure from 200 to 400 MPa increases the cooking loss from 2–8 % to 10–22 %. The authors conclude that pressure treatment is a viable tool in producing healthier reduced-salt-beef-batter products. In a similar study, Crehan et al. (2000) also noted that pressurization of raw minced beef at 150 MPa for 5 min significantly decreases the cook loss (0.7 %) of frankfurters but pressurization at 300 MPa does not change the cooking loss of frankfurters compared to non-pressurized sample. Jiménez Colmenero et al. (1997) observed that pressurization at 100 MPa for 5 and 20 min insignificantly affects the cooking loss (cooking out fluid) and water released from low- and high-fat sausages. However, pressurization at 300 MPa for 5 and 20 min significantly increases the cooking loss and water released about 3.1–4.6 times in low-fat sausage and about 3.1–4.6 times in high-fat sausages compared to non-pressurized samples. The pressurization at 300 MPa may denature myofibrillar proteins inducing protein aggregation and decreasing the ability of meat emulsions to retain moisture and fat content (Jiménez Colmenero et al. 1997).

High-pressure treatment can also improve the cooking loss of meat patty products with low-salt content. Macfarlane and McKenzie (1984) observed that pressurization at 150 MPa in the presence of 1 % and 3 % NaCl decreases the cooking loss of beef patties but not at 150 MPa in the presence of 0.5 % NaCl. Treatment at 50 MPa at 0–3 % NaCl does not change the cooking loss of beef patties. Iwasaki et al. (2006) reported that in the absence of NaCl, pressurization at 200–400 MPa for 10–20 min increases the cooking loss of pork meat patty from 13.7 % to 23.6–26.2 %. However, in the presence of 1 % NaCl, the same pressurization reduces the cooking loss of the meat patty from 13.0 % to 8.5–10.8 %. In the presence of 2 % NaCl, the cooking loss is further reduced from 8.6 % to 5.7 % by pressurization at 300 MPa. However, pressurization at 200 and 400 MPa increases the cooking loss of the pork patty about 10–12 % in the presence of 2 % NaCl (Iwasaki et al. 2006). Sikes et al. (2009) also noted that at concentrations of NaCl from 0.5 % to 2.0 %, high-pressure treatment at 200 MPa significantly decreases the cooking loss of beef-sausage batters from 35 % to 7 %. The concentration of 1 % NaCl in beef-sausage-batter results in the greatest marked reduction in the cooking loss by high pressure compared to non-pressurized samples. The sausage batter with 1 % NaCl exhibits overall good quality in terms of reduced cooking loss and improved texture compared to the other levels of added NaCl. Solubilization of myosin and actomyosin by pressurization at low ionic strength and disaggregation and unfolding of proteins by high pressure may contribute to the reduction of the cooking loss of meat products (Macfarlane 1974; Cheftel and Culioli 1997).

The reduction in cooking loss of meat products by high pressure is affected by pH. Macfarlane and Mckenzie (1984) reported that beef patties exhibit the greatest cooking loss at a pH near the isoelectric point (pH 4.6) of muscle, while patties exhibit the lowest cooking loss at a pH 7.2–9.2. At a pH between 4.2 and 4.7, high-pressure

treatment at 150 MPa for 20 min can further increase the cooking loss of beef patties compared to non-pressure-treated patties. However, at a pH between 4.7 and 6.2, high-pressure treatment decreases the cooking loss of beef patties, showing greatest differences between pressure-treated and non-pressure-treated patties in cooking loss at pH 5.2 and 5. At pH above 6.8, little difference in the cooking loss of beef patties between pressure-treated and non-pressure-treated samples is observed (Macfarlane and Mckenzie 1984). In addition, at pH between 5.9 and 6.8, the presence of tetrasodium pyrophosphate (TSPP, 0.5 %) further decreases the cooking loss in both pressure- and non-pressure-treated beef batters. Fernández-Martin et al. (2002) also found that pork batters with added sodium tripolyphosphate (TPP) (0.3 %) and NaCl (1.5 %) exhibit a reduction in weight loss 10 % greater than the batters with only 1.5 % NaCl after pressurization at 400 MPa (70 °C, 30 min). The effects of pH on the cooking loss of meat patties by high pressure may be associated with the increased solubility of myofibrillar proteins by high pressure (Macfarlane 1974). Although the presence of TSPP or TPP can slightly increase the pH of meat products and also promotes solubilization of proteins, the mechanisms by which high pressure combined with TSPP or TPP reduces the cooking loss of meat products remain to be elucidated (Macfarlane and Mckenzie 1984; Fernández-Martin et al. 2002).

A combination of high-pressure and heat treatment can improve the cooking loss of meat products. Jiménez Colmenero et al. (1998) reported that heat treatment of chicken batters containing 1.5 % and 2.5 % salt at 70 and 80 °C results in a cooking loss of 2.7–4.2 % and 7.4–7.6 %, respectively. However, combining high pressure (200, 400 MPa) with heat at 70 and 80 °C reduces the cooking loss of chicken batters to 0.57–0.77 % and 1.39–1.59 %, respectively. Jiménez Colmenero et al. (1998) also observed that the combination treatment results in a similar result with pork batters with 2.5 % salt but not pork batters with 1.5 % salt. Mor-Mur and Yuste (2003) also noted that pressurization at 500 MPa and 65 °C for 5 and 15 min reduces the weight loss of cooked sausage significantly compared to heat treatment at 80–85 °C for 40 min. The pressure- and heat-treated sausage has about 1.0 % of weight loss, while the heat-treated sausage exhibits about 1.8 % weight loss; pressure- and heat-treated sausages are less firm and more juicy than the heat-treated sausages. Fernández-Martin et al. (2002) also observed a combination pressure (400 MPa) with heat (70 °C, 30 min) reduces the weight loss of the pork batters (1.5 % NaCl) by 85 % and batters (1.5 % NaCl and 0.3 % TPP) by 93 % as compared to the treatment at 70 °C for 30 min. Fernández-Martin et al. (2002) reported that the solubility of salt-soluble proteins increases three times after the treatment of combined pressure with heat as compared to the treatment with heat alone. The combined treatment of pressure with heat (400 MPa, 70 °C, 30 min) may induce partially denatured proteins through stabilizing hydrogen bonds, which enhance the water-holding capacity of meat products (Fernández-Martin et al. 2002).

High-pressure treatment alters myofibril structure and depolymerizes and solubilizes myofibrillar proteins, affecting the water-holding capacity of meat and its comminuted products (Cheftel and Culioli 1997; Sun and Holley 2010). The presence of salt in comminuted meat products promotes the solubilization of myofibrillar proteins. A combination treatment of pressure and heat at low temperature

(1–10 °C) or high temperature (up to 70 °C) may favor partial denaturation of meat proteins. An increase in soluble protein content and partial denaturation of soluble proteins by high pressure benefit gelation and water binding of muscle proteins, improving WHC and reducing cooking loss (Macfarlane 1974; Macfarlane and Mckenzie 1984; Sikes et al. 2009). With a combination of temperature and selection of ionic strength and pH, high pressure is a promising tool to not only improve yield but also produce reduced-salt meat products with good or equivalent quality to high-salt meat products. To improve the yield and quality of meat products, the selection of high-pressure treatment conditions should be based on distinct sources of fish and meat as well as specific formulation of meat products.

18.7 Effects of High Pressure on Nutritional Values of Food Proteins

18.7.1 Digestibility

Due to rigid conformational structure, some native proteins such as intact globular proteins (β-Lg) and legume proteins are poorly digestible under gastric conditions (Kitabatake and Kinekawa 1998; Nielsen et al. 2002). Protein modification by high pressure may improve protein digestibility by loosening protein structure and enhancing accessibility for proteolytic enzymes. To increase nutritional values of proteins, it is important to know how high pressure can improve protein digestibility for better intestinal absorption.

High-pressure treatment exhibits a great improvement in digestibility of milk and soybean whey proteins. Zeece et al. (2008) observed that β-Lg after high-pressure treatment at 600–800 MPa exhibits rapid digestion with pepsin in less than 1 min under simulated gastric condition. More than 90 % of the digested peptides of pressure-treated β-Lg are less than 1500 Da (Zeece et al. 2008). Vilela et al. (2006) reported that high-pressure treatment at 550 MPa for one cycle or 400 MPa for three cycles improves the digestibility of whey protein isolates by pepsin and pancreatin and alters peptide profile of hydrolysates (Vilela et al. 2006). Penas et al. (2004) also reported that high-pressure treatment at 100 MPa or 200 MPa for 15 min at 37 °C increases hydrolysis of soybean whey proteins by trypsin and pepsin or by chymotrypsin. The soybean whey hydrolysates have five peptides smaller than 14 kDa by trypsin and chymotrypsin and 11 peptides by pepsin (Penas et al. 2004).

However, for certain legumes, high-pressure treatment does not result in a significant improvement of in vitro protein digestibility. Han et al. (2007) reported that soaking selected legumes (lentils, chickpeas, peas, and soybean) under high pressure at 621 MPa for 0.5 or 1 h results in an inconsistent or insignificant increase in in vitro protein digestibility as compared to simply soaking the legume beans for 12 h. Yin et al. (2008) examined the effect of pressure treatment at 200–600 MPa at 25 °C on vicilin-rich red kidney bean isolates and observed that while protein solubility

increases slightly at 400 MPa or greater, the trypsin digestibility of red kidney bean concomitantly decreases. Soluble protein aggregates formed under pressure were suggested to be responsible for the observed decrease in digestibility.

Based on these studies, high-pressure treatment can greatly improve the digestibility of milk and soybean whey proteins. However, the improvement of digestibility for proteins by high pressure depends on sources of proteins, conditions of pressure treatment, and types of enzymes.

18.7.2 Allergens

Many food-related allergies are associated with milk, soy, and egg white proteins. To tackle protein allergens, enzymatic hydrolysis is a technique that can be used to reduce the allergenicity of food proteins or to make hypoallergenic ingredients or foods. For example, some infant formulas include fully digested protein ingredients to avoid allergens in children. High-pressure modification of allergenic proteins may provide new sites or targets for enzymatic hydrolysis. Therefore, it is important to determine whether hydrolysis under high pressure can reduce allergenicity of proteins or eliminate protein epitopes.

High-pressure treatment promotes a rapid hydrolysis of milk proteins, reducing allergenic epitopes. Chicon et al. (2008b) reported that the hydrolysis of β-Lg, an important milk allergen, by chymotrypsin and trypsin can be achieved in 10–20 min under high pressure at 100–400 MPa. The hydrolysates exhibit a decrease in binding to immunoglobulin G (IgG) greater than hydrolysates produced in 8 h by chymotrypsin or 48 h by trypsin at atmospheric pressure. However, the hydrolysates retain some residual IgE-binding properties (Chicon et al. 2008b). Chicon et al. (2009) further reported that under high pressure at 400 MPa, WPI hydrolyzed by pepsin and chymotrypsin for 10 min exhibit as loss of α-Lac and β-Lg with production of large hydrophobic peptides. The hydrolysates have reduced human IgG- and IgE-binding properties (Chicon et al. 2009). However, high-pressure treatment prior to the hydrolysis exhibits different effects in reducing allergenicity of milk proteins. Chicon et al. (2008a, b) observed that β-Lg and WPI treated at 200 or 400 MPa without hydrolysis exhibit an increase in IgG binding but no increase in IgE binding. The WPI treated at 400 MPa exhibits an increase in hydrolysis by pepsin, but the hydrolysates are not reduced in IgE-binding properties (Chicon et al. 2008a, b).

Hydrolysis under high pressure also reduces the allergenicity of soy proteins and egg white proteins. Penas et al. (2006a) reported that 200 and 300 MPa treatment promotes hydrolysis of soybean whey protein by alcalase, neutrase, corolase 7089, and corolase PNL. The hydrolysates of soybean whey exhibit a decrease of immunochemical response to anti-Gly m 1 monoclonal antibodies (Penas et al. 2006b). Penas et al. (2006b) also observed that hydrolysates obtained by alcalase and neutrase treatment at atmospheric pressure or under 100 and 200 MPa do not show residual antigenicity. Hydrolysates obtained under 300 MPa by alcalase, neutrase, and corolase PNL do not exhibit residual antigenicity (Penas et al. 2006b).

Under 400 MPa, pepsin hydrolyzes egg ovalbumin protein in minutes with large and hydrophobic peptides. Some fragments in the hydrolysates are identified as carrying identified IgE-binding epitopes. Although the antigenicity of the hydrolysates is lower than those made by hydrolysis for hours at atmospheric pressure, the hydrolysates retained residual IgG- and IgE-binding properties (López-Exposito et al. 2008).

Protein allergen epitopes are parts of the amino acid sequence and potentially part of hydrolyzed proteins. Although allergenic proteins are significantly hydrolyzed under high pressure, the hydrolyzed peptides still retain the ability to can bind IgE or IgG. Extensive hydrolysis of protein can destroy allergen epitopes. According to recommendations of the American Academy of Pediatrics, extensively hydrolyzed milk proteins with fragments less than 1000 Da or amino acid formulations can be used for infants with cow's milk allergy. However, extensive hydrolysis greatly affects the protein palatability, nutritional properties, and functional properties (foaming, emulsifying, gelation). It is challenging to apply high pressure in conjunction with enzymatic hydrolysis to produce hypoallergenic foods and, at the same time, retain protein palatability and nutritional and functional properties to meet the food quality requirements.

High pressure can promote protein enzymatic hydrolysis and reduces antigenicity of protein by unfolding native protein structure and exposing new regions including allergen epitopes to be attacked by proteinases. Chicon et al. (2009) reported that WPI hydrolysates with low antigenicity obtained by pepsin at 400 MPa had improved emulsion activity at pH 7.0 and heat stability at isoelectric point. An appropriate selection of pressure-treatment conditions (pressure level, durations, pH, and temperature), types of enzymes, and hydrolysis conditions may result in hydrolysates with not only reduced allergenicity for non-sensitizing patients or used as ingredients for hypoallergenic foods with good functional properties (Chicon et al. 2009).

18.7.3 Bioactive Peptides

Bioactive peptides are peptides with certain amino acid sequences exerting physiological activities with health benefits. Enzymatic hydrolysis of food proteins can release bioactive peptides. For example, bioactive peptides in the primary sequence of whey proteins can be released by protein hydrolysis. The type of bioactive peptides obtained from proteolysis depends on the amino acid sequence and three-dimensional structure of proteins. High pressure unfolds proteins, exposes new cleavage sites, and promotes enzymatic hydrolysis producing different molecular-weight peptides. Therefore, enzymatic hydrolysis under high pressure is a new tool to produce bioactive peptides for health benefits. However, research reports in this area are limited.

Vilela et al. (2006) reported that 550 MPa at 26–27 °C for one cycle and at 400 MPa for three cycles improve digestibility and produces an altered peptide profile of whey protein isolates by pepsin and pancreatin (Vilela et al. 2006). Vilela et al. (2006) found that the peptides with a molecular weight less than 1 kDa

increases the levels of reduced GSH and total GSH in mutant CFRT (cystic fibrosis transmembrane conductance regulator) cells (human tracheal epithelial cells) compared to the peptides from native WPI hydrolysates (Vilela et al. 2006). The results suggest that bioactive peptides produced from pressurized whey protein hydrolysates may improve the function as anti-inflammatory agents via inhibition of IL8 release in cells with the mutant CFRT condition.

Quiros et al. (2007) studied the release of bioactive peptides from ovalbumin under high pressure at 100–400 MPa with chymotrypsin, trypsin, and pepsin. Compared to the control at atmospheric pressure, high-pressure treatment prior to hydrolysis does not improve the release of bioactive peptides—YAEEFRYPIL, FRADHPFL, and RADHPFL—which have angiotensin converting enzyme (ACE)-inhibitory properties. However, under high pressure, the release of bioactive peptides is accelerated. The amount of these bioactive peptides under high-pressure treatment for one hour is greater than those with the treatment at atmospheric pressure for eight hours. The authors suggest that hydrolysis under high pressure can be used to promote the proteolysis of ovalbumin for the quick production of specific bioactive peptides (Quiros et al. 2007).

18.8 Prospectives of High-Pressure Processing on Food Proteins

High pressure is a relative new technological tool applied to modification of food proteins. Under certain pressure conditions, food proteins exhibit improved properties such as solubility, gelation, emulsification, foaming, coagulation, water-holding capacity, digestibility, reduction of food allergen, and release of bioactive peptides. However, in other conditions, high-pressure treatment can have opposite impact on protein functionality such as emulsifying and foaming properties. Based on available studies, pressure-modified proteins have not exhibited an improvement in flavor-binding properties. It is a challenge to process protein ingredients eliminating allergen epitopes during hydrolysis under high pressure while retaining desirable functional properties. However, high-pressure treatment exhibits great potential to produce low-salt meat products.

Protein functional properties depend on the conformational structure of native or modified proteins. The conditions of high-pressure treatment determine the structures and functional properties of modified proteins. To improve food protein functional properties by high-pressure technology, it is essential to further understand structures of various native proteins, changes of proteins under or after high-pressure treatment, denaturation of proteins by a combination of high pressure and temperature, and effects of environmental factors such as pH, ionic strength, and other food components on protein modifications during high-pressure treatment.

Compared to thermal processing, high pressure is an emerging technology for improving quality of protein-based food products. It is crucial to better understand the elliptic phase diagram in the area of protein denaturation and inducing a stable

intermediate state of protein with improved functional properties using combinations of pressure and temperature. In addition, there is a need to further understand how to combine high pressure with proteolysis to improve digestibility of plant proteins, produce rich bioactive peptide ingredients, and produce hypoallergenic protein ingredients and food products.

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Chapter 19
Effects of High Pressure on Enzymes

Indrawati Oey

Abstract This book chapter describes the catalytic activity of enzymes and the underlying mechanism and associated kinetics of enzyme inactivation during high hydrostatic pressure combined with temperature treatment. Effects of combined pressure and temperature on enzyme stability for various food quality-related enzymes such as amylase, lipoxygenase, myrosinase, polygalacturonase, pectin-methylesterase, peroxidase, polyphenoloxidase and protease are discussed. Since enzymes have different pressure and temperature sensitivities, selective combinations of pressure, temperature and time can be identified to create novel properties of food products.

Keywords Enzymes • Stability • Catalytic activity • Kinetics • Inactivation • Modelling • Pressure • Temperature • Protein structure • Process design

19.1 Introduction

Effects of pressure (HP) and temperature (*T*) on protein stability have been studied for more than a half century (e.g. Suzuki and Kitamura 1960; Brandts et al. 1970; Hawley 1971; Zipp and Kauzmann 1973; Masson and Balny 1990; Mozhaev et al. 1994; Hayashi et al. 1998; Scirè et al. 2010, Ying et al. 2012). In general, the pressure and temperature stability of protein can be depicted as an elliptical contour (Fig. 19.1) (Suzuki and Kitamura 1960; Brandts et al. 1970; Hawley 1971) that implies possibilities to achieve protein denaturation by lowering temperature, elevating pressure, elevating temperature or a combination of these factors. Since enzymes are complex globular proteins, knowledge on the pressure and temperature stability of protein is used as a basis to understand the underlying mechanisms and kinetics of enzyme stability and activity under pressure.

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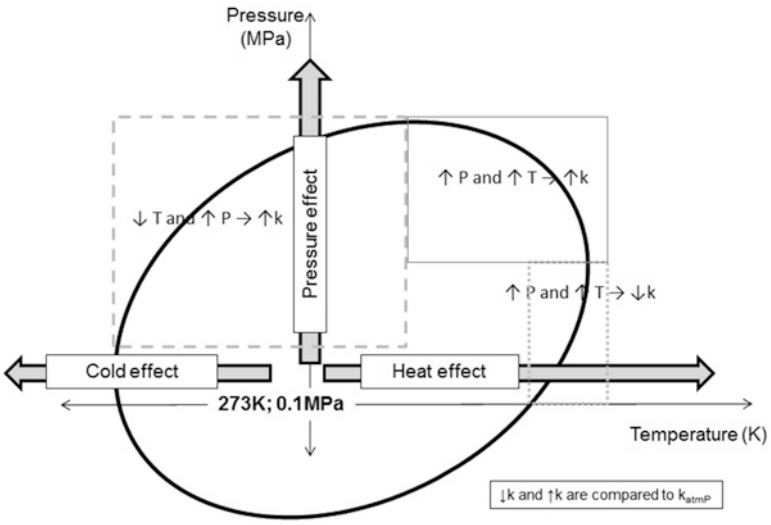

Fig. 19.1 Schematic pressure and temperature diagram of enzyme inactivation (*P* pressure, *T* temperature; *k* inactivation rate constants)

19.2 Understanding Protein Stability Under Pressure

Protein folding is one of the most prominent parameters to determine the degree of protein denaturation. At low pressure (<200 MPa), a reversible protein denaturation followed by slow refolding process after pressure release often occurs (conformational drift combined with hysteric behaviours also observed after pressure release), whilst at pressure beyond 300 MPa irreversible protein denaturation takes place and results in chemical modifications or unfolding of single-chain proteins (Cheftel 1991; Heremans 1992; Masson 1992). During high hydrostatic pressure treatment combined with temperature (HP/T), changes of protein structure are governed by *Le Chatelier* principle stating that pressure favours the reactions with negative volume changes. The principle of microscopic ordering also dictates that the degree of molecule ordering is increased by elevating pressure level (Cheftel 1991; Heremans 1992; Masson 1992; Mozhaev et al. 1994).

Protein has a complex three-dimensional conformational structure composing of four different hierarchies of protein structure. *Primary protein structure* composes of amino acids in a sequential order together with covalent peptide bonds and disulphide bridges (if any). One of the uniqueness of HP/T treatments is a relative pressure inability to alter covalent bonds. As a result, pressure has no or limited effects on the primary structure of protein due to perpetuation of covalent bonds in contrast to temperature effect at ambient pressure (Cheftel 1991; Heremans 1982, Mozhaev et al. 1994).

Secondary structure of protein is rather stable under pressure. This spatial protein structure composes of polypeptide chain forming α-helices and β-structures such as β-bends and β-pleated sheets, linked together with intra- or inter chain molecular hydrogen bonds. At extreme pressure level (>700 MPa and ambient temperature), this structure can be altered when the hydrogen bonds which maintain the secondary structure are disrupted leading to irreversible protein denaturation (Balny and Masson 1993). Scirè and co-workers (2010) have observed that α-helices and β-sheets have different stability under pressure. α-Helices (an ordered and particularly stable structure with a high density that restricts interactions with other molecules) are more sensitive towards pressure lower than 500 MPa compared to β-sheets (a zigzag structure, more stretched than the α-helix), whilst at pressure above 500 MPa (up to 900 MPa) the sensitivity of β-sheets is reversed. The structure of α-helices and β-sheets changes at pressure above 1000 MPa. Yi et al. (2012) have observed in mushroom polyphenoloxidase that increasing pressure level up to 1600 MPa decreased the percentage of α-helix. This change could explain the partial inactivation of PPO under pressure since α-helix is vital for its catalytic activity. In this study, the surface sulphydryl group of PPO under pressure up to 1000 MPa was similar to native enzymes; however, elevating pressure up to 1200 MPa significantly increases the surface sulphydryl group, indicating the occurrence of protein unfolding. The proportion of α-helix and β-structure also determines the pressure and temperature stability of protein. For example, the secondary structure of *Aspergillus aculeatus* pectinmethylesterase/PME which consists of a substantial amount of β-helix conformation needs pressure level greater than 1000 MPa to induce changes of this protein structure (Dirix et al. 2005). This phenomenon could explain the extreme pressure stability of PME such as carrot PME (Ly-Nguyen et al. 2003a), green bell PME (Castro et al. 2005), orange PME (Sampedro et al. 2008) and peach PME (Boulekou et al. 2010).

The three-dimensional arrangement of *tertiary* (a polypeptide chain containing regions of well-defined structure composing of α-helices, β-bends and β-pleated sheets or random coil secondary structures stabilized by hydrogen bonds, hydrophobic interactions, electrostatic interactions and van der Waals interactions) and *quaternary* (associated with non-covalent bonds of protein units stabilized by hydrogen bonds and hydrophobic and electrostatic interactions including disulphide bonds) structure is mostly lost due to pressure (Heremans 1993; Mozhaev et al. 1996). Pressure beyond 150–200 MPa disturbs non-covalent bonds, including hydrophobic bonds and electrostatic interactions (Cheftel 1991; Balny and Masson 1993) leading to conformational changes of these protein structures which results in imperfect packing of the protein molecule, dissociation of oligomeric enzymes to subunits and protein unfolding with large volume changes (Cheftel 1991; Balny and Masson 1993; Silva and Weber 1993; Mozhaev et al. 1994). Readers can also refer to Chap. 18 for more details on pressure effects on proteins.

19.3 Effects of HP/T Treatment on Enzyme Inactivation

Conformational alteration of protein structure (mostly referred to as "denaturation") could entail a reversible or an irreversible loss of its ability to perform a certain biological function such as loss in catalytic activity of enzymes which mostly refers to "inactivation" (enzyme inactivation). However, it should be aware that changes in interactions between macromolecules and modification of functional groups with or even without conformational changes could also result in loss of catalytic activity of enzymes. Effects of HP/T treatment on inactivation (based on residual enzyme activity after HP/T treatment at ambient pressure *post factum*) of food-related enzymes have been investigated in different enzyme systems, ranging from (partial) purified enzymes dissolved in buffer solution and food juices to enzymes endogenously present in intact food matrices (in situ) as summarized in Table 19.1.

Generally increasing the level of pressure and temperature enhances the inactivation of enzymes (Fig. 19.1, full line frame), for example, alkaline phosphatase/ALP, amylase, hydroperoxide lyase/HPL, lipoxygenase/LOX, myrosinase/MYR, pectinmethylesterase/PME, peroxidase/POD, polygalacturonase/PG, polyphenoloxidase/PPO, β-glucosidase, trimethylamine-N-oxide demethylase and xylanase (Table 19.1). In most studies, enzymes can be irreversibly inactivated due to HP/T treatment. The rank of pressure and temperature stability is very much dependent on enzyme types and sources. The level of enzyme inactivation during HP/T treatment is affected by process intensity (e.g. pressure level, temperature level and pressure-temperature holding time), enzyme concentration, enzyme purity, complexity of environmental condition such as pH, salt concentrations, molarity, buffer type, presence of inhibitor/activator, composition of food medium, food matrix, etc. Some studies also reported a reversible enzyme inactivation due to HP/T treatment, for example, carrot POD (Soysal et al. 2004), and pressure-induced enzyme release from cell membrane resulting in higher enzyme activity such as PPO in apple juice (Buckow et al. 2009) and POD in green beans (Akyol et al. 2006).

At high temperature (close to the temperature of thermal inactivation at ambient pressure), some enzymes such as pectinmethylesterase, polyphenoloxidase, milk alkaline phosphatase and soybean lipoxygenase show reverse stability at elevated pressure (mostly observed at pressure up to 200 MPa) compared to that at ambient pressure (Fig. 19.1, dotted line frame). The rate of enzyme inactivation (referred to as loss of enzyme activity at ambient pressure) at high temperature is slowed down when pressure level is increased above 0.1 MPa. In literature, this phenomenon is mostly called as "antagonistic" effect of pressure on thermal inactivation. Increasing temperature (>70 °C) at atmospheric pressure mostly disturbs the stability of covalent and non-covalent bonds leading to changes in protein structure due to aggregation, incorrect folding or chemical alteration. Thermodynamically, protein denaturation due to heating at atmospheric pressure leads to very large changes in entropy (less ordered conformational structure) surpassing the absolute value of enthalpy change resulting in a negative change in Gibbs free energy making the denaturation favourable. At elevated pressure the degree of protein molecule order-

Table 19.1 Combined pressure-thermal effect on enzyme inactivation*

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Alkaline phosphatase (ALP)				
Milk ALP	Raw bovine milk[a]	25–63 °C**; 0.1–700 MPa; kinetic study[b] within max. time frame of 14–300 min	• Irreversible inactivation • First-order inactivation kinetics • $P > 400$ MPa: $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value) • $P \leq 400$ MPa; 55–63 °C: antagonistic P effect on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Ludikhuyze et al. (2000a)
α-Amylase				
Bacillus amyloliquefaciens	Commercial enzyme dissolved (100 g/L) in Tris–HCl buffer (0.1 M; pH 8.6)	10–45 °C; 150–680 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value)	Grauwet et al. (2010)
B. subtilis	Commercial enzyme dissolved (1 g/L) in MES (50 mM; pH 5.0)	10–50 °C, 0.1–750 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value)	Grauwet et al. (2009)
B.subtilis	Commercial enzyme dissolved (15 mg/mL) in Tris–HCl buffer (10 mM; pH 8.6)	30–50 °C; 550 MPa and 40 °C; 450–650 MPa; kinetic study within time frame of 81 min	• Enzyme denaturation using gel electrophoresis • First-order denaturation kinetics • $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value)	Ludikhuyze et al. (1997)
Aspergillus niger	Commercial glucoamylase dissolved (0.5 mg/mL) in ACES buffer (0.1 M)	40–95 °C; up to 1400 MPa; kinetic study	• Two fractions observed: pressure labile (79 %) and stable (21 %) fractions • Irreversible inactivation • Biphasic model based on first-order inactivation kinetics • $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value)	Buckow et al. (2005)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
β-Amylase				
Barley	Commercial β-amylase dissolved (0.0625 mg/mL) in ACES buffer (0.1 M; pH 5.6)	20–70 °C; up to 700 MPa; kinetic study	• Irreversible inactivation • n^{th}-order inactivation kinetics ($n = 1.4$) • $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value)	Heinz et al. (2005)
Hydroperoxide lyase (HPL)				
Tomato HPL	Tomato juice	25–90 °C; 100–650 MPa; 15 min (including 3 min of equilibration time)	• Irreversible inactivation • <300 MPa/25 °C/15 min: 20 % inactivation • 650 MPa/25 °C/15 min: 80 % inactivation • $\uparrow P$ and T resulted in ↑ inactivation	Rodrigo et al. (2007)
Glucosidase				
Almond β-glucosidase	Purified enzyme dissolved in McIlvaine buffer pH 5.0	30–70 °C; 50–600 MPa; kinetic study up to 120 min	• Irreversible inactivation • First-order inactivation kinetics • $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value)	Terefe et al. (2013)
Lipoxygenase (LOX)				
Tomato LOX	Partially purified[c] tomato LOX dissolved in MOPS/KOH (10 mM; pH 6.8)	10–60 °C; 100–650 MPa; kinetic study within time frame of 1 h	• Irreversible inactivation • First-order inactivation kinetics • $T \geq 20$ °C: $\uparrow P$, T and time resulted in ↑ inactivation ($\uparrow P$ and $T \rightarrow \uparrow k$ value) • $T < 20$ °C: $\downarrow T$, $\uparrow P$ and ↑time-enhanced inactivation ($\downarrow T$ and $\uparrow P \rightarrow \uparrow k$ value) • <550 MPa; 50–60 °C: antagonistic P effect on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Rodrigo et al. (2006a)
	Tomato juice	25–90 °C; 100–650 MPa; 15 min (including 3 min of equilibration time)	• Irreversible inactivation • 550 MPa/25 °C/15 min: complete inactivation • $\uparrow P$ and T resulted in ↑ inactivation	Rodrigo et al. (2007)

	Tomato pericarp tissue	(−26)–20 °C; 100–500 MPa; 13 min	• Irreversible inactivation • Combined *P* and subzero temperature: ↑inactivation • ≤500 MPa/20 °C: no inactivation • 400–500 MPa/−10 and −20 °C/13 min: complete inactivation • 100–500 MPa/−26 °C/13 min: max. 25 % inactivation	Van Buggenhout et al. (2006)
Soybeans LOX	Commercial purified soybean LOX dissolved Tris–HCl buffer (0.4 mg/mL 10 mM; pH9)	(−15)–68 °C; 0.1–650 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 30 °C: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑ k value) • T < 30 °C: ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑k value) • <200 MPa/65 °C: antagonistic P effect on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Indrawati et al. (1999)
	Soybean milk and crude extract centrifugated from soybean milk	5–60 °C; 0.1–650 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 10 °C: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • T < 10 °C: ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑*k* value)	Wang et al. (2008)
Green beans LOX	Green beans juice	(−10)–60 °C; 200–700 MPa; kinetic study within max. time frame of 210 min	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 20 °C: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • T < 20 °C: ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑*k* value)	Indrawati et al. (2000a)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
	Green beans juice	(−10)–70 °C; 50–650 MPa; kinetic study within max. time frame of 210 min	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 10 °C: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • T < 10 °C: ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑k value)	Indrawati et al. (2000b)
	Green beans	(−10)–70 °C; 50–550 MPa; kinetic study within max. time frame of 150 min	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 10 °C: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • T < 10 °C: ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑*k* value)	Indrawati et al. (2000b)
Green peas LOX	Green pea juice	(−15)–70 °C; 50–625 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 10 °C, ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • T < 10 °C, ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑k value) • <200 MPa/>60 °C: antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Indrawati et al. (2001)
	Green peas	(−10)–70 °C; 100–500 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • T ≥ 10 °C, ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • T < 10 °C, ↓T, ↑P and ↑time-enhanced inactivation (↓T and ↑P → ↑k value) • <200 MPa/>60 °C: antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Indrawati et al. (2001)

Myrosinase (MYR)

Mustard seeds MYR	Partially purified dissolved in broccoli juice (pH adjusted to 6.5)	40–60 °C; ≤ 700 MPa; max. treatment time =2 h	• High pressure stability • No inactivation at 55 °C and 600 MPa for 2 h.	Van Eylen et al. (2008a)
Broccoli MYR	Partially purified dissolved in phosphate buffer (0.1 M; pH 6.55)	20 °C/350–500 MPa and 35 °C/150–450 MPa; kinetic study within max. time frame of 80 min	• Irreversible inactivation • <350 MPa/35 °C: antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa) • Inactivation described by consecutive step model based on first-order kinetics	Ludikhuyze et al. (1999)
	Broccoli heads	15–60 °C; 50–500 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • ≥150 MPa/15–50 °C: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • ≤100 MPa; 55–60 °C: antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Van Eylen et al. (2008b)
	Broccoli juice	10–60 °C; 100–600 MPa; kinetic study	• 3.4–86.8 % inactivation after pressure build-up • T ≤ 40 °C, ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • <200 MPa/50–60 °C: antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Van Eylen et al. (2007)
Green cabbage MYR	Partially purified using ammonium precipitation, lyophilized and afterwards diluted in deionized water (25 mg/mL)	35–50 °C; 100–400 MPa; kinetic study up to 60 min	• Irreversible inactivation • At 10 °C, 300–450 MPa, inactivation can be described by consecutive step model based on first-order kinetics • 100–400 MPa/35–50 °C: inactivation described by first-order kinetics	Ghawi et al. (2012)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Pectinmethylesterase (PME)				
Apple PME	Golden delicious and Judaine apples	15–65 °C; 200–600 MPa; kinetic study within max. time frame of 10.5 min	• Irreversible inactivation • Incomplete inactivation (mostly only inactivation of pressure labile isozymes)	Baron et al. (2006)
Orange PME	Commercial purified orange peel PME; clear apple juice	25 °C; 200–400 MPa; kinetic study within max. time frame of 180 min	• Irreversible inactivation • 400 MPa/25 °C/25 min: highest enzyme inactivation (1 log unit reduction)	Riahi and Ramaswamy (2003)
	Greek navel orange juice	30–60 °C; 100–700 MPa; kinetic study within max. time frame of 30 min	• Irreversible inactivation of pressure labile isozymes • ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) but not at high temperature and low pressure • Antagonistic effect of pressure on thermal inactivation	Polydera et al. (2004)
	Valencia and Navel orange juice	20 °C; 600 MPa; 60 s	Incomplete inactivation	Bull et al. (2004)
	Orange juice	300, 350, 400 MPa; 1–3 pressure cycles; 20–120 min	• Irreversible inactivation • Pressure cycling had statistically less significant effects on PME inactivation rather than pressure level, temperature and pressure holding time	Basak et al. (2001)
	Orange juice	25, 37.5, 50 °C; 400, 500, 600 MPa; kinetic study within max. time frame of 30 min	• Less than 1 log unit irreversible inactivation	Nienaber and Shellhammer (2001)

	Valencia Navel orange juice-milk-based beverage	25–65 °C; 0.1–700 MPa; 2–75 min	• Irreversible inactivation • Two fractions observed: pressure labile and stable fractions • 7 % pressure stable fraction • T ≤ 55 °C/P ≤ 550 MPa, only inactivation of pressure labile fraction • P ≥ 550 MPa and constant temperature, k value for inactivation of pressure labile fraction was higher (more pressure sensitive) than that of pressure stable fraction • PME in orange juice-milk beverage was more stable than in orange juice alone (without milk)	Sampedro et al. (2008)
Tomato PME	Purified with affinity chromatography; dissolved in citrate buffer (50 mM; pH 4.4)	25–66 °C; 550–700 MPa; kinetic study	• Pressure stable • Antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated *P* was lower than at 0.1 MPa)	Fachin et al. (2002a)
	Purified by affinity chromatography and followed by cation exchange chromatography; dissolved in citrate buffer (0.1 M; pH 6)	20 and 40 °C; 100–800 MPa; kinetic study within max. time frame of 30 min	• Irreversible inactivation • One fraction of pressure labile isozyme found • First-order kinetics • 600 MPa/40 °C/6 min: one log unit of inactivation • 600 MPa/20 °C/18 min: one log unit of inactivation	Plaza et al. (2007)
	Tomato pericarp tissue	(−26)–20 °C; 100–500 MPa; 13 min	No/limited inactivation	Van Buggenhout et al. (2006)
	Tomato juice	60–75 °C; 0.1–800 MPa	At 75 °C, antagonistic effect of pressure on thermal inactivation (inactivation at elevated *P* was lower than at 0.1 MPa)	Stoforos et al. (2002)
	Tomato juice	• 50 °C; 550, 600, 700 MPa; kinetic study within max. time frame of 60 min • 62 °C; 500 MPa; kinetic study within max. time frame of 70 min	• Very high resistance towards elevated pressure • Antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Fachin et al. (2002b)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
	Tomato juice	4, 25, 50 °C; 100–500 MPa; 10 min	• High resistance towards elevated pressure • 200 MPa/25 °C/10 min: 27.8 % inactivation	Hsu (2008)
Strawberry PME	Purified with affinity chromatography; dissolved in Tris–HCl buffer (20 mM; pH 7)	10 °C; 850–1000 MPa; kinetic study within max. time frame of 600 min	• High resistance towards elevated pressure • Pressure labile (90 %) and stable (10 %) fractions observed • Only pressure labile fraction inactivated • ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) for pressure labile fractions	Ly-Nguyen et al. (2002a)
White grapefruit PME	Purified with affinity chromatography; dissolved in Tris buffer (20 mM; pH7)	10–62 °C; 100–800 MPa; kinetic study	• Irreversible inactivation • High resistance towards elevated pressure • Pressure labile (80 %) and stable (20 %) fractions observed • Only pressure labile fraction inactivated • ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • Antagonistic effect of low pressure (up to 200 MPa) on thermal inactivation	Guiavarc'h et al. (2005)
Green pepper PME	Crude extract and purified with affinity chromatography; dissolved in citrate buffer (pH 5.6)	25–60 °C; 400–800 MPa; 15 min	• Pressure stable • PME in crude extract is less pressure stable than purified PME in citrate buffer	Castro et al. (2005)
	Purified with affinity chromatography; dissolved in citrate buffer (pH 5.6)	10–62 °C, 100–800 MPa; kinetic study	• Irreversible inactivation • High resistance towards elevated pressure • Pressure labile and stable fractions observed • Effective inactivation only in the pressure labile fraction • At 800 MPa/10–30 °C, inactivation of pressure stable fraction was observed • ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • ≥350 MPa; T>54 °C: antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Castro et al. (2006a)

Green bell PME	Green bell pepper and pepper puree	25, 40 and 60 °C; 0.1–500 MPa; 15 min	• At 25 °C and ↑P: residual PME activity increased • At 40 °C and ↑P up to 300 MPa, residual PME activity increased and decreased by further pressure increase • At 60 °C, around 40 % inactivation • PME in puree was more stable than intact tissue	Castro et al. (2005)
	Green bell pepper	Room temperature (18–20 °C); 100 and 200 MPa; 10 and 20 min	Pressure-induced enzyme extractability resulting in higher residual enzyme activity	Castro et al. (2008)
Carrot PME	Purified with affinity chromatography; dissolved in Tris buffer (20 mM; pH7)	10 °C; 600–700 MPa; kinetic study within max. time frame of 20 h	• Irreversible inactivation • High resistance towards elevated pressure • Pressure labile (90–95 %) and stable (5–10 %) fractions observed • Only pressure labile fraction effectively inactivated • ↑P, *T* and time resulted in ↑ inactivation (↑*P* and *T*→ ↑*k* value)	Ly-Nguyen et al. (2002b)
	Purified with affinity chromatography; dissolved in Tris buffer (20 mM; pH7)	10–65 °C; 100–825 MPa; kinetic study	• Irreversible inactivation • Pressure labile (94–95 %) and stable (5–6 %) fractions observed • Only pressure labile fraction effectively inactivated • ↑P, T and time resulted in ↑ inactivation (↑P and T→ ↑k value) • ≤300 MPa; > 50 °C: antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Ly-Nguyen et al. (2003a)
Banana PME	Purified with affinity chromatography; dissolved in Tris buffer (20 mM; pH7)	10 °C; 600–700 MPa; kinetic study	• Irreversible inactivation • Pressure labile (92 %) and stable (8 %) fractions observed • Only pressure labile fraction effectively inactivated • ↑P, T and time resulted in ↑ inactivation (↑P and T→ ↑k value)	Ly-Nguyen et al. (2002c)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Mango nectar PME	Mango pulp	<17 °C (not identified clearly – initial or processing temperature), 275, 345 and 414 MPa and times for 1, 2 and 4 min with come-up time of approx. 3–4 min	• No inactivation • Increase in enzyme activity at 346 and 414 MPa. after 4 min	Bermúdez-Aguirre et al. (2011)
Plum PME	Purified with affinity chromatography	25 °C; 650–800 MPa; kinetic study	• First-order kinetic inactivation	Nunes et al. (2006)
Peach PME	Peach pulp	30–70 °C; 100–800 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • ≤600 MPa; 70 °C: antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa) • ↑P, T and time resulted in ↑ inactivation (↑P and $T \rightarrow \uparrow k$ value)	Boulekou et al. (2010)
Peroxidase (POD)				
Kiwifruit POD	Partially purified kiwi POD	10–50 °C; 200–500 MPa; kinetic study within max. time frame of 30 min	• Irreversible inactivation • Different isozymes have different resistance towards pressure • At 30 and 50 °C, synergistic effect of increases in pressure and temperature on inactivation ↑P and T resulted in ↑ inactivation • At constant pressure and 50 °C, prolonging treatment time remarkably enhances the inactivation (↑P and $T \rightarrow \uparrow k$ value) • 600 MPa/50 °C/30 min: max. 70 % inactivation	Fang et al. (2008)
Horseradish POD	Commercial purified horseradish POD; dissolved in Tris buffer (50 mM; pH7) with H_2O_2 (50 mM) and guaiacol (0.23 M)	25–40 °C; 100–500 MPa; kinetic study within max. time frame of 5 min	• Irreversible inactivation	García et al. (2002)

Carrot POD	Carrot	(−26)–20 °C; 100–500 MPa; 13 min	No/limited inactivation	Van Buggenhout et al. (2006)
	Carrot	20–50 °C; 250–450 MPa; kinetic study within max. time frame of 60 min	At 20 °C, no clear tendency of inactivation was observed by increasing pressure	Akyol et al. (2006)
	Carrot	25–45 °C; 0.1–600 MPa; 15 min	• Reversible inactivation found at 506 MPa/25, 35, 40 °C for 15 min treatment • 91 % irreversible inactivation at 600 MPa/40 °C for 15 min treatment	Soysal et al. (2004)
Green beans POD	Green beans	20–50 °C/250–450 MPa; kinetic study within max. time frame of 60 min	350 MPa/50 °C: enzyme activity increased from 55 % to 84 % by prolonging treatment time from 30 to 60 min	Akyol et al. (2006)
Green bell pepper POD	Green bell pepper	Room temperature (18–20 °C); 100 and 200 MPa; 10 and 20 min	70 % enzyme inactivation	Castro et al. (2008)
Green peas POD	Green peas	20–50 °C; 250–450 MPa; kinetic study within max. time frame of 60 min	• No effective inactivation was observed • 30 °C/300 and 350 MPa: higher enzyme activity was found (probably due to enzyme extractability)	Akyol et al. 2006
Lychee POD	Lychee	20, 40, 60 °C; 200, 400, 600 MPa; 10 and 20 min	• At 200 MPa, increase in enzyme activity after HP treatment. *P* treatment at 40 °C gave greater effect than at 20 and 60 °C • 600 MPa/60 °C/20 min: 50 % inactivation	Phunchaisri and Apichartsrangkoon (2005)
Red bell pepper POD	Red bell pepper	Room temperature (18–20 °C); 100 and 200 MPa; 10 and 20 min	40 % enzyme inactivation	Castro et al. (2008)
Strawberry POD	Strawberry puree ("Festival" and "Aroma" cultivars)	24–90 °C, 100–690 MPa; kinetic study between 5 and 15 min	• Irreversible inactivation • First-order inactivation kinetics • ↑*P* and *T* resulted in ↑ inactivation (↑*P* and *T*→ ↑*k* value)	Terefe et al. (2010)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Lactoperoxidase	Milk and whey	20–65 °C; ≤ 700 MPa; kinetic study within max. time frame of 140 min	• No effective inactivation was observed • 700 MPa; 15–65 °C: no inactivation • 73 °C; 150–750 MPa: extreme antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa) • First-order inactivation kinetics • 30 °C/300 and 350 MPa: higher enzyme activity was found (probably due to enzyme extractability) • Higher stability in milk than in whey	Ludikhuyze et al. (2001)
Polygalacturonase (PG)				
Tomato PG	Purified with cation exchange chromatography; dissolved in Na-acetate buffer (40 mM; pH 4.4)	25 °C; 500–800 MPa; 15 min	• PG2 had a higher resistance towards pressure than PG1 • β-Subunit was pressure stable protein	Peeters et al. (2004)
	Purified with cation exchange chromatography; dissolved in Na-acetate buffer (40 mM; pH 4.4)	25 °C; ≤ 500 MPa; 17 min (including 2 min equilibration time)	• ≤300 MPa, no inactivation of PG1 and PG2 • 300–500 MPa: PG1 and PG2 inactivation found • Complete inactivation of PG1 at mild pressure treatment • No influence of tomato varieties on PG pressure stability	Rodrigo et al. (2006b)
	Partially purified tomato PG; dissolved in NaCl solution (0.5 M)	5–50 °C; 300–600 MPa; kinetic study	• Irreversible inactivation • Pressure labile and stable fractions found • Only pressure labile fraction effectively inactivated • ↑P, T and time resulted in ↑ inactivation (↑P and $T \rightarrow \uparrow k$ value)	Fachin et al. (2002b)
	Tomato pericarp tissue	(−26)–20 °C; 100–500 MPa; 13 min	• >300 MPa; 20 °C: ↑P, T and time resulted in ↑ inactivation • 500 MPa/20 °C/13 min: 89 % inactivation	Van Buggenhout et al. (2006)
			• 100–500 MPa/−10 to −26 °C/13 min: no/limited inactivation	

	Tomato juice	5–55 °C; 200–550 MPa; kinetic study within max. time frame of 210 min	• Irreversible inactivation • First-order inactivation model • ↑*P*, *T* and time resulted in ↑ inactivation (↑*P* and *T* → ↑*k* value)	Fachin et al. (2003)
	Tomato juice and pieces	25 °C; 0.1–500 MPa; 17 min (including 2 min of equilibration time)	• PG in tomato juice had higher pressure resistance towards pressure than in tomato pieces • 400 MPa/25 °C/17 min: 70 % inactivation • 600 MPa: complete inactivation found • *P* resulted in dissociation of stable PG fraction (PG1) • Different behaviour of PG inactivation under pressure compared to thermal treatment at 0.1 MPa	Peeters et al. (2004)
	Tomato juice	4, 25, 50 °C; 100–500 MPa; 10 min	• Irreversible inactivation • Beyond 400 MPa/4 and 25 °C/10 min: max. 90 % inactivation • 100–500 MPa/50 °C/10 min: max. 30 % inactivation	Hsu (2008)
Polyphenoloxidase (PPO)				
Apple PPO	Partially purified golden delicious apple PPO; dissolved in phosphate buffer (0.1 M, pH 6)	25 °C/800 and 900 MPa; kinetic study within max. time frame of 210 min	• Irreversible inactivation • First-order inactivation model • ↑P, T and time resulted in ↑ inactivation • *Va* value: −35.62 cm^3/mol	Weemaes et al. (1998)
	Cloudy apple juice from Boskop apples pretreated at 400 MPa/20 °C/5 min	20–80 °C/0.1–700 MPa; kinetic study within max. time frame of 60 min	• 200–500 MPa/room T and 0.1 MPa/45–55 °C: 65 % increase in PPO activity • Inactivation kinetics described by n^{th}-order reaction model with n = 2.2 • ≥300 MPa: ↑P, T and time resulted in ↑ inactivation (↑P and T → ↑k value) • <300 MPa/60–75 °C: antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated *P* was lower than at 0.1 MPa)	Buckow et al. (2009)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Avocado PPO	Partially purified South African avocado PPO; dissolved in phosphate buffer (0.1 M, pH 7)	25 °C/800 and 900 MPa; kinetic study within max. time frame of 210 min	• Irreversible inactivation • First-order inactivation model • ↑P, T and time resulted in ↑ inactivation • *Va* value: −22.38 cm^3/mol	Weemaes et al. (1998)
Grape PPO	Partially purified white grape PPO; dissolved in phosphate buffer (0.1 M, pH 7)	25 °C/700 and 850 MPa; kinetic study within max. time frame of 210 min	• Irreversible inactivation • First-order inactivation model • ↑*P*, *T* and time resulted in ↑ inactivation • *Va* value: −28.13 cm^3/mol	Weemaes et al. (1998)
	Partially purified grape PPO; dissolved in McIlvaine buffer (pH 4 and 5) and grape juice	10–60 °C; 400–800 MPa; 17 min (including 2 min equilibration time)	• ↑*P* and *T* resulted in ↑ inactivation • Different pressure stability of PPO in buffer (pH 4 and 5) and in grape juice	Rapeanu et al. (2006)
	Partially purified Victoria grape PPO; dissolved in McIlvaine buffer (pH 3–6)	25 °C; 400–800 MPa; 15 min	• Increasing pH from 3 to 6, increased pressure resistance	Rapeanu et al. (2005a)
	Partially purified Victoria grape PPO; dissolved in McIlvaine buffer (pH 3–6)	10–55 °C; 0.1–800 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • ↑*P*, *T* and time resulted in ↑ inactivation (↑*P* and *T* → ↑*k* value) • ≤600 MPa; > 45 °C: antagonistic effect of low pressure on thermal inactivation (*k* value for inactivation at elevated *P* was lower than at 0.1 MPa)	Rapeanu et al. (2005a)
	Victoria grape must	20–70 °C; 100–800 MPa; kinetic study within max. time frame of 120 min	• Irreversible inactivation • Inactivation of labile and stable fractions • Kinetics described by a biphasic model based on first-order model • ↑*P*, *T* and time resulted in ↑ inactivation (↑*P* and *T* → ↑*k* value) • Antagonistic effect of low pressure on thermal inactivation (*k* value for inactivation at elevated *P* was lower than at 0.1 MPa)	Rapeanu et al. (2005b)

Lychee PPO	Lychee	20, 40, 60 °C; 200, 400, 600 MPa; 10 and 20 min	• Remarkable pressure inactivation at 60 °C • 600 MPa/60 °C/20 min: 90 % inactivation	Phunchaisri and Apichartsrangkoon (2005)
Mango puree PPO	Mango puree	Room temperature; 207, 345, 483 and 552 MPa; 2 s and 0, 1, 3, 5, 10 and 15 min	Limited inactivation	Guerrero-Beltrán et al. (2006)
Pears PPO	Partially purified Durondeau pears PPO; dissolved in phosphate buffer (0.1 M, pH 7)	25–60 °C/900 MPa; kinetic study within max. time frame of 180 min	• Irreversible inactivation • First-order inactivation model • At 900 MPa, ↑T and time resulted in ↑ inactivation	Weemaes et al. (1998)
Red bell pepper PPO	Red bell pepper	Room temperature (18–20 °C); 100 and 200 MPa; 10 and 20 min	High pressure resistance (no/limited inactivation)	Castro et al. (2008)
Green bell pepper PPO	Green bell pepper	Room temperature (18–20 °C); 100 and 200 MPa; 10 and 20 min	50 % inactivation	Castro et al. (2008)
Plum PPO	Partially purified red beauty plum PPO; dissolved in phosphate buffer (0.1 M, pH 7)	25–50 °C /900 MPa; kinetic study within max. time frame of 180 min	• Irreversible inactivation • First-order inactivation model • No inactivation at 25 °C • At 900 MPa, ↑T and time resulted in ↑ inactivation	Weemaes et al. (1998)
Plum PPO	Plum puree "Songold" cultivar	Initial temperature of 10 °C; 400 and 600 MPa; 7 min	• No inactivation. • Increase in residual enzyme activity	González-Cebrino et al. (2012)
Potato PPO	Potato	(−26)–20 °C; 100–500 MPa; 13 min	No/limited inactivation	Van Buggenhout et al. (2006)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Strawberry PPO	Partially purified strawberry PPO; dissolved in phosphate buffer (0.1 M; pH 7)	10–50 °C; 100–750 MPa; kinetic study	• Irreversible inactivation • Pressure labile and stable fractions observed • Complete inactivation of pressure labile fraction • 19–81 % inactivation due to pressure build-up and adiabatic heating • ↑P, T and time resulted in ↑ inactivation of pressure stable fraction (↑P and T→ ↑k value) • ≤200 MPa; >50 °C: antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Dalmadi et al. (2006)
Strawberry PPO	Strawberry puree ("Festival" and "Aroma" cultivars)	24–90 °C, 100–690 MPa; kinetic study between 5 and 15 min	• 16–23 % inactivation	Terefe et al. (2010)
Strawberry PPO	Ripe strawberries puree ("Camarosa" cultivars)	Initial temperature settings of 40, 50 and 60 °C for 200 MPa and 600 MPa resulted in the average processing temperature of 50, 58 and 65 °C and 57, 62 and 71 °C, respectively, during the holding pressure phase	• At <30 °C, 13 % and 80 % inactivation after 200 and 600 MPa, respectively, for 15 min • At 600 MPa and various temperatures ranging from 29 to 71 °C for 5 min, 35–91 % inactivation	Sulaiman and Silva (2013)
PPO	Fruit smoothies (mixture of whole apple, apple juice from concentrate, strawberry, banana and orange with ratio of 29.5, 29.5, 21.0, 12.0 and 8.0 g per 100 g of smoothies, respectively)	20 °C, 450 and 600 MPa, 5 and 10 min, respectively	• Irreversible inactivation • 66 % inactivation at 450 MPa/20 °C/5 min • 16 % inactivation at 600 MPa/20 °C/10 min • After 10-h storage at 4 °C, the enzyme activity decreased up to 30 % for 450 MPa/20 °C/5 min and 10 % for 600 MPa/20 °C/10 min	Keenan et al. (2012)

Protease				
Cathepsin D	Ovine milk	400 and 650 MPa; 40 and 55 °C; 10 min	• Irreversible inactivation • Max. 60 % inactivation	Moatsou et al. (2008)
	Bovine milk	40, 50 and 60 °C; 300–450 and 600 MPa; 0, 5, 10 and 15 min	• High resistance towards pressure • Higher stability in raw milk than in pasteurized milk	Bilbao-Sáinz et al. (2009)
	Commercial bovine spleen cathepsin D dissolved in Bis-Tris buffer (10 mM; pH 6.0)	20–75 °C; 0.1–650 MPa; kinetic study	• Irreversible inactivation • First-order inactivation kinetics • ↑P, T and time resulted in ↑ inactivation (↑P and $T\rightarrow\uparrow k$ value) • 50–65 °C; low pressure (between 100 and 200 MPa): antagonistic effect of low pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa)	Buckow et al. (2010)
Aminopeptidase	*Lactobacillus delbrueckii* subsp. *bulgaricus* ACA-DC 0105	20–40 °C; 100–700 MPa; kinetic study within max. time frame of 40 min	• ≤450 MPa: increase in enzyme activity • 600 and 750 MPa; 20, 30 and 40 °C; 10 min complete enzyme inactivation. ↑P, T and time resulted in ↑ inactivation (↑P and $T\rightarrow\uparrow k$ value) • First-order inactivation kinetics	Katsaros et al. (2009a)
Ficin	Commercial enzyme (0.05 mg/mL) dissolved in phosphate buffer (pH 7)	50–80 °C; 500–900 MPa; kinetic study within max. time frame of 40 min	• Max. 50 % enzyme inactivation. • ↑P, T and time resulted in ↑ inactivation (↑P and $T\rightarrow\uparrow k$ value) • First-order inactivation kinetics	Katsaros et al. (2009b)
Papain	Commercial enzyme (0.05 mg/mL) dissolved in phosphate buffer (pH 7)	50–80 °C; 500–900 MPa; kinetic study within max. time frame of 40 min	• Max. 80 % enzyme inactivation • ↑P, T and time resulted in ↑ inactivation (↑P and $T\rightarrow\uparrow k$ value) • First-order inactivation kinetics	Katsaros et al. (2009b)

(continued)

Table 19.1 (continued)

Enzymes	Enzyme system	Process condition	Effect on enzyme stability	References
Actinidin	Kiwifruit juice	25–50 °C; 200–800 MPa; kinetic study within max. time frame of 20 min	• ≤600 MPa; 45–50 °C and ≤800 MPa; 40 °C: antagonistic effect of pressure on thermal inactivation (k value for inactivation at elevated P was lower than at 0.1 MPa) • <40 °C: ↑P, T and time resulted in ↑ inactivation (↑P and $T \rightarrow \uparrow k$ value) • First-order inactivation kinetics	Katsaros et al. (2009c)
Myofibril-bound serineproteinases	Silver carp (*Hypophthalmichthys molitrix*): crude enzyme extract and myofibrils	20 °C; 200–500 MPa; kinetic study for 30 min	• First-order inactivation kinetics • ↑P and time resulted in ↑ inactivation (↑$P \rightarrow \uparrow k$ value) • Va value: −12.40 cm^3/mol and 10.43 cm^3/mol for crude enzyme extract and myofibrils, respectively	Qiu et al. (2013)
Trimethylamine-N-oxide demethylase				
	Squid	20 °C; 300 MPa; 0, 5, 10, 20 min	• Irreversible inactivation • Max. 75 % inactivation	Gou et al. (2010)
Xylanase	*Thermomyces lanuginosus* xylanase diluted (80–40 U/mL) in phosphate–citrate buffer (0.1 M; pH 6.5)	50–70 °C; 100–600 MPa; kinetic study	• First-order inactivation kinetics • ↑P, T and time resulted in ↑ inactivation (↑P and $T \rightarrow \uparrow k$ value)	Gogou et al. (2010)
	Xylanase B from *Thermotoga maritima* in sodium acetate buffer (0.2 M; pH 5.0) with 4 M urea	100–115 °C; 500–700 MPa; kinetic study	• First-order inactivation kinetics • ↑ T and time resulted in ↑ inactivation (at constant P, ↑$T \rightarrow \uparrow k$ value)	Vervoort et al. (2011)

*Effects of HP process on enzyme inactivation were evaluated based on the comparison between the residual enzyme activity before and after HP processing (*post factum*) measured at atmospheric pressure (0.1 MPa)

**Processing temperature otherwise specifically identified in the table

[a]Endogenous enzyme of the corresponding food matrix and no purification was done

[b]The study was carried out at different treatment time intervals to follow the effect of HP and T on enzyme inactivation

[c]Partially purification was done using ammonium sulphate precipitation

ing could be increased which reassemble the protein structure suitable for partial/complete recovery of enzyme activity.

Combined elevating pressure and lowering temperature also results in enzyme inactivation. At low and subzero temperature (Fig. 19.1, dashed line frame), most enzymes are stable but some are inactivated such as lipoxygenase (LOX) (Indrawati et al. 1998; Van Buggenhout et al. 2006). The rate of LOX inactivation is enhanced by elevating pressure and lowering temperature (Indrawati et al. 1999, 2000a, b, 2001; Rodrigo et al. 2006a; Van Buggenhout et al. 2006). This "cold denaturation/inactivation" could occur due to changes in the interactions between protein molecules and adjacent water (Meersman et al. 2008). Some studies (Privalov 1990; Da Poian et al. 1995; Weber 1995; Silva et al. 1996) have observed that at low temperature water molecule forms a shield around the adjacent non-polar molecules of protein in different entities resulting in loss of the ability to interact with each other (less hydrophobic interactions). Under pressure, non-polar interaction is more affected even though higher ordered protein molecule (low change in entropy) is obtained due to higher compressibility.

19.4 Kinetics of Enzyme Inactivation during HP/T Treatment

19.4.1 Time Dependence of Enzyme Inactivation during HP/T Treatment

An analogous scheme of thermal inactivation at ambient pressure previously proposed by Lumry and Eyring (1954) was found for pressure inactivation of enzymes by Zipp and Kauzmann (1973) described as follows:

$$\text{Native enzyme} \underset{k_f}{\overset{k_u}{\Leftrightarrow}} \text{Reversibly unfolded enzyme} \xrightarrow{k_i} \text{Inactivated enzyme}$$

where k_u, k_f*and* k_i are respectively reaction rate constants (k value) for denaturation, renaturation and subsequent irreversible inactivation processes.

Simple inactivation scheme to calculate the inactivation rate can be expressed by Eq. (19.1).

$$v = k_i[U] \tag{19.1}$$

where v represents inactivation rate and $[U]$ concentration of unfolded enzymes. The observed, experimentally measured inactivation rate constant (k_{obs}) can be mathematically described as Eq. (19.2).

$$k_{obs} = \frac{k_i K}{1+K} \tag{19.2}$$

where K is the concentration ratio between unfolded and native enzymes. At "high" temperature or pressure, where $K \gg 1$, the k_{obs} value is equal to k_i value. In this case, the influence of reversible (folding and unfolding) denaturation can be neglected, and the inactivation rate constant is determined based on the irreversible inactivation reaction.

The decrease of enzyme activity (A) as a function of inactivation time (t) can be written as n^{th}-order reaction equation (19.3).

$$\frac{dA}{dt} = -k_{obs} A^n \tag{19.3}$$

where k_{obs} and n represent the observed inactivation rate constant and reaction order, respectively. Under pressure, a non-first-order inactivation behaviour of enzymes has been reported such as inactivation of apple PPO (Buckow et al. 2009).

Under constant extrinsic and intrinsic conditions (such as isobaric and isothermal condition), Eq. (19.3) can be integrated to Eq. (19.4).

$$A_t = (A_o^{1-n} + (n-1)k_{obs}t)^{\frac{1}{1-n}} \tag{19.4}$$

where A_o and A_t refer to the enzyme activity at $t=0$ and at time t under isobaric isothermal condition.

Enzyme inactivation by temperature, pressure or cold under pressure can often be described by a first-order kinetic inactivation model ($n=1$). For $n=1$, Eq. (19.3) can be transformed to Eq. (19.5).

$$\ln\left(\frac{A_t}{A_o}\right) = -k_{obs}t \quad \text{or} \quad A_t = A_0 \cdot \exp(-k_{obs}t) \tag{19.5}$$

Some studies (Table 19.1) have observed enzyme fractions having different pressure and temperature stability. If each enzyme fraction is inactivated in parallel following first-order reaction kinetics, Eq. (19.5) can be modified to Eq. (19.6) to describe such inactivation process.

$$A_t = \sum_i A_i \exp(-k_{obs,i}t) \tag{19.6}$$

In this case A_t represents the experimentally measured enzyme activity which is not differentiated based on the i^{th} fractions, A_i the enzyme activity of the ith fraction at $t=0$ and $k_{obs,i}$ its inactivation rate constant. In literature, two fractions, i.e. stable and labile fractions, are mostly reported such as inactivation of *Aspergillus niger* α-amylase (Buckow et al. 2005); PME from orange (Sampedro et al. 2008), tomato (Fachin et al. 2002a), white grapefruit (Guiavarc'h et al. 2005), green pepper (Castro et al. 2006a) and carrot (Ly-Nguyen et al. 2002b, 2003a); PG from tomato (Fachin et al. 2002b; Peeters et al. 2004); and PPO from grapes (Rapeanu et al. 2005b) and

strawberry (Dalmadi et al. 2006) (Table 19.1). In case of two fractions having different pressure and temperature stability, Eq. (19.6) can be transformed to Eq. (19.7).

$$A_t = A_L \exp(-k_{obs,L} t) + A_S \exp(-k_{obs,S} t) \tag{19.7}$$

where A_L and A_S are respectively the enzyme activities of the labile and stable fractions at $t=0$ and k_L and k_S their corresponding inactivation rate constants. When the stable fraction has a constant non-zero residual enzyme activity upon long heating or pressurization, Eq. (19.7) can be simplified to be Eq. (19.8), which is mostly called as a (first-order) fractional conversion model.

$$A_t = A_\infty + (A_o - A_\infty) \exp(-k_{obs} t) \tag{19.8}$$

where A_o and A_S are respectively initial enzyme activity and constant non-zero residual enzyme activity.

When a serial (e.g. two) irreversible inactivation step following first-order reaction, for example, an irreversible conversion of the native enzyme to an intermediate enzyme form with lower enzyme activity and afterwards the conversion of the intermediate to an inactive enzyme form, occurs irreversibly, a consecutive model (Eq. 19.9) can be applied. In literature, this model has been used, for example, to describe the inactivation kinetics of broccoli myrosinase under pressure (Table 19.1).

$$\begin{aligned} A = {} & (A_{int} - A_{irr}(\frac{k_{obs,int}}{k_{obs,int} - k_{obs,irr}})) \exp(-k_{obs,int} t) \\ & + (A_{irr}(\frac{k_{obs,int}}{k_{obs,int} - k_{obs,irr}})) \exp(-k_{obs,irr} t) \end{aligned} \tag{19.9}$$

where A_{int} and A_{irr} respectively represent enzyme activities at intermediate state and the irreversible inactivated enzyme and $k_{obs,int}$ and $k_{obs,irr}$ the inactivation rate constants for the irreversible conversion from native to intermediate enzymes and the irreversible conversion of the intermediate to inactivated enzymes, respectively.

19.4.2 Temperature Dependence of Enzyme Inactivation Rate Constants during HP/T Treatment

Under pressure, enzyme inactivation rate constants (k value) are increased by elevating temperature ($\uparrow P$ and $\uparrow T \rightarrow \uparrow k$ value, Table 19.1 and Fig. 19.1 full line frame). At constant pressure, Arrhenius relation is used to describe temperature dependency of inactivation rate constants. Activation energy (E_a value)—a kinetic parameter

describing temperature dependence of k values—can be estimated based on Eq. (19.10) when the relation between the reciprocal values of temperature and the natural logarithm (ln) of k values is linear.

At low temperature and elevated pressure, Arrhenius plot could deviate from linearity, for example, lipoxygenase from soybean (Indrawati et al. 1999; Wang et al. 2008), green beans (Indrawati et al. 2000a, b), green peas (Indrawati et al. 2001) and tomato (Rodrigo et al. 2006a). This non-linearity is observed in the temperature region under pressure where decreasing temperature enhances enzyme inactivation (Fig. 19.1, temperature area between full and dashed line frame).

$$\ln(k) = \ln(k_{T_{\text{ref}}}) + \left(\frac{E_{\text{a}}}{R}\left(\frac{1}{T_{\text{ref}}} - \frac{1}{T}\right)\right) \tag{19.10}$$

where R is the universal gas constant, T temperature (K) and $k_{T_{\text{ref}}}$ inactivation rate constants at reference temperature (T_{ref}).

19.4.3 Pressure Dependence of Enzyme Inactivation Rate Constants during HP/T Treatment

At constant temperature, Eyring relationship is used to describe pressure dependency of inactivation rate constants. Activation volume (V_{a} value)—a kinetic parameter describing pressure dependence of k values—can be estimated using Eq. (19.11) when the relation between pressure and k values is linear.

$$\ln(k) = \ln(k_{P_{\text{ref}}}) - \left(\frac{V_{\text{a}}}{RT}(P - P_{\text{ref}})\right) \tag{19.11}$$

where R is the universal gas constant, T temperature (K) and $k_{P_{\text{ref}}}$ inactivation rate constants at reference pressure (P_{ref}).

The Eyring plot is not always linear in the whole pressure and temperature area studied, for example, soybean LOX inactivation (Indrawati et al. 1999). In this case, empirical mathematical models were developed to describe pressure dependency of inactivation rate constants [$k = function(P)$] or the V_{a} values are estimated using Eq. (19.11) only in the pressure and temperature area where the Eyring relation is valid.

19.4.4 Combined Pressure and Temperature Dependence of Enzyme Inactivation Rate Constants during HP/T Treatment

In order to describe the elliptical contour diagram of pressure-temperature dependence of enzyme inactivation, different approaches have been carried out. Empirical mathematical approaches have been introduced to describe pressure-temperature dependence of enzyme inactivation rate constants, for example, elliptical equation or modified Arrhenius relation (Van den Broeck et al. 2000; Polydera et al. 2004) or modified Eyring relation (Ludikhuyze et al. 1998). The latter two approaches are restricted only to the pressure and temperature range where Arrhenius or Eyring relations are valid. Therefore, it limits their usage as a general basic approach for kinetic modelling.

Indrawati et al. (1999) have suggested for the first time the concept relating thermodynamics of protein unfolding to kinetics of enzyme inactivation. This suggestion is inspired based on a similarity in the pressure and temperature dependence of denaturation (Heinisch et al. 1995) and inactivation (loss of enzyme activity) of soybean LOX. This kinetic model is constructed based on the thermodynamic theory where the occurrence of phase transition is based on the contribution of changes in volume (ΔV) and entropy (ΔS) to the free energy (ΔG) function (Eq. 19.12) (Hawley 1971; Morild 1981).

$$\mathrm{d}(\Delta G) = -\Delta S \mathrm{d}T + \Delta V \mathrm{d}P \tag{19.12}$$

The entropy change (ΔS) and the volume change (ΔV) vary with pressure and temperature (Eqs. 19.13 and 19.14). Under isobaric and isothermal conditions, Eq. (19.12) can be integrated to yield Eq. (19.15).

$$\mathrm{d}(\Delta S) = \left(\frac{\partial \Delta S}{\partial T}\right)_P \mathrm{d}T + \left(\frac{\partial \Delta S}{\partial P}\right)_T \mathrm{d}P \tag{19.13}$$

$$\mathrm{d}(\Delta V) = \left(\frac{\partial \Delta V}{\partial T}\right)_P \mathrm{d}T + \left(\frac{\partial \Delta V}{\partial P}\right)_T \mathrm{d}P \tag{19.14}$$

$$\begin{aligned} \Delta G = {} & \Delta G_\mathrm{o} + \Delta V_\mathrm{o}(P - P_\mathrm{o}) - \Delta S_\mathrm{o}(T - T_\mathrm{o}) + \frac{1}{2}\Delta\kappa(P - P_\mathrm{o})^2 \\ & + \Delta\zeta(P - P_\mathrm{o})(T - T_\mathrm{o}) - \Delta C_\mathrm{p}\left[T\left(\ln\frac{T}{T_\mathrm{o}} - 1\right) + T_\mathrm{o}\right] \end{aligned} \tag{19.15}$$

where ΔC_p is the heat capacity change ($T\delta\Delta S/\delta T)_P$, $\Delta\zeta$ the thermal expansibility factor ($(\delta\Delta V/\delta T)_P = -(\delta\Delta S/\delta P)_T$) and $\Delta\kappa$ the compressibility factor ($\delta\Delta V/\delta P)_T$.

This thermodynamic model is converted into a kinetic model with an assumption that enzyme inactivation is accompanied by the formation of a metastable

activated state ($^{\neq}$) which exists in equilibrium with the native enzyme (transition state theory of Eyring). This conversion is made by substituting Eqs. (19.16) and (19.17) in Eq. (19.15):

$$\Delta G^{\neq} = -R_t T \ln(K^{\neq}) \tag{19.16}$$

$$K^{\neq} = \frac{kh}{rk_B T} \tag{19.17}$$

yielding Eq. (19.18).

$$\begin{aligned} \ln(k) = \ln(k_0) - \frac{\Delta V_0^{\neq}}{R_t T}(P - P_o) + \frac{\Delta S_0^{\neq}}{R_t T}(T - T_o) - \frac{1}{2}\frac{\Delta \kappa^{\neq}}{R_t T}(P - P_o)^2 \\ - \frac{\Delta \zeta^{\neq}}{R_t T}(P - P_o)(T - T_o) + \frac{\Delta C_p^{\neq}}{R_t T}[T(\ln\frac{T}{T_o} - 1) + T_o] \end{aligned} \tag{19.18}$$

In the last decade, this approach has been used as a general basic approach for kinetic modelling to describe pressure and temperature dependence of k values for various food enzymes. In some studies, this model has been modified to obtain high accuracy of the estimated parameters, for example, by implementing second-order approximation (Eq. 19.19, Smeller 2002) leading to formation of second-degree polynomial (elliptical), implementing higher-order term (Ly-Nguyen et al. 2003b) or omitting some terms (Grauwet et al. 2009). This modification makes the thermodynamic terms meaningless. Therefore, this modified model is rewritten as polynomial equation (Buckow and Heinz 2008).

$$[T(\ln\frac{T}{T_o} - 1) + T_o] = \frac{(T - T_o)^2}{2T_o} \tag{19.19}$$

According to Morild (1981), estimation of the unknown parameters of Eq. (19.18), by means of computerized curve fitting, might require data from rather large pressure and temperature intervals, approximately in the range of 500 MPa and 100 °C. This requirement might be unrealizable resulting in a limited pressure and temperature range explored. It therefore could explain the poor estimation of thermodynamic kinetic parameters (Eq. 19.18) as reported previously.

19.4.5 Use of Kinetic Information for Process Design and Assessment

Kinetic information is indispensable for design, optimization and assessment of HP/T processing. When the pressure and temperature dependence of enzyme inactivation can be mathematically described, pressure and temperature combination

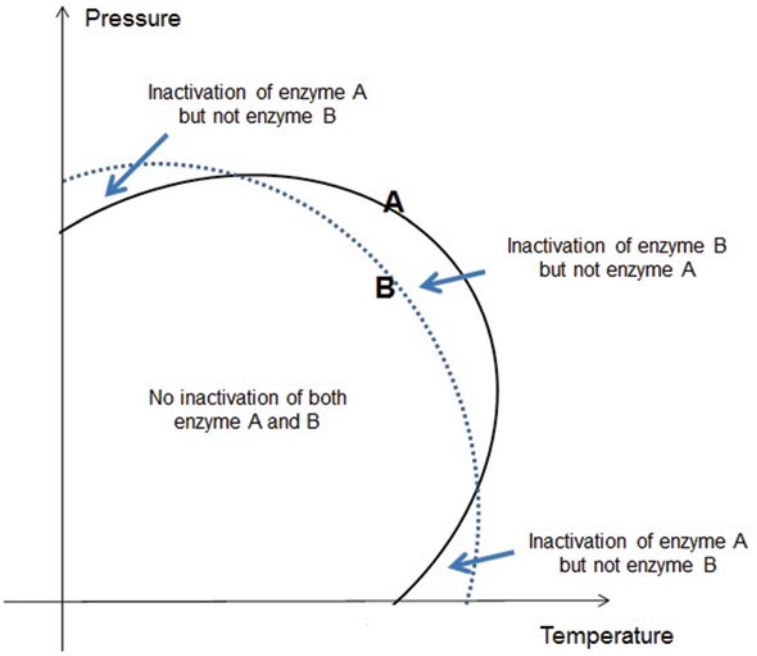

Fig. 19.2 Schematic pressure and temperature diagram illustrating the same inactivation for enzymes having different pressure and temperature stability (*full line*, enzyme A; *dashed line*, enzyme B)

resulting in a certain level of enzyme inactivation can be predicted. Figure 19.2 schematically illustrates enzymes with different pressure and temperature stability. If one would inactivate enzyme A whilst maintaining enzyme B, two strategies of process design can be established, i.e. first strategy using low/ambient temperature combined with high pressure or the second one using heating combined with low pressure. If one would maintain these enzymes, processing can be designed at pressure and temperature combinations under the curves of enzyme inactivation. Furthermore, combining kinetic information of enzymes and microbial inactivation with quality retention/modification can be applied to intelligently design a HP/T processing resulting in safe food products with tailored quality.

Kinetics of enzyme inactivation under pressure can be manipulated by changing the intrinsic factors of enzyme systems such as pH, molarity, enzyme concentration, buffer solution, etc. This unique behaviour opens opportunities to use enzymes as an indicator to map the process uniformity during HP/T processing and to evaluate the process impact. Recently, the use of enzymes for pressure-temperature time indicator (pTTI) combined with solvent engineering has been demonstrated, for example, using *Bacillus subtilis* α-amylase (Grauwet et al. 2009), *Bacillus amylo-*

liquefaciens α-amylase (BAA) (Grauwet et al. 2010) and *Thermomyces lanuginosus* xylanase (Gogou et al. 2010).

Since enzymes have different pressure and temperature sensitivities (Table 19.1 and Fig. 19.2), selective processing condition (combination of pressure, temperature and time) can be identified to allow in situ creation of various specific population for active endogenous enzymes. This potential has been demonstrated in tomato matrix (Houben et al. 2013a). Different percentages of residual enzyme activity in tomato matrix, namely, pectinmethylesterase (50 %), polygalacturonase (2 %), β-galactosidase (64 %) and α-arabinofuranosidase (32 %), can be created after HP/T treatment at 25 °C/550 MPa/10 min. As a comparison, cold-break process (10 min, 65 °C, 0.1 MPa) in this study resulted in different percentages of pectinmethylesterase (74 %), polygalacturonase (12 %), β-galactosidase (2 %) and α-arabinofuranosidase (12 %), and hot-break process (8 min, 95 °C, 0.1 MPa) inactivated the enzymes. This strategy has also been demonstrated in the mixture of different vegetables, for example, broccoli and carrot puree (Houben et al. 2013b). This study opens possibilities to create novel structural properties of the food products by selecting appropriate pressure, temperature and time combinations.

19.5 Catalytic Activity of Enzymes During HP/T Treatment

Based on knowledge on pressure and temperature stability of enzymes, HP/T process combination (i.e. pressure, temperature and time) to achieve targeted food quality and microbial safety (inactivation of vegetative cells) can be determined. Instead of pressure-enhanced enzyme inactivation, pressure can also retard or enhance the rate of enzymatic and chemical reactions depending on the resulting volume change (*Le Chatelier* principle) during HP/T treatment (Mozhaev et al. 1996).

Catalytic activity of enzymes under pressure has been investigated in simple model systems (i.e. enzyme and its substrate exposed together in buffer solution with predefined medium composition) and in complex systems such as in intact food matrices in which enzymes and their substrates are situated in their natural locations. Up to now the underlying mechanisms of enzyme activity during HP/T treatment are not yet completely understood due to simultaneous occurrence of complex phenomena under pressure. Reversible or irreversible conformational alterations and possible changes in ion charges of the protein molecule during HP/T treatment influence the stability of enzyme molecules which could affect its catalytic activity, substrate affinity, enzyme interactions with activators, inhibitors, cofactors and so on. Similarly, the stability of other (bio)molecules such as enzyme substrates (protein, starch, pectin, etc.) and their secondary products could also be affected under pressure leading to changes in the susceptibility of substrates towards enzymatic reactions, ion charges, substrate degradation, etc. Therefore, most studies (Table 19.2) can only estimate the overall catalytic activity of enzymes

Table 19.2 Catalytic activity of enzymes during combined pressure-thermal treatment[a]

Enzymes	Enzyme, substrate and matrices	Processing condition	Effects on enzyme activity	References
Amylase				
α-Amylase	Commercial *Aspergillus niger* glucoamylase dissolved (0.5 mg/mL) in ACES buffer (0.1 M), maltose monohydrate solution (10 µg/mL in ACES buffer (0.1 M, pH 4.5))	40–80 °C*; ≤ 600 MPa; 30 min	318 MPa/84 °C: optimal enzyme activity (sugar yield is more than twice compared to that at 0.1 MPa and 65 °C)	Buckow et al. (2005)
β-Amylase	Commercial barley β-amylase dissolved (0.0625 mg/mL) in ACES buffer (0.1 M, pH 5.6) mixed (1:1 ratio) with soluble starch (3.33 mg/mL in ACES buffer (0.1 M, pH 5.6))	20–65 °C; 100–400 MPa; 30 min	106 MPa/63 °C: optimal enzyme activity	Heinz et al. (2005)
Lactoperoxidase	Acid whey diluted 1/5 in phosphate buffer (0.1 M; pH 6.4) containing 10 mM-ABTS and 10 mM-H_2O_2	20–80 °C; 0.1–300 MPa; 15 min	Under pressure, optimal enzyme catalytic activity was found around 30–40 °C	Ludikhuyze et al. (2001)
Myrosinase (MYR)				
Broccoli MYR	Partially purified broccoli MYR, commercial sinigrin, phosphate buffer (0.1 M; pH 6.55)	20–50 °C; 50–200 MPa	• At all temperature, activity slightly increased with increasing pressure up to 50–75 MPa • No activity found at 150 and 200 MPa most probably due to enzyme inactivation	Ludikhuyze et al. (2000b)
	Endogenous MYR, commercial sinigrin, broccoli juice	20–50 °C; 50–250 MPa; 2, 10 and 15 min	• 100–150 MPa/45 °C: optimal activity • At all *P* levels, the enzymatic catalytic activity enhanced by elevating *P* up to 100 MPa and decreased with further pressure increase	Van Eylen et al. (2008b)
	Endogenous MYR and glucosinolates, intact broccoli florets	20–50 °C; 50–250 MPa; 2, 10 and 15 min	250 MPa/40 °C: optimal activity	Van Eylen et al. (2008b)

(continued)

Table 19.2 (continued)

Enzymes	Enzyme, substrate and matrices	Processing condition	Effects on enzyme activity	References
	Endogenous MYR and glucosinolates, intact broccoli florets	20–40 °C;100–500 MPa; 15 and 30 min	Glucosinolate hydrolysis and the conversion of glucosinolates to isothiocyanates and indole oligomers were promoted by elevating *P*	Van Eylen et al. (2009)
Mustard seed MYR	Partially purified, commercial sinigrin, broccoli juice (pH adjusted to 6.5)	20–60 °C; 100–400 MPa;3 and 13 min	200 MPa/60 °C: optimal activity	Van Eylen et al. (2008a)
	Partially purified, commercial sinigrin, Bis-Tris buffer (2 mM; pH6.5)	20–60 °C; 100–600 MPa; 3 and 13 min	• 200 MPa/40 °C: optimal activity	Van Eylen et al. (2008a)
Pectinmethylesterase (PME)				
Carrot PME	Endogenous PME, endogenous pectin, intact shredded carrots (in situ)	30–60 °C; ≤ 600 MPa; total treatment time = 18 min (including 3 min of equilibration time)	• 200–400 MPa/50 °C: most pronounced increase in PME catalytic activity • 380 MPa/50 °C: optimal enzyme activity • At constant *T*, ↑released methanol with ↑*P*, followed by a plateau and a decline	Sila et al. (2007)
	Endogenous PME, endogenous pectin, carrot pieces (in situ)	30–60 °C; ≤ 500 MPa; 15 min including equilibration time	• 100–400 MPa/60 °C: most pronounced increase in PME catalytic activity • 380 MPa/50 °C: optimal enzyme activity • At constant *T*, ↑released methanol with ↑*P*, followed by a plateau	Sila et al. (2007)
Apple PME	Endogenous PME, commercial apple pectin (75 % degree of methylation); golden delicious and Judaine apple juices	15–65 °C; 200–600 MPa; various treatment time up to 10.5 min	Reaction rate constants of methanol release ranged from 6.2 to 12.4 ± 1.0 mM/day for HP treated juices.	Baron et al. (2006)

Tomato PME	Purified with affinity chromatography (1.1–1.2 U/mL), commercial apple pectin, Na-acetate buffer (0.1 M; pH 4.4 containing 117 mM NaCl)	30–70 °C; 0.1–500 MPa; 20 min	• In presence of tomato PG, PME catalyzed hydrolysis of pectin ↑ with ↑ *P* up to 300 MPa and ↑ *T* up to 60 °C and afterwards ↓ • In absence of tomato PG, ↑ *P* up to 400 MPa and *T* up to 70 °C ↑PME catalyzed hydrolysis of pectin • ≤300 MPa/≤60 °C: tomato PME was more active in the presence of tomato PG than in the absence of PG	Verlent et al. (2007)
	Purified with affinity chromatography, commercial apple pectin, Tris–HCl (0.1 M; pH 8, 0.4 M NaCl) and Na-acetate buffer (0.1 M; pH 4.4; 0.4 M NaCl)	*pH 8*: 30–65 °C; 100–500 MPa; up to 35 min *pH 4.4*: 35–65 °C; 150–600 MPa; 35 min	• Catalytic PME activity at both pH values and HP was higher than at 0.1 MPa • 300 MPa/55 °C: optimal activity at pH 8 • 450 MPa/47 °C: optimal activity at pH 4.4 • Catalytic activity at pH 8 was higher than pH 4.4 under HP/T treatment	Verlent et al. (2004a)
Green bell pepper PME	Purified with affinity chromatography, commercial apple pectin, citrate buffer (20 mM; pH 5.6 containing 400 mM NaCl)	30–60 °C; 200–600 MPa; various treatment time	200 MPa/55 °C: optimal activity	Castro et al. (2006b)
Polygalacturonase (PG)				
Tomato PG	Purified with cation exchange chromatography, polygalacturonic acid (PGA), Na-acetate buffer (0.1 M; pH 4.4)	25–65 °C; 100–500 MPa; various reaction time intervals	• Elevating *P* retarded PG-catalyzed PGA depolymerization • Lower enzymatic activity at elevated pressure that at 0.1 MPa • Pressure sensitivity of the reaction rate ↑ with ↑ *T* • Temperature sensitivity of the reaction rate ↓with ↑ *P*	Verlent et al. (2004b)
	Purified with cation exchange chromatography, commercial apple pectin with different patterns of methyl esterification, Na-acetate buffer (0.1 M; pH 4.4)	30–65 °C, 100–300 MPa; various reaction time intervals of 5 min (0–35 min)	• Pectin deesterified by tomato PME (block wise) resulted in higher catalytic activity of tomato PG under pressure compared to that deesterified by fungal PME (random) • At all *T* and ↑ *P*, catalytic enzyme activity decreased	Verlent et al. (2005)

(continued)

Table 19.2 (continued)

Enzymes	Enzyme, substrate and matrices	Processing condition	Effects on enzyme activity	References
	Purified with cation exchange chromatography (0.65–0.8U/mL), commercial apple pectin, Na-acetate buffer (0.1 M; pH 4.4; 117 mM NaCl)	30–70 °C; 0.1–400 MPa; 20 min	• ≤300 MPa and ≤ 50 °C: PG-catalyzed pectin depolymerization accelerated in the presence of tomato PME • >300 MPa and > 50 °C: depolymerization decreased with increasing both process parameters	Verlent et al. (2007)
Protease	Commercial bovine spleen cathepsin D dissolved in Bis-Tris buffer (10 mM; pH 6.0) Substrate: biotinylated fluorescent peptide (0.0125 mM fluorescence resonance energy transfer (FRET)); 0.5 mM DTT in Bis-Tris buffer (10 mM; pH 6.0)	20–60 °C; 100–400 MPa; 30 min	• Catalytic activity was reduced at 100–400 MPa up to 50 % • Optimum substrate cleavage under pressure around 60 °C	Buckow et al. (2010)

*Processing temperature under isobaric isothermal condition

[a]It refers to the catalytic activity of enzyme to convert its substrate during HP/T treatment. The studies were carried out by applying enzyme together with its substrate in the pressure chamber (in situ reaction). After the HP/T treatment, the enzyme activity was ceased by heat shock to inactivate the enzymes whilst maintaining the stability of the substrate conversion products, and the substrate conversion products were identified and quantified at 0.1 MPa

based on the formation of enzymatic reaction products or the reduction of substrate concentration after HP/T treatment (*post factum*).

As summarized in Table 19.2, studies have shown that pressure and temperature combination of optimum enzyme activity varies dependent on enzyme species and sources, types of substrates, composition of enzyme-substrate mixtures, complexity of the medium and food matrices where enzyme and substrate are exposed to, etc. For example, α-amylase has optimal enzyme activity at relatively higher temperature (Buckow et al. 2005) than other enzymes such as β-amylase, lactoperoxidase, myrosinase, polygalacturonase and pectinmethylesterase. Different types of substrates (e.g. pectin and polygalacturonic acid) and the existence of other enzymes (such as PME and PG) participating in the same enzymatic reaction pathway affect the pressure and temperature combination of optimal enzyme activity (Verlent et al. 2004a, 2007). It has been reported that different food preparation also influences the optimal enzyme catalytic activity during HP/T processing even though the same intact food matrices are used. For example, different optimum pressure and temperature region for endogenous PME activity has been observed in carrot pieces and shredded carrots (Sila et al. 2007). Moreover, in intact food matrices, formation of different hydrolysis products can be directed during HP/T processing as observed in broccoli myrosinase (Van Eylen et al. 2009).

19.6 Conclusion

HP/T treatment offers a wide range of potentials not only limited to inactivation of enzymes and vegetative cells whilst maintaining high premium sensorial and nutritional quality but also activation of enzymes. It should be taken into account that the effects of HP/T on biomolecules such as enzymes are also unique and different compared to ambient pressure. By understanding the underlying mechanisms and kinetics on enzyme behaviour under pressure, HP/T technique can be used to tailor food quality by scientifically engineering the occurrence of enzymatic and chemical reactions in situ (in intact food products).

One should be aware that enzymes and their substrates are natural ingredients complementarily available in fresh food materials, for example, in dairy products, meats, fishes, cereals, fruit and vegetables, etc. This potential offers an interesting solution to current consumers' demand for (processed) food products with no/less artificial additives and becomes a next challenge for food technologists. Hereto, further investigation and more integrated research in this area are needed to have a better understanding of enzyme behaviour in food materials and composites during HP/T processing.

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Chapter 20
Pressure Gelatinization of Starch

Kazutaka Yamamoto and Roman Buckow

Abstract Starch, which is conventionally processed by heat, is a key food component and an industrial raw material. High hydrostatic pressure (HHP) can induce gelatinization of starch without heating. Spontaneous retrogradation can be observed immediately after HHP-induced gelatinization, depending on the starch content and temperature. In HHP treatment of starch systems, it should be differentiated whether it is anisotropic or isotropic compression, each of which results in different properties of the obtained starches. Physicochemical changes of various starches due to thermal or HHP treatment have been studied intensively by various methods, and the behavior of starch gelatinization under combinations of heat and HHP has been systematically revealed in recent years. In this paper, trends in the study of HHP-treated starch are reviewed from the viewpoints of fundamental and application approaches.

Keywords Starch • Gelatinization • Retrogradation • High hydrostatic pressure • High pressure • State diagram

Abbreviations

CP/MAS ^{13}C NMR	Cross-polarization/magic angle spinning ^{13}C nuclear magnetic resonance
DSC	Differential scanning calorimetry (-meter)
DTA	Differential thermal analysis
DTG	Differential thermal gravimetry
FTIR	Fourier transform infrared
HHP	High hydrostatic pressure

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ΔH_{gel}	Enthalpy change upon gelatinization measured by DSC
ΔH_{retro}	Enthalpy change upon melting of retrograded starch measured by DSC
RVA	Rapid visco-analyzer
SEM	Scanning electron microscopy
T_{gel}	Onset temperature of gelatinization measured by DSC
TG	Thermal gravimetry
UV–Vis	Ultraviolet visible

20.1 Introduction

20.1.1 Starch and Gelatinization

Starch is a granular storage material found in terrestrial plants abundantly and universally, and it is also important for humans as a key food component (Zobel 1984). When heated in the presence of water, the intermolecular bonds of starch molecules are broken down, allowing hydrogen bonding sites (hydroxyl hydrogen and oxygen) to bind with water. Disruption of this structure is usually referred to as heat gelatinization or, simply and conventionally, gelatinization, which is essential in all kinds of industrial and culinary utilization of starch. It is characterized by a loss in crystallinity and birefringence, solubilization of amylose, and irreversible swelling of the granules (Waigh et al. 2000). Thus, starch is utilized as a texture modifier in the food industry and as an adhesive agent in other industries.

Starch is primarily composed of amylose and amylopectin, which are homoglycans comprised only of α-D-glucose. Amylose is an α-1,4-linked linear glucose polymer, and amylopectin is an α-1,6-branched tuft of α-1,4-linked linear glucose oligomers (branched chains) and/or polymers. The chemical structures of amylose and amylopectin depend on the botanical origin of starch and other environmental factors during plant growth. Starch granules appear in different shapes depending on its botanical origin. In general, each of starch granules has a porous core called "hilum," which is the ignition point of growth rings, i.e., alternate concentric ellipsoidal lamellar structure. It is speculated that some pores exist on the granular surface, and these pores are connected to the hilum through channels. The crystalline layers of the granule consist of ordered regions composed of double helices formed by the short chains of amylopectin, most of which are further ordered into crystalline structures known as crystalline lamellae. Amorphous regions of the semicrystalline layers and amorphous layers are composed of amylose and non-ordered amylopectin branches (Waigh et al. 1997). Glucose monomers in amylopectin are oriented radially in the starch granule, with the nonreducing ends of the chains toward the granule surface. As the radius increases, so does the number of branches required to fill the space, with the consequent formation of concentric regions of alternating amorphous and crystalline structure.

Physicochemical properties of starch are strongly affected by the length and number of amylose molecules and/or amylopectin branched chains. Starch with deficiency of amylose may form sticky gels, being referred to as waxy starch. It is currently understood that two branched chains of amylopectin form a double strand, and the double strands further form tufts comprising microcrystalline domains. Wide-angle X-ray diffractometry classifies these diffraction patterns into three categories. Type A is often observed in cereal starch such as maize, wheat, and rice (Hizukuri et al. 1983; Parker and Ring 2001). Type B is typical for high-amylose (>50 %) cereal starches and for tuber starches such as potato, lily, tulip, lotus, and canna (Hizukuri et al. 1983; Parker and Ring 2001). Type C represents intermediate superimposed patterns of types A and B and can be found in tropical plants and legume starches such as peas and beans (Sugimoto and Watsuji 2006) and chestnuts (Iwaki and Sugimoto 2004). Diffraction patterns have not yet been revealed based on currently available information on the chemical structure of amylopectin, although information on these patterns has been intensively accumulated (Hizukuri 1985; Jane et al. 2003; Seetharaman and Bertoft 2013). Although some models have been suggested, there is no conclusive three-dimensional structure model for starch granules (Pérez et al. 2009).

20.1.2 Pressure-Induced Gelatinization

The effect of HPP on starch gelatinization has recently been studied intensively. Although the mechanism of HPP-induced gelatinization is likely to be different from heat gelatinization, both temperature and pressure are, as in the case of protein denaturation, crucial parameters when changes in starch structures are intended to be brought about in food processing (Knorr et al. 2006).

Starch can change into gel or paste irreversibly due to order–disorder transition when heated in the presence of water. During heat gelatinization, starch granules absorb water and swell, and the growth rings and crystalline features are lost (Fig. 20.1). For a complete hydration and gelatinization of (potato) starch, more

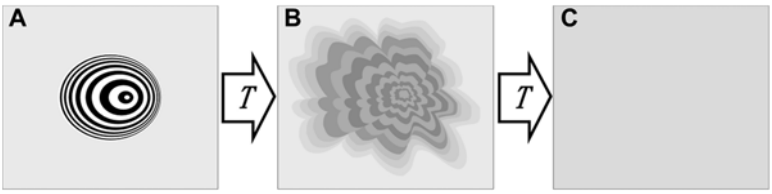

Fig. 20.1 Schematic diagram of heat gelatinization of a starch granule. (**a**) Starch granule suspended in water: cross section of the granule presents lamellar structure of concentric ellipsoids. (**b**) Heating induces swelling and disruption of the granule. Molecular motion is accelerated by heating and lamellar structure is lost by swelling. (**c**) Further heating leads to complete gelatinization where the granular shape is not observed. (**a**–**c**) Figures reprinted from Yamamoto, K.; Fukami, K.; Kawai, K.; and Koseki, S. 2006. Pressure gelatinization of starch. *Food and Packaging* (*Shokuhin to Youki*) 47: 448–456, with copyright permission of the Institute of Canning Technology, ©2006

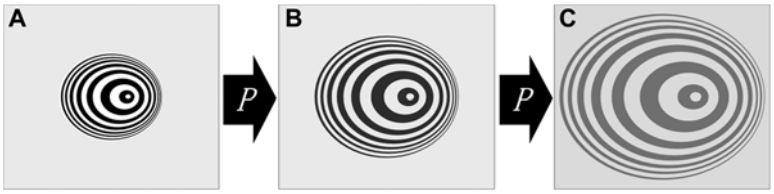

Fig. 20.2 Schematic diagram of pressure gelatinization of a starch granule as described by Yamamoto et al. (2006). (**a**) Starch granule suspended in water. (**b**) Starch granule can be partially gelatinized by HHP with swelling of the granule induced. (**c**) Completely pressure-gelatinized starch granule can retain granular shape

than 14 water molecules per one glucose unit are required (Donovan 1979). When gelatinized starch is stored, for instance, in a refrigerator, new starch crystals are formed and its texture becomes stiff. This hardening phenomenon is referred to as retrogradation (Hoover 1995). Heat-gelatinized starch shows increased enzymatic susceptibility and, thus, high degradability by amylases, while retrograded heat-gelatinized starch becomes resistant to amylase digestion.

On the other hand, starch gel or paste can be obtained when a mixture of starch and water is treated with HHP. This phenomenon is referred to as pressure gelatinization. In pressure gelatinization, starch granules can swell while often maintaining their granular shape and lamellar structure (Fig. 20.2) (Stute et al. 1996; Stolt et al. 2001; Fukami et al. 2010). Knorr et al. (2006) suggested that under pressure the disintegration of the macromolecule is incomplete, since the pressure stabilization of hydrogen bonds favors the helix conformation (Fig. 20.3). Crystalline conversion from A to B isomorph under pressure has also been reported (Katopo et al. 2002).

20.1.3 Anisotropic Versus Isotropic Compressions of Starch

In HHP treatment of relatively dry starch samples in powder form, special attention should be paid to the mode of compression, either isotropic or anisotropic. In the case where starch granules are suspended in a liquid pressure medium such as water in a pouch, external pressure compresses the pouch and pressure is transmitted isotropically to individual starch granules through the medium (Fig. 20.4a). On the other hand, in the case where starch granules without a medium are put in a cylinder and directly and one dimensionally compressed by a piston, the granules are compressed anisotropically until the granules are distorted and completely packed (Fig. 20.4b).

Fig. 20.4 (continued) water of lower density would permeate the starch granules of higher density and uniformize the density. (**B**) to (**b**) Anisotropic compression: when starch granules in the absence of water are compressed, especially one dimensionally, the granules are first packed due to the high compressibility of air surrounding the granules. Pressure is then transmitted anisotropically via loosely packed granules. Once the granules are tightly packed while the granules are distorted to fill the space among the granules, pressure is isotropically transmitted via tightly packed starch granules as pressure media

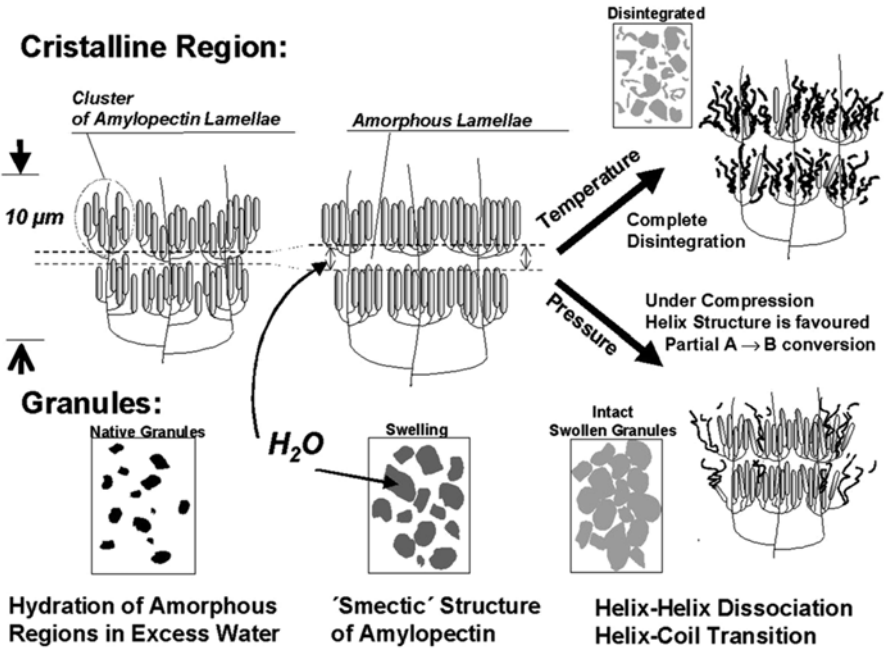

Fig. 20.3 Scheme of starch gelatinization under pressure or at high temperature in excess water. Figure reprinted from Knorr et al. (2006)

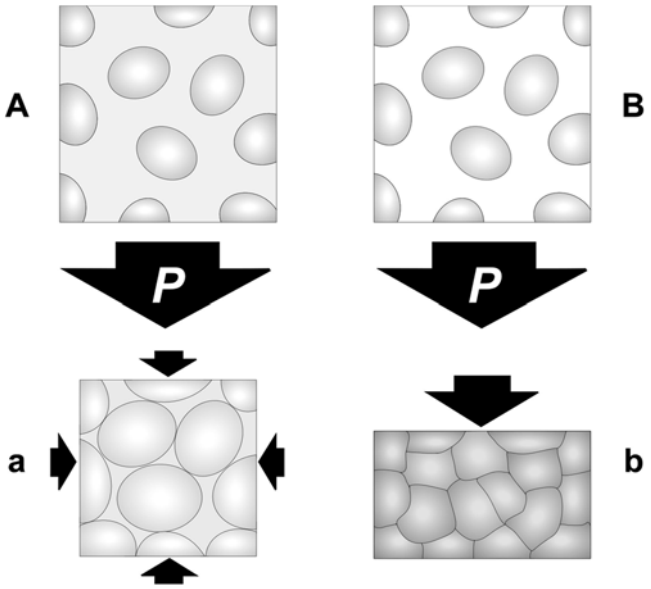

Fig. 20.4 Isotropic and anisotropic compressions of starch as described by Yamamoto et al. (2006). (**A**) to (**a**) Isotropic compression: starch granules suspended in water (pressure medium) can be compressed isotropically and homogeneously via the pressure medium. It can be indicated that

In early studies on HHP treatment of starch, there was no viewpoint to utilize HHP actively for gelatinization: neither the utilization of HHP-treated starch as a novel food ingredient in food industry nor the study of pressure gelatinization behavior in HHP-treated starchy foods was the focus of such studies. In fact, the objective in these early studies was to study the effect of pressure on the mechanical damages of starch in the milling process. For instance, in ball milling of starch, anisotropic high pressure can be generated when heavy balls impact the starch granules on the inner surface of the rotating vessel (Brown and Heron 1879).

One of the oldest descriptions of HHP treatment of starch could be the following: *Stärke, bei 20,000 atm. einem gleitenden Druck ausgesetzt, verliert ihr Röntgenogramm* (Starch exposed to a sliding pressure of 20,000 atm loses its X-ray diffractogram) (Meyer et al. 1929). Although no detailed experimental procedure including the origin of starch was described in this literature, it is probable that the starch was subjected to pressure anisotropically under a sliding condition. Mercier et al. (1968) compressed several moistened starches anisotropically at 588.4 MPa by using a piston and investigated the microscopic morphology, X-ray diffraction, iodine-binding capacity, water solubility, ethanol solubility, solubilized glucose, and enzymatic degradations by α- and β-amylases. Due to anisotropic compression, compressed starch granules under a microscope appeared as flat ellipsoids which were observed in milling as well (Jones 1940). It is a matter of interest that this compression trial aimed to improve starch availability by damaging native starches. After direct compression at 0.8–1.2 GPa, air- or oven-dried starches (Kudta and Tomasik 1992), and dry and wet starches in the presence of metal salts (Kudla and Tomasik 1992) were analyzed by methods including differential thermal analysis. Liu et al. (2008) carried out direct compression on several starches (approximately 14 % moisture) at up to 1500 MPa for 24 h. Differential scanning calorimetry (DSC) and scanning electron microscopy indicated that gelatinization temperature and enthalpy change were slightly lowered and starch granule shape and surface appearance were changed after the high pressure treatment. However, X-ray diffraction patterns and birefringence of HHP starches were not changed.

Another method of compression is high pressure extrusion (Kim and Hamdy 1987). Starch colloidal solutions (0.5–2.5 % w/v) were extruded via an orifice of a French pressure cell while maintaining high hydraulic pressure at 90, 138, or 276 MPa. A combination of high pressure homogenization (22.0 MPa, 67.5 °C for 11 min) and spray drying results in a starch product that is similar in terms of morphology but less enzymatically digestible than that produced by a combination of heat gelatinization (5 % w/w starch–water suspension, gelatinized at 121 °C for 20 min) and spray drying (Le Thanh-Blicharz et al. 2012).

Recently, HHP treatment of starch has principally been carried out in isotropic ways. In many cases, starch gelatinization is studied in the presence of water, and a starch–water mixture is transferred into a pouch which is placed in an HHP cylinder filled with pressure medium enabling isotropic compression (Fig. 20.5).

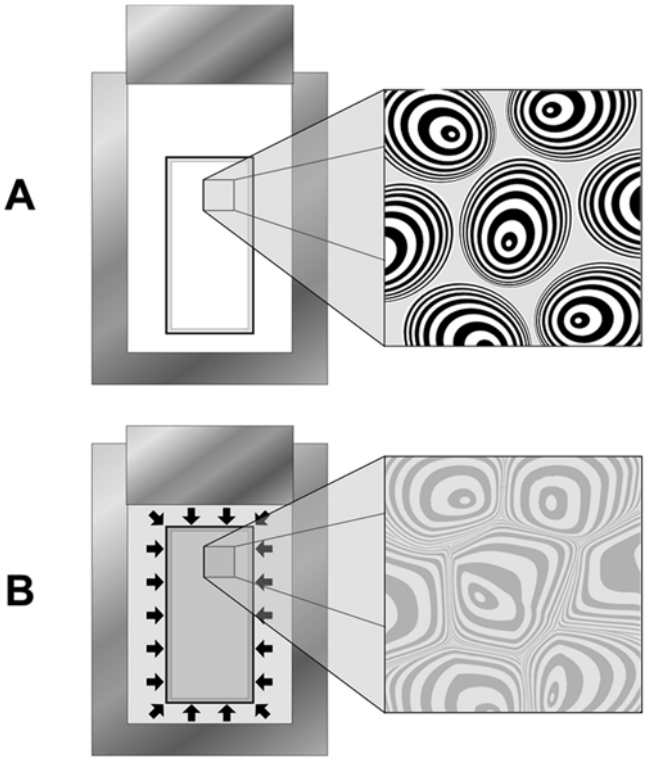

Fig. 20.5 Pressure gelatinization of a starch–water mixture by isotropic compression as described by Yamamoto et al. (2006). (**a**) Pouched starch–water mixture. (**b**) Pressure-gelatinized starch granules by isotropic compression via liquid pressure medium retain lamellar structure while the granules are tightly packed with distortion. At high starch content (i.e., low water content), granules are swollen and packed tightly. At low starch content (i.e., high water content), granules are swollen with granular shape retained, as shown in Fig. 20.2c, although there are some exceptions

20.1.4 Characteristics of Pressure-Gelatinized Starch

Early studies reported an increase in the gelatinization temperature of a dilute suspension of potato starch (0.4 %) after HHP treatment at up to 253.3 MPa for 4 min (Thevelein et al. 1981). In contrast, Muhr and Blanshard (1982) reported that HHP treatment (200–1500 MPa) decreased gelatinization temperature. High pressure differential thermal analysis was carried out for further investigation on wheat, potato, and pea starches, indicating that starch gelatinization temperature first increases by a few degrees and decreases when pressure exceeds 150–250 MPa (Muhr et al. 1982).

Since HHP was suggested as a means of food processing in Japan (Hayashi 1987), pressure gelatinization has been studied intensively. It was reported that potato starch was more pressure resistant than wheat and maize starches (Hayashi and Hayashida 1989). Thereafter, pressure resistance was discussed in terms of starch crystalline types (Ezaki and Hayashi 1992): B-type starches such as potato and lily starches were more resistant to pressure than A-type starches such as maize starch, while C-type starches such as sweet potato starch had intermediate pressure resistance between A- and B-type starches. The effect of pressure holding time on gelatinization enthalpy change and gel properties was investigated using barley starch (Stolt et al. 2001). Retrogradation was observed immediately after HHP treatment of starch (Hibi et al. 1993; Stute et al. 1996; Katopo et al. 2002). Hu et al. (2011) compared the retrogradation behaviors between pressure- and heat-gelatinized rice starches, demonstrating that the retrogradation rate of pressure-gelatinized rice starch was slower than that of heat-gelatinized starch. Other studies indicated that pressure holding times between 1 and 66 h did not affect enthalpy changes upon pressure gelatinization and melting of retrograded potato starch (Kawai et al. 2007a). HHP treatment induced swelling and gelatinization of starches but retained the granular shapes (Stute et al. 1996; Stolt et al. 2001).

Properties of HHP-treated starch are different from those of heat-treated starch. Amylose is released from heat-gelatinized starch but little from HHP-treated starch (Douzals et al. 1998; Oh et al. 2008b) or not at all (Stute et al. 1996). HHP treatment induces the swelling of starch granules while retaining their granular shapes (Stolt et al. 2001; Fukami et al. 2010). HHP treatment of barley starch and waxy maize starch showed that rheological properties, microstructure, birefringence, and enthalpy change upon gelatinization were dependent on holding time and holding pressure (Stolt et al. 1999, 2001; Buckow et al. 2007). However, further studies are necessary to clarify whether prolonged HHP treatment could complete the gelatinization of partially gelatinized starch treated at lower pressures. In addition, one report discussing the relationship between structure and pasting properties of HHP-treated (690 MPa) various starches having different chemical structures has been presented (Katopo et al. 2002).

20.1.5 Methods of Analysis for Pressure Gelatinization

When starch granules are observed under polarized light, raw starch granules show hilum-centered birefringence, which refracts light in an anisotropic material in two slightly different directions to form two rays and basically corresponds to crystallinity, while gelatinized granules lose the hilum and the birefringence (Zobel 1984). This method has been found to be suitable to detect very low degrees of pressure gelatinization and has been used by several authors (Thevelein et al. 1981; Muhr and Blanshard 1982; Stute et al. 1996; Douzals et al. 1998; Stolt et al. 2001; Bauer and Knorr 2004, 2005). The number of birefringent granules is counted and the

gelatinization degree can be evaluated from the ratio of birefringent to total (birefringent and non-birefringent) granules. The quantitative performance of judging the degree of gelatinization by birefringence loss was calibrated with differential scanning calorimetry (DSC) results, indicating that the method often slightly overestimates the gelatinized fraction in comparison with DSC measurements (Douzals et al. 2001). On the other hand, Bauer and Knorr (2004) showed that there was a good linear relationship between degree of gelatinization by birefringence loss and that by electrical conductivity.

DSC measurement is widely used for quantitative analysis of the degree of gelatinization. Depending on the botanical source of starch, an endothermic peak can be observed at around 60–80 °C in the presence of excess water, although the difference in thermal properties among starches of different botanical origins has not yet been clarified. The peak area is calculated as enthalpy change upon gelatinization (or simply gelatinization enthalpy: ΔH_{gel}), which is used as an index of pressure gelatinization. ΔH_{gel} assumes a maximum value when the starch is intact, and it becomes zero when completely gelatinized. Characteristic temperatures of the peak such as the onset temperature (T_o), the peak top temperature (T_p), and the conclusion temperature (T_c) may vary after HHP treatment (Thevelein et al. 1981; Douzals et al. 2001; Kawai et al. 2007a).

Analysis of moist (10.0–34.0 %) barley starch samples by DSC under pressure (pressurized by nitrogen gas up to 2.5 MPa) showed that less water is required for initiation of gelatinization under the pressurized condition than under atmospheric pressure (Vainionpää et al. 1993). Recently, high sensitivity DSC was introduced to analyze dilute suspensions (0.5 %) of waxy wheat, waxy potato, waxy maize, and high amylose maize starches and mixtures of waxy and high-amylose maize starches after HHP treatment (Blaszczak 2007; Blaszczak et al. 2007a, b).

Pressure gelatinization can also be evaluated by X-ray diffractometry. When pressure-gelatinized, the intensities of the characteristic peaks on a broad halo in the diffractogram of the starch are reduced, indicating loss of crystallinity. Completely pressure-gelatinized starch shows only an amorphous halo (Hibi et al. 1993; Katopo et al. 2002; Blaszczak et al. 2005b). For a quantitative evaluation of pressure gelatinization, a crystallinity index can be used (Hibi et al. 1993). However, attention should be paid to evaluating pressure gelatinization and retrogradation of B-type starch (Stute et al. 1996), since the diffraction pattern of B-type crystallinity can be superimposed by that of retrograded starch in the pressure gelatinization. Therefore, X-ray diffraction should be used for evaluation of HHP-treated B-type starch in combination with other methods such as DSC.

Compressibility of HHP-treated starch was calculated by measuring volume changes (cross-sectional area of the pressure chamber multiplied by the plunged length of the piston) of 16 % w/w (dry matter basis) aqueous suspension of wheat starch and pure water under varying pressure conditions (Douzals et al. 1996a, b). During compression below 300 MPa, compressibility of starch suspension was close to that of pure water, while reduction of volume was higher for starch suspension at higher pressures due to starch gelatinization. Interestingly, compressibility

of starch suspension was higher during decompression than during compression, and starch gels treated with HHP remained compressed after decompression. These results indicated that total volume was reduced due to starch melting and that water binding to starch under pressure is strong.

NMR analysis of HHP-treated starch has been performed using cross-polarization/magic angle spinning (CP/MAS) ^{13}C NMR which is a powerful tool to analyze the structure of solid organic materials (Blaszczak et al. 2005a, b). HHP-treated potato starch presents two resonances (Blaszczak et al. 2005b), which are characteristic to amorphous starch (Gidley and Bociek 1985).

Snauwaert and Heremans (1999) monitored in situ the pressure gelatinization of potato starch by optical microscopy facilitated with a video camera in a diamond anvil cell (DAC). Once pressure gelatinization was initiated under pressure, swelling did not stop until the pressure was reduced to below the initiation pressure. From the swelling constant, the activation volume was calculated to be −18 cm^3/mol, and elliptical starch granules appeared to have a much lower swelling threshold pressure than spherical ones. Swelling of wheat starch granules under HHPs of up to 300 MPa was also observed under an optical microscope by Bauer et al. (2004). Similarly, Buckow et al. (2007) observed in situ maize starch gelatinization in aqueous solution (5 % w/w) in a high pressure cell at HHPs of up to 650 MPa (Fig. 20.6).

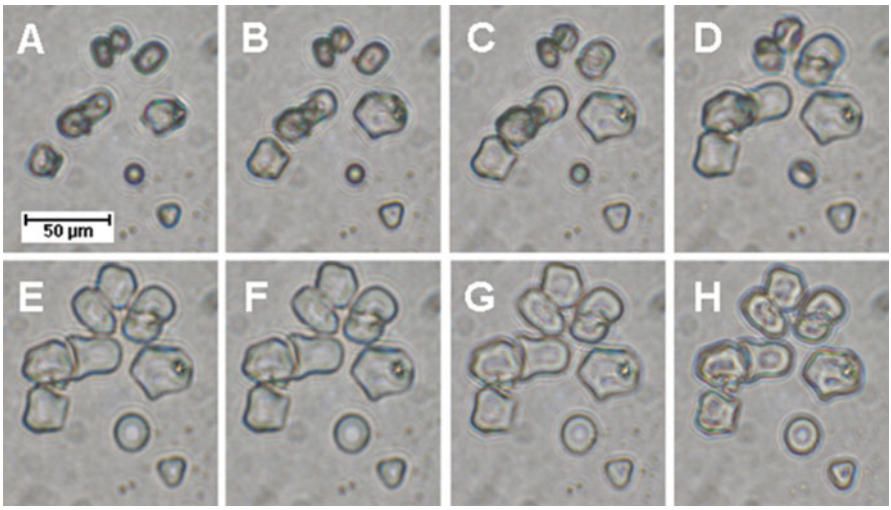

Fig. 20.6 In situ observation of maize starch granule gelatinization in aqueous solution (5 % w/w) at 30 °C and 0.1 MPa. (**a**) Pressure increases up to 650 MPa (**b**), after 1 min at 650 MPa (**c**), after 2 min at 650 MPa (**d**), after 5 min at 650 MPa (**e**), after 10 min at 650 MPa (**f**), after 20 min at 650 MPa (**g**), and after pressurization at 650 MPa for 20 min and pressure release to ambient pressure (**h**). Figure reprinted from Buckow et al. (2007)

Pressure gelatinization behaviors of rice, potato, maize, waxy maize, pea, and tapioca starches were studied in situ in a DAC by Fourier transform infrared (FTIR) spectroscopy (Rubens et al. 1999; Rubens and Heremans 2000). The characteristic absorptions observed with amorphous and crystalline features of the above-mentioned starches were specified, and the ratio of specific absorption intensities synergistically changed upon heating or pressurization. In addition, the changes upon heating and pressurization were not synergistic but monotonous in the cases of the aqueous suspensions of amylose, amylopectin, and their mixture (1:1), suggesting the importance of imperfect packings of amylose and amylopectin in starch granules (Rubens and Heremans 2000). The data on the pressure gelatinization by the in situ measurements was thermodynamically analyzed as in the case of other biopolymers (Smeller 2002).

As a novel technique, scanning transitiometry of starch–water emulsion is of great interest (Randzio and Orlowska 2005). Thermal and volumetric properties upon gelatinization of wheat starch were studied at a pressure range from 0.1 to 100 MPa and a temperature range from 10 to 157 °C (283 to 430 K).

20.1.6 Pressure Gelatinization and Enzymatic Digestibility

Amylase digestibility of starch increased after HHP treatments of starch–water mixture at 100–600 MPa and elevated temperatures (45 and 50 °C) (Hayashi and Hayashida 1989). This tendency was also observed with potato and wheat starches which were HHP-treated (0.1–650 MPa) at 10 °C and digested by α-amylase (Noguchi et al. 2003). HHP-gelatinized starch shows increased enzymatic susceptibility (digestibility) similar to heat-gelatinized starch (Hayashi and Hayashida 1989). Gomes et al. (1998) pressure-treated wheat or barley flour suspension at up to 800 MPa and evaluated the level of glucose produced by inherent α- and β-amylases during HHP treatment. The degree of gelatinization increased with increasing pressure. However, glucose productivity decreased as HHP was further raised due to the inactivation of the amylases. *Bacillus amyloliquefaciens* alpha-amylase (BAA) in a buffer solution with or without a substrate (soluble starch) was treated with HHPs of up to 400 MPa. The activation volumes for HHP inactivation of the enzyme were evaluated from the rates of hydrolysis as -13.8 ± 2.1 (with substrate) and -28.4 ± 2.2 cm^3/mol (without substrate), respectively (Raabe and Knorr 1996). It was indicated that the pressure resistance of the enzyme could be increased in the presence of the substrate due to binding of the substrate to the enzyme. Furthermore, a retarded enzymatic hydrolysis of starch by BAA under pressures of up to 400 MPa at 25 °C was detected, indicating a reversible inhibition of the reaction but irreversible inactivation of the enzyme. Similarly, enhanced maize starch hydrolysis by glucoamylase from *Aspergillus niger* was found with increasing HHP (Buckow 2006), but pressures of up to 300 MPa can induce changes in product composition accompanying the hydrolysis of maltooligosaccharides by porcine

pancreatic α-amylase (Matsumoto et al. 1997; Baks et al. 2008b). However, applying HHPs of up to 600 MPa at elevated temperatures (e.g., 60–80 °C) can significantly increase enzyme activity and starch digestibility (Heinz et al. 2005).

20.1.7 Roles of Water in Pressure Gelatinization

Water is indispensable for both heat gelatinization and pressure gelatinization. In heat gelatinization, 14 molecules of water per one glucose unit are required for complete hydration upon gelatinization (Donovan 1979). However, a systematic understanding of the effect of water content on pressure gelatinization has not yet progressed sufficiently, as most studies on pressure gelatinization have been carried out under limited conditions in terms of water content.

HHP treatment of starch with low water content, i.e., dry or low moist starch, has been carried out by anisotropic compression in many reports. Enzymatic digestibility of potato, wheat, and maize starches at low water content (2–36 %) was measured after piston compression, and digestibility showed minimums for potato starch at a water content of about 19 % and for wheat and maize starches at about 14 %, respectively (Mercier et al. 1968). Reducibility of air- or oven-dried potato starch at a water content of 15.1 % increased after pelletization by plunger compression (Kudla and Tomasik 1992, 1992). The authors suggested that water might act as a Lewis base and hydrolyze the glucosidic bonds with the help of applied compression energy which was estimated to be in the order of energy of covalent bonds. In addition, depolymerization of starch was observed after high pressure extrusion (Kim and Hamdy 1987). On the contrary, several papers have reported that the chemical bonds of starch molecules were not influenced by HHP treatment (Hibi et al. 1993; Katopo et al. 2002).

At higher water content (≥70 %), a few phase transition diagrams (gelatinization vs. pressure vs. temperature) have been presented (Douzals et al. 2001; Knorr et al. 2006; Buckow et al. 2007, 2009) and an example of complete gelatinization of barley malt, wheat, tapioca, normal maize, and potato starch slurries (5 % w/w) after 15-min processing at isothermal/isobaric conditions can be seen in Fig. 20.7.

Pressure gelatinization and other related experiments were often carried out at a fixed water content. However, some studies have investigated pressure gelatinization of starch at several water contents (Yamamoto et al. 2009). Stute et al. (1996) reported that HHP gelatinization requires at least a water content of 50 % as evaluated in the DSC measurements of HHP-treated (600 MPa, 20 °C, 15 min) starch samples at water contents of 42 %, 56 %, and 71 %. Katopo et al. (2002) treated starches of various botanical origins at water contents of 50 % and 67 % at 690 MPa and room temperature for 5 min and concluded that the degree of gelatinization was higher in starches at 67 % than in those at only 50 %. As indicated above, pressure gelatinization has been studied at limited water contents, and, therefore, a systematic understanding on the role of water in pressure gelatinization of various starches is not yet possible.

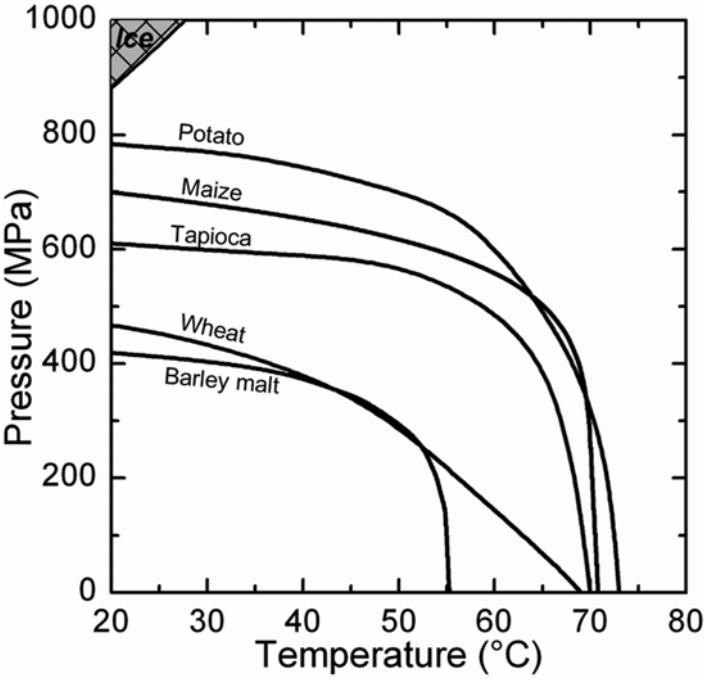

Fig. 20.7 Phase diagram for complete gelatinization of barley malt, wheat, tapioca, normal maize, and potato starch slurries (5 % w/w) after 15-min processing at isothermal/isobaric conditions. Isolines were taken from Knorr et al. (2006)

20.1.8 Effect of Water Content on Pressure Gelatinization

Potato starch has been reported to be more pressure resistant than cereal starches such as maize, wheat, and rice starches (Muhr et al. 1982; Ezaki and Hayashi 1992; Katopo et al. 2002; Oh et al. 2008a, b).

Kawai et al. (2007b) treated potato starch–water mixtures at water contents of 30–90 % w/w with pressures of 400–1200 MPa at 40 °C for 1 h andpresented a state diagram. Pressure gelatinization and pressure-induced retrogradation were evaluated from endothermic peaks by differential scanning calorimetry (DSC). As shown in Fig. 20.8, an endothermic peak, which corresponds to the enthalpy change upon gelatinization (ΔH_{gel}), can be observed at approximately 75 °C, whereas another peak at approximately 58 °C corresponds to the enthalpy change upon melting of retrograded starch (ΔH_{retro}). The value of ΔH_{gel} decreased upon pressure gelatinization, and increased gelatinization was achieved with increasing pressure in starches with higher water content (Fig. 20.9). On the other hand, retrogradation was observed with completely or partially pressure-gelatinized starch, and the value of ΔH_{retro} tended to increase with decreased water content of the starch samples and with increased treatment pressure (Fig. 20.10). Taking into account that the non-treated potato starch

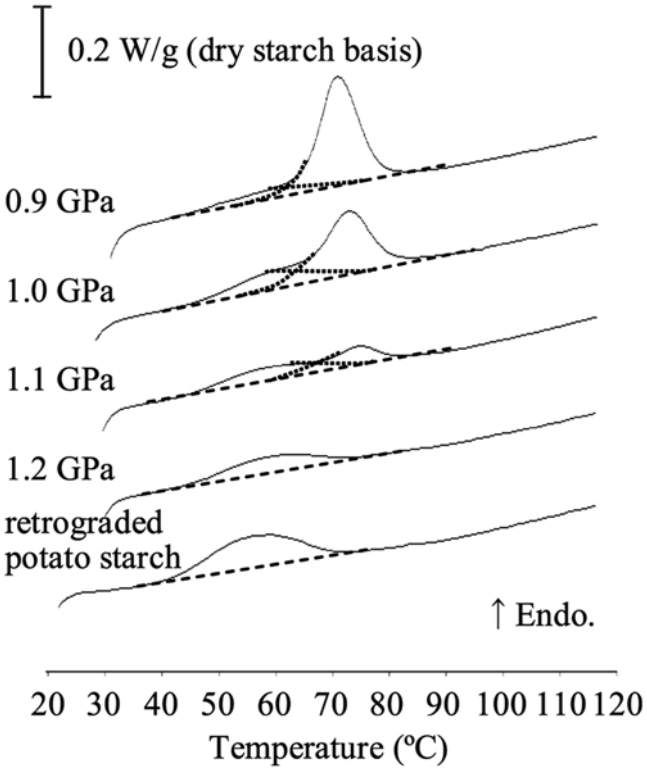

Fig. 20.8 DSC thermograms of the potato starch–water mixture (water: 50 % w/w) treated with HHP (0.9–1.2 GPa) and the retrograded potato starch–water mixture (water: 30 % w/w) gelatinized completely with heat and stored at 4 °C for 7 days, as described by Kawai et al. (2007a)

showed $H_{gel} = 20 \pm 2$ J/g (dry starch basis), the state of the HHP-treated potato starches was classified into five categories: complete gelatinization ($\Delta H_{gel} = 0$ J/g), complete gelatinization with retrogradation ($\Delta H_{gel} = 0$ J/g and $\Delta H_{retro} > 0$ J/g), partial gelatinization ($\Delta H_{gel} < 18$ [=20 – 2] J/g and $\Delta H_{retro} = 0$ J/g), partial gelatinization with retrogradation ($\Delta H_{gel} < 18$ J/g and $\Delta H_{retro} > 0$ J/g), and thermodynamically no change ($\Delta H_{gel} \geq 18$ J/g and $\Delta H_{retro} = 0$ J/g). The classification was presented as a state diagram (treatment pressure vs. starch content) (Fig. 20.11). Data for depicting the state diagrams were physicochemically analyzed using mathematical models and were compared with those of wheat starch–water mixtures (5–80 % w/w) published by Baks et al. (2008a). Thereafter, the state diagram of potato starch–water mixtures at water contents of 30–90 % w/w and pressures of 400–1000 MPa (Fig. 20.12) was extended in terms of treatment temperature (20–70 °C) (Kawai et al. 2012). With increased temperature and/or water content, the pressure required for complete gelatinization decreased. Retrogradation was observed at starch contents ranging from 20 % to

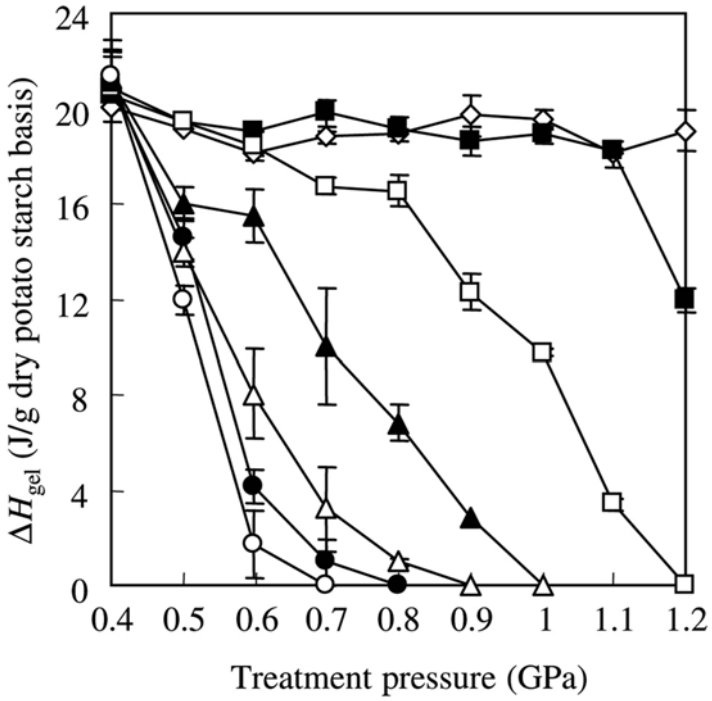

Fig. 20.9 Dependence of ΔH_{gel} on treatment pressure and starch content of potato starch–water mixture, as described by Kawai et al. (2007a). *Open circle*, 10 %; *closed circle*, 20 %; *open triangle*, 30 %; *closed triangle*, 40 %; *open square*, 50 %; *closed square*, 60 %; *open diamond*, 70 %

70%w/w, which is wider than the reported general range of 30–60 % w/w observed after heat gelatinization at ambient pressure (Hoover 1995). At a water content of 80 %, retrogradation was observed at relatively low temperatures of 20 °C and 30 °C, while at a water content of 30 %, retrogradation only occurred at relatively high temperatures of 60 °C (only at 1000 MPa) and 70 °C (at 400–1000 MPa).

20.1.9 Effect of Treatment Time on Pressure Gelatinization

Only a few reports have examined pressure gelatinization of starch as a function of pressure holding time (Stolt et al. 2001; Buckow et al. 2007; Kawai et al. 2007a). Kawai et al. (2007a) reported on the effect of treatment time (1, 18, and 66 h) on gelatinization and retrogradation of potato starch–water mixtures (water content: 30–90 % w/w) treated at 600–1000 MPa. The values of ΔH_{gel} and ΔH_{retro} were dependent on the water content and were not affected by treatment time in the tested range (Fig. 20.13). However, the onset temperature of gelatinization (T_{gel})

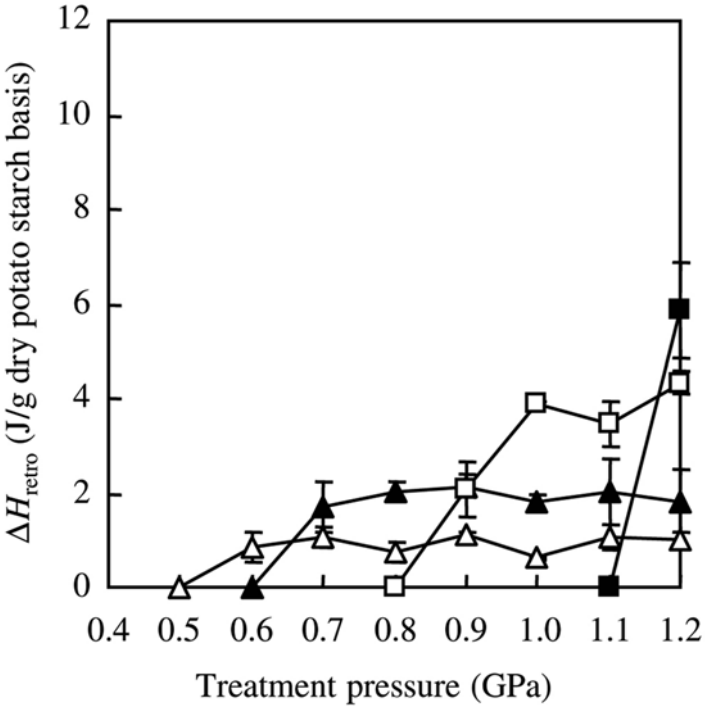

Fig. 20.10 Dependence of ΔH_{retro} on treatment pressure and starch content of potato starch–water mixture, as described by Kawai et al. (2007a). *Open triangle*, 30 %; *closed triangle*, 40 %; *open square*, 50 %; *closed square*, 60 %

increased with increased water content and treatment time (Fig. 20.14). Although long-time HHP treatment is impractical from the viewpoint of running cost in the food and starch industries, it is of fundamental importance to understand gelatinization and retrogradation properties of HHP-treated starch, especially from the viewpoint of annealing.

20.1.10 Effect of Amylose on Pressure Gelatinization

Amylose is considered to play an important role in pressure gelatinization. Fukami et al. (2010) pointed out that amylose maintains the granular structure of HHP-treated (600 MPa and 40 °C for 1 h) normal and waxy maize starches. When completely gelatinized (as confirmed by DSC and birefringence), the granules of waxy maize starch lost their granular structure, while those of normal maize starch maintained their granular shape although they were swollen (Fig. 20.15). Buckow et al. (2009) also demonstrated that temperature and pressure stabilities of high amylose maize starches are significantly higher than those of waxy and normal starches. Blaszczak et al. (2007c) subjected waxy maize, amylopectin wheat, and

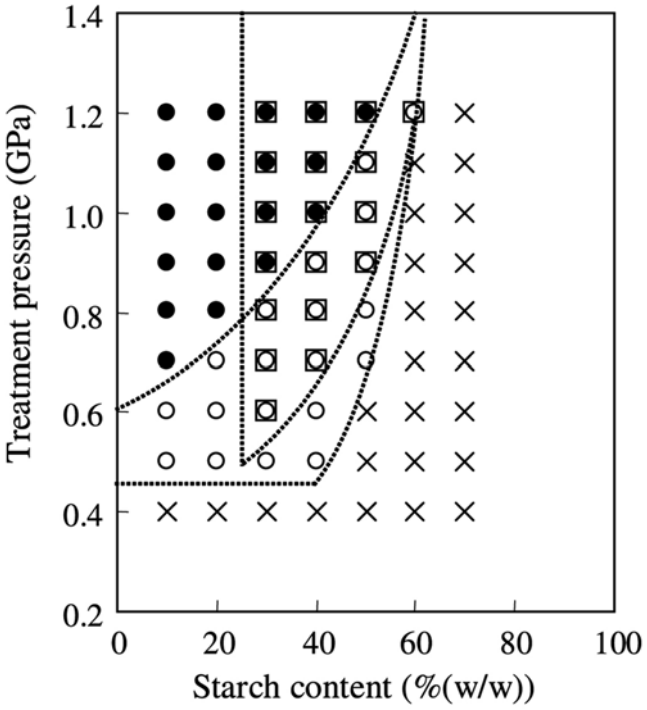

Fig. 20.11 State diagram (treatment pressure vs. starch content) of HHP-treated potato starch–water mixtures, as described by Kawai et al. (2007a). *Closed circle*, complete gelatinization; *closed circle in square*, complete gelatinization with retrogradation; *open circle*, partial gelatinization; *open circle in square*, partial gelatinization with retrogradation; *cross*, thermodynamically unchanged

amylopectin potato starches in excess water to 650 MPa for 9 min and measured the relaxation time constants of the starch gels. Two different relaxations were observed, indicating different mobility of water molecules due to differences in the structure of the waxy or amylopectin starch gels.

20.1.11 Toward Practical Applications of Pressure-Gelatinized Starches

For utilization of pressure-gelatinized starch, a recent publication on starch-based hydrogels is of interest (Szepes et al. 2008). Gels were prepared using potato starch via HHP treatment with the aim of drug formulation. The effect of HHP treatment on the binding of odorants to starch was studied by using maize starches (Blaszczak 2007; Blaszczak et al. 2007c). Yamada et al. (1998) reported on some trials introducing fatty acids to ball-milled starch granules with HHP treatment. However, they focused on ball mill treatment rather than HHP treatment, and the effect of HHP on the fatty acid introduction was not clearly described.

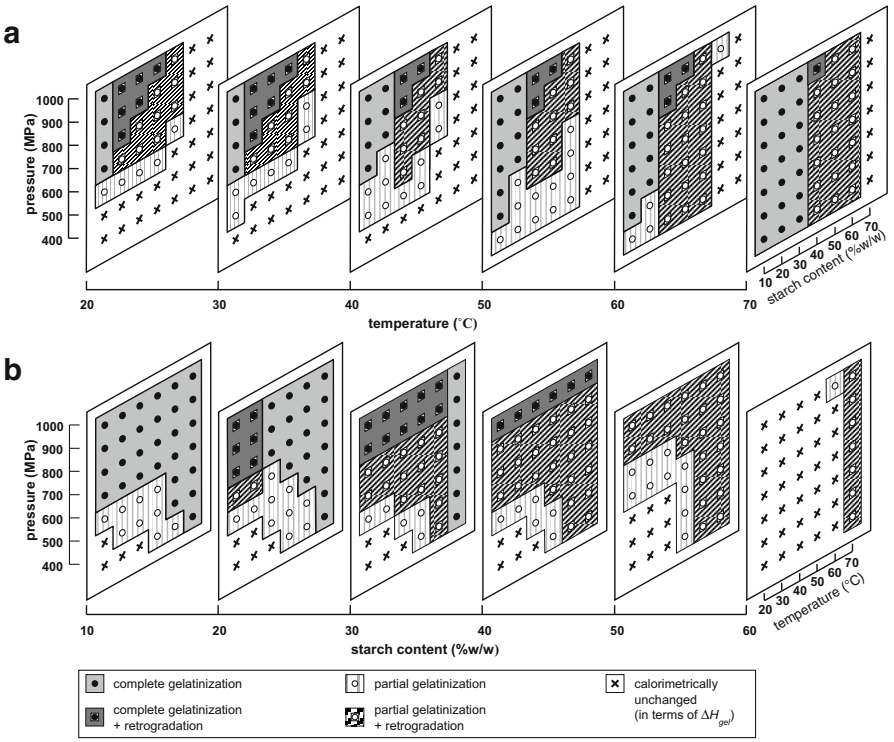

Fig. 20.12 State diagrams of potato starch–water mixtures (10–60 % w/w) HHP treated (400–1000 MPa) in a temperature range between 20 and 70 °C, as described by Kawai et al. (2012). (**a**) Diagrams (treatment pressure vs. starch content) are arranged along the axis of treatment temperature; (**b**) diagrams were replotted (treatment pressure vs. treatment temperature) and arranged along the axis of starch content. *Closed circle*, complete gelatinization; *closed circle in square*, complete gelatinization with retrogradation; *open circle*, partial gelatinization; *open circle in square*, partial gelatinization with retrogradation; *cross*, thermodynamically unchanged

HHP treatment has been applied to study its effect on food or its model system. The influence of potato starch on HHP-treated surimi gels (400 and 650 MPa, 10 min) was compared with heat-treated gels (90 °C, 40 min) (Tabilo-Munizaga and Barbosa-Cánovas 2005). The applied pressures seem to be insufficient for gelatinization, although the water holding capacity of the HHP-treated surimi gels was higher than for heat-treated gels. Huttner et al. (2009) evaluated impacts of HHP on oat batters, and Barcenas et al. (2010) reported microbial, physical, and structural changes in HHP-treated wheat dough. Vallons and Arendt (2010) studied HHP-induced rheological changes of wheat flour–water suspensions. Ten-min treatment of starch-gluten suspension at 400 or 600 MPa led to starch gelatinization and formation of protein network, which promoted strengthening of the flour structure. However, gelatinization of starch due to HHP treatment was the main cause of the enhanced viscoelastic properties.

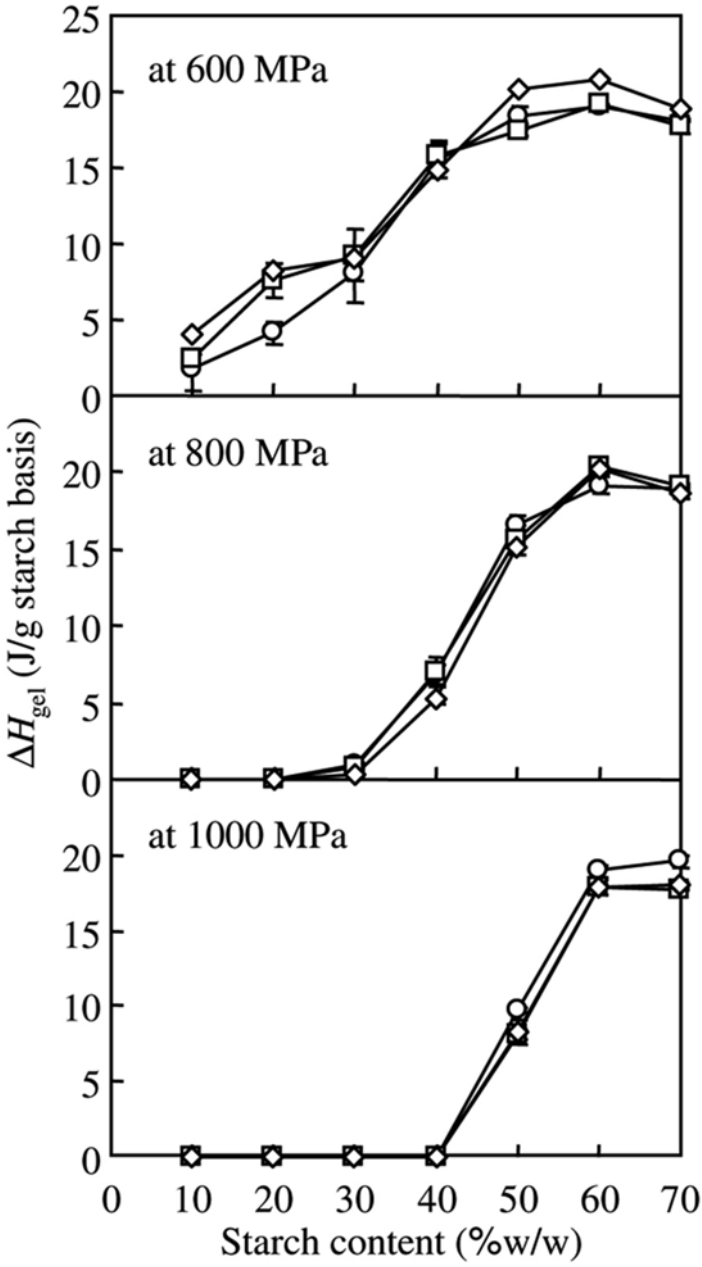

Fig. 20.13 Dependence of ΔH_{gel} on treatment time (1, 18, and 66 h), treatment pressure, and starch content of potato starch–water mixture, as described by Kawai et al. (2007b). *Circle*, 1 h; *square*, 18 h; *diamond*, 66 h

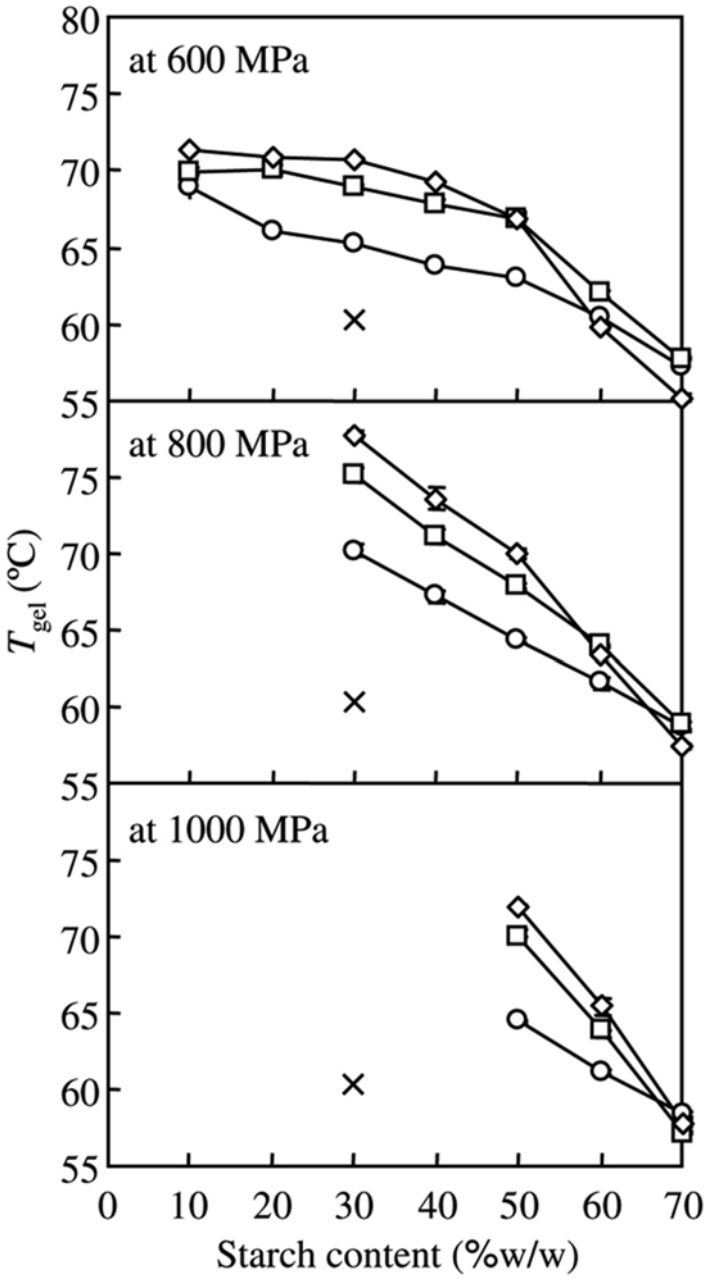

Fig. 20.14 Dependence of T_{gel} (onset temperature) on treatment time (1, 18, and 66 h), treatment pressure, and starch content of potato starch–water mixture, as described by Kawai et al. (2007b). *Circle*, 1 h; *square*, 18 h; *diamond*, 66 h. The *cross* indicates the T_{gel} of native potato starch–water mixture at the starch content of 30 % w/w

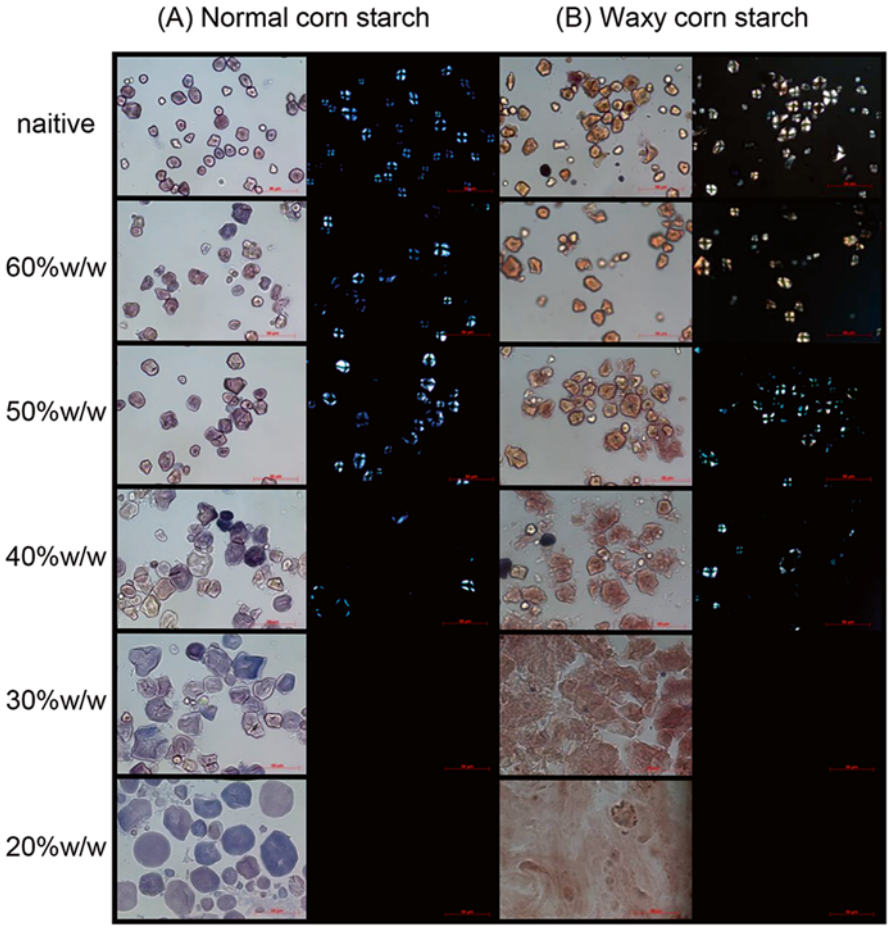

Fig. 20.15 Micrographs of (**a**) normal and (**b**) waxy maize starches treated at 600 MPa and 40 °C for 1 h at a range of starch contents, as described by Fukami et al. (2010). *Left*, bright-field microscopy; *right*, polarized microscopy; bar, 50 μm

Watanabe et al. (1991) found that the cooking properties of aged rice grains were improved by HHP treatment and that optimum pressure was 100 MPa in treatments at 20 °C for 10 min. Increasing pressure during HHP treatment at 0.1–600 MPa and 20–70 °C for up to 2 h facilitated gelatinization and improved water uptake and moisture equilibrium of Thai glutinous rice during soaking (Ahromrit et al. 2006, 2007). Basmati rice flour slurry and extracted rice starch were completely HHP-gelatinized after 15 min at 650 MPa and 550 MPa (approximately at room temperature), respectively (Ahmed et al. 2007). Lille and Autio (2007) evaluated the size and number of ice crystals in HHP-frozen starch gels using size and total area of pores in microscopic images of thawed gel. This study showed that average size and total area occupied by the pores were clearly reduced by HHP freezing. Kweon

et al. (2008) indicated that sodium chloride and sucrose have solute-induced barostablizing (or piezostabilizing) effects on HHP gelatinization (600 MPa at 25 °C for 15 min) of maize starch. Application of HHP to chemical conversion of starch into chemically engineered starches has been reported recently. For example, HHP can enhance the decrease of thermally generated radicals (Blaszczak et al. 2008), acid hydrolysis (Lee et al. 2006; Choi et al. 2009b), cross-linking (Hwang et al. 2009; Kim et al. 2012a, b), phosphorylation (Blaszczak et al. 2010, 2011), acetylation (Choi et al. 2009a; Kim et al. 2010), and hydroxypropylation (Kim et al. 2011) of maize and potato starches. Blaszczak et al. (2011) reported a decrease in thermally generated radicals in phosphorylation of maize starches with various amylose content after HHP treatment. HHP-assisted chemical conversion of starch has been reviewed by Kim et al. (2012b).

For industrial applications of HHP-treated starch, it is necessary to predict and control the process condition of gelatinization and retrogradation while demonstrating possible ways of commercialization of HHP-treated starches. An approach depicting state diagrams of HHP-gelatinized starches of various botanical origins would promote prediction and control of the process. New applications of HHP treatment to chemical modifications and other industrial process may be of great interest. It is also expected that analysis of HHP-treated starch through various and novel approaches will contribute to better understanding of starch granular structures.

20.2 Conclusions

The effect of HHP treatment on the behavior of starch has been studied since the (possibly) first description by Meyer et al. (1929), and experiments have been carried out by anisotropic and isotropic compressions and high pressure extrusion.

It has been revealed that starch in the presence of water can be gelatinized by HHP treatment. Functional and rheological properties of HHP-treated starches such as viscosity, pasting properties, retrogradation, and enzymatic digestibility often differ from the properties of heat-gelatinized starches. One difference is that regular (non-waxy) starch granules can be swollen and gelatinized by HHP but still retain their granular shapes. These phenomena may be affected by amylose content. Nonetheless, new insights into the mechanics of HHP-induced starch hydration and gelatinization could be achieved through in situ studies with diamond anvil cells, which permit the use of infrared and Raman spectroscopies, X-ray diffraction, and optical microscopy techniques.

Systematic studies on different types of starches at a wide range of pressures, temperatures, times, and water content are still rare. Investigations of microstructural changes of different starches during HHP treatment would be of interest in order to gain a better understanding of the interplay of amylose and amylopectin. Similarly, a comparison of crystallinity and molecular order within granules of HHP-treated and heat-treated starches can provide new information on the underlying mechanisms of starch granule swelling under different physical conditions.

A detailed study on the effect of water content on pressure gelatinization of potato starch showed that the state diagram (treatment pressure vs. water content) can classify the state of HHP-treated starch into five categories: complete gelatinization into five categories: complete gelatinization, complete gelatinization with retrogradation, partial gelatinization, partial gelatinization with retrogradation, and thermodynamically no change. Such state diagrams can be of practical use and, thus, remain important to the food industry for preparing HHP-treated starches and foods with defined properties.

Sugars and salts are common co-solutes in starchy foods, and thus the effects of such additives on pressure gelatinization have been investigated from the viewpoint of their solute-induced stabilization. However, further studies on the influence of saccharides, fibers, fats, and proteins, for example, on gelatinization under pressure may be needed to make this process of practical interest in the food industry. Recent studies also suggest that HHP treatment can enhance flour properties post-milling by structure modification of proteins and partial swelling of the starch granules.

Finally, HHP can also achieve targeted gelatinization degrees in starch, possibly resulting in a product with defined enzymatic digestibility and glycemic response. HHP-treated starch also gives unique retrogradation patterns, which can be attractive in terms of food functionality as nondigestible starch and/or fat replacers in low-energy formulations.

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Chapter 21
Reaction Chemistry at High Pressure and High Temperature

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Abstract Conventional thermal processing technologies, widely used to increase food safety and stabilize foods, can cause extensive chemical changes in foods. The food industry has been improving conventional and developing new technologies in response to consumers demand for high safety standards and close-to-fresh quality foods with high nutritional value. Pressure-assisted thermal processing (PATP), also called pressure-assisted sterilization (PATS) if the desired level of bacterial spore inactivation is achieved, is an alternative technology based on applying high temperature under high pressure. In the specific case of conduction-heating foods, adiabatic compression heating facilitates reaching temperatures lethal to microorganisms, which, in combination with fast decompression cooling, lowers quality degradation to levels below conventional thermal processing. However, its implementation requires advances in the analysis of reaction kinetics at high pressure and elevated temperature. Unfortunately, very few studies have focused on PATP effects on chemical reaction rates of quality factors at the temperature and pressure levels required for the sterilization of low-acid foods. Even fewer studies have focused on the effect of pressure on thermal degradation reactions known to form toxic compounds. At present, it is not possible to predict whether pressure will increase or decrease the rate for the degradation of quality factors and the formation of toxic compounds unless its activation volume value (V_a) is determined experimentally. Reactions with negligible rate under conventional pressure are of particular interest because they could become important in PATP-treated foods if their V_a value is negative and large. Such reactions will be greatly accelerated by pressure and could

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constitute a significant cause of safety risk and quality degradation. The evaluation of the safety risk in PATP-treated foods is therefore necessary but this need is still largely ignored by funding agencies in the USA. For example, temperatures in excess of 120 °C are required for a detectable formation of acrylamide in foods treated conventionally, but if this reaction had a large negative V_a value, significant levels could be formed at lower temperatures and high pressure. Although a recent model solution study conducted in Europe demonstrated that acrylamide formation is actually inhibited by pressure, this favorable finding must be confirmed with experiments in actual foods.

Keywords High-pressure processing • Reaction chemistry • Activation volume

21.1 Introduction

A significant recent development in the food processing industry has been the commercialization of high-pressure processing (HPP) at room or refrigeration temperature. However, HPP provides only a pasteurization effect since the inactivation of bacterial spores by pressure alone is not feasible (Torres et al. 2010; Torres and Velazquez 2008; Mújica-Paz et al. 2011; Serment-Moreno et al. 2015). An emerging and particularly innovative conservation technology, pressure-assisted thermal processing (PATP) or pressure-assisted thermal sterilization (PATS), could facilitate meeting the demand for more healthy, nutritious, varied, and convenient processed foods. This development is based on the combined application of high pressure and high temperature, typically in excess of 600 MPa and 100 °C (Shao et al. 2010; Valdez-Fragoso et al. 2011; Serment-Moreno et al. 2014). PATP promises to yield shelf-stable foods of high quality and reduce the loss of constituents with desirable health benefits (Torres et al. 2009b; Pérez Lamela and Torres 2008a, b; Escobedo-Avellaneda et al. 2011). However, PATP processing conditions can break covalent bonds and thus losses of nutrients, color pigments, and flavor compounds should be expected. Furthermore, under PATP conditions, the formation of some toxic compounds may reach rates not observed in conventional thermal processes (Segovia-Bravo et al. 2012). Therefore, the severity of PATP conditions achieving the inactivation of bacterial spores and baroresistant enzymes must be approached carefully to meet microbial safety and shelf-life goals. Unfortunately, very few comprehensive reports have been published on PATP effects on the kinetics of chemical changes in foods (e.g., Ramirez et al. 2009; Saldaña and Martinez-Monteagudo, 2014).

Typical HPP processing conditions include pressure levels of 500–600 MPa, initial temperature below 40 °C, and a holding time of 1–5 min. HPP products currently in the market include refrigerated products such as fruit juices, deli meats, seafood, ready-to-eat meals, and salsas (Torres et al. 2009a; Torres and Velazquez 2008; Ulloa-Fuentes et al. 2008a, b). Consumer perceptions that HPP products are closer to “natural” and have a high retention of nutrients including health-enhancing

components with high market value have facilitated the market acceptance of these products (Cruz et al. 2011). On the other hand, PATP is not yet a commercial technology and will require more complex safety validation procedures than HPP, particularly for the production of shelf-stable low-acid foods (pH>4.5). However, there is an important commercial interest in this new process alternative as reflected by the availability of commercial 35–55 L PATP prototypes being used for research on process and product development in Europe and the USA. In PATP processing, foods are first preheated to 70–90 °C, after which the temperature increases to levels lethal to bacterial spores due to the adiabatic heating of the food and the pressurizing fluid upon pressurization. Subsequent decompression reduces product temperature to values below those causing significant thermal degradation. In addition, it is possible to identify pressure and temperature combinations accelerating spore inactivation rate. These two effects reduce the severity of thermal treatments, particularly for conduction-heating foods, resulting in large quality improvements without compromising food safety.

The analysis of chemical reactions in PATP-treated foods is necessary when marketing foods in countries regulated by novel food laws. In the European Union (EU), novel foods were originally defined as foods and food ingredients not used for human consumption to a significant degree prior to May 1997 (Hepburn et al. 2008). In the EU and other countries following similar regulations (e.g., Canada), the safety evaluation of PATP-treated foods must follow the comparative principle of "substantial equivalence," i.e., they must be compared with comparable products obtained by conventional technologies. If undesirable compounds are detected in the PATP product, a detailed risk assessment of these compounds must be carried out including hazard identification, characterization, and evaluation of the consumer exposure (Tritscher 2004). By contrast, in the USA, a PATP sterilization process approved for mashed potatoes required no such characterization of toxicity risk (Anonymous 2009).

21.2 Effect of Extrinsic and Intrinsic Factors on Chemical Reactions under High Pressure

Information on the retention of vitamins, pigments, and flavor compounds under PATP conditions ensuring microbial and enzyme inactivation will require substantially more research. Although the maximum pressure covered in published studies on chemical changes in foods and model systems under PATP conditions is ~600–850 MPa, temperatures above 100 °C required to achieve the sterilization of low-acid foods are rarely included. Also, studies are needed to evaluate the effect of dissolved oxygen on the chemical degradation of flavor compounds and nutrients in PATP-treated foods since Oey et al. (2006) demonstrated that PATP degradation rates can increase with the dissolved oxygen concentration.

Serment-Moreno et al. (2014) reviewed recently a very large number of primary and secondary kinetic models available to design PATP treatments. However, the reliability of values predicted by these models will depend also on the availability

and quality of the experimental data. Unfortunately, missing data such as sample temperature, pressurization rate, insufficient sample characterization including dissolved oxygen concentration and at least initial and posttreatment pH which has not been measured while these foods are under high pressure and high temperature, limitations of applying gas reaction chemistry to chemical reactions under PATP conditions, and incorrect applications of physical principles can be found in publications on high-pressure research. For example, the Le Chatelier principle predicts a displacement of the equilibrium point for chemical reactions and is associated with the reaction molar volume change ΔV, defined as the difference between the partial molar volume of products and reactants. This value can be estimated from the partial derivative with respect to pressure of the reaction equilibrium constant K (Torres et al. 2009b, 2010):

$$V = -RT\left(\frac{\partial \ln K}{\partial p}\right)_T \tag{21.1}$$

One of the most important aspects frequently overlooked in the analysis of PATP treatments is the pressure-induced pH change. Water ionization changes under high temperature-high pressure resulting in a pH decrease. Marshall and Franck (1981) estimated the water ionization constant ($-\log K_w$) and predicted a drop of 1.09–1.46 pH units when water is pressurized in the 400–700 MPa and 70–100 °C range. Even though during depressurization pH might return to its original value, the pressure-induced pH shift while the food is under high pressure will affect the rate of chemical reactions and the inactivation rate of enzymes and microorganisms (Paredes-Sabja et al. 2007). Interpreting pH effects in food systems under pressure is challenging because the parameters to predict the pressure-induced pH shift (Eq. 21.2, El'Yanov and Hamann 1975; Neuman et al. 1973) have not been determined for foods. However, the reaction molar volume change for organic acids and buffers frequently found in biological system has been determined by spectrophotometric methods. Anionic solutions are usually accompanied by a negative change, whereas cationic and zwitterionic solutions show a slight pH increase (Gayán et al. 2013; Hayert et al. 1999; Kunugi 1992; El'yanov and Hamann 1975; Neuman et al. 1973):

$$(\mathrm{p}K_a)_p = (\mathrm{p}K_a)_0 + \frac{p(\ V^0)}{RT(1+bp)} \tag{21.2}$$

where:

$(\mathrm{p}K_a)_p$ = pressure-shifted dissociation constant
$(\mathrm{p}K_a)_0$ = dissociation constant at the reference pressure (0.1 MPa)
p = pressure (MPa)
ΔV^o = reaction molar volume change ΔV of the dissociating acid at 0.1 MPa (m^3/mol)
R = universal gas constant, 8.31446 (Pa m^3)/(K mol)
T = absolute temperature (K)
$b = 9.2 \times 10^{-4}$ MPa (assumed constant for all acids)

The limited consideration in published works of the food pH changes induced by pressure can be justified by the lack of practical and widely available instruments to

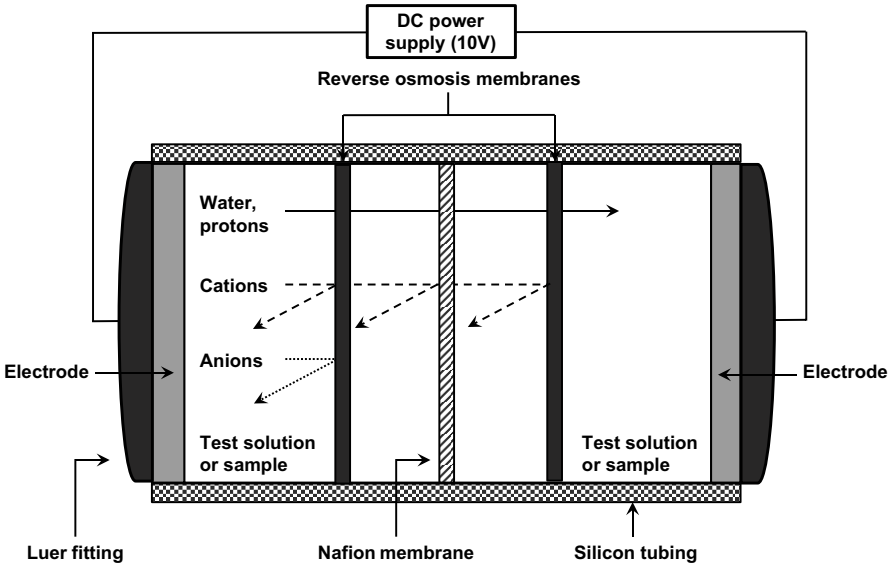

Fig. 21.1 Schematic view of a pH sensor capable of measuring pH of semiliquid and liquid food samples up to 800 MPa and 25 °C. Modified from Samaranayake and Sastry (2013)

measure them by direct methods. Although Samaranayake and Sastry (2010, 2013) developed recently an in situ pH sensor for use at pressures up to 825 MPa at room temperature (25 °C), the device needs to be further validated and tested at high temperature. The pH sensor consists of a Nafion membrane placed between two reverse osmosis membranes that selectively isolate the movement of hydrogen ions from the test solution when a 10 V (DC) voltage is applied to the two chromel wires acting as electrodes (Fig. 21.1). The membrane assembly and test solution are enclosed in silicon tubing allowing pressure transmission and sealed with luer fittings. The electrical connections are insulated with a Teflon tube sealed with epoxy resin. The test solutions consisted of a buffer (0.05 M biphthalate at pH 4.01 or 0.025 M phosphate at pH 6.01) and diluted HCl (0.1 M at pH = 1.35) mixture used to correct pH measurements since its ion concentration is known considering that HCl is fully dissociated in water. Finally, the pressurized solution pH was modeled (Eq. 21.3) by relating the density (ρ) and proton conductivity (σ^+) ratios at atmospheric pressure condition taken as a reference (subindex 0) (Samaranayake and Sastry 2008, 2010):

$$(\mathrm{pH})_0 - (\mathrm{pH})_\mathrm{P} = \log(\rho_\mathrm{P} / \rho_0) + \log(\sigma_\mathrm{P}^+ / \sigma_0^+) \tag{21.3}$$

The pH sensor developed by Samaranayake and Sastry (2010) has been used to determine the pressure-induced pH shift in buffer systems and in semiliquid and liquid foods at 0.1–785 and 0.1–800 MPa, respectively (Samaranayake and Sastry 2013). The pH of organic (acetate, biphthalate, phosphate, and sulfanilate) and biological (ACES, citrate, HEPES, MES, and TRIS) buffers decreased by 0.05–0.32 units at 785 MPa ($p < 0.05$), although the acidity of the solutions remained fairly

Table 21.1 Pressure effect on the pH of commercial food products (adapted from Samaranayake and Sastry 2013)

Food sample	pH_0 (0.1 MPa)	pH (800 MPa)	Steepest pH decline (Pressure range)
Liquids			
Deionized water	5.50	4.81	−0.69 ± 0.07 (0.1–800 MPa)
Fruit juices (apple, grapefruit, orange, tomato)	3.30–4.30	3.00–4.00	<0.3 (100–500 MPa)
Milk	6.60	6.30	<0.3 (100–500 MPa)
Chicken broth (99 % fat-free)	5.80	5.19	−0.37 ± 0.02 (0.1–100 MPa)
Semiliquids			
Guacamole	3.90	3.50	−(0.3–0.4) (0.1–100 MPa)
Ranch dressing	3.35	2.95	−(0.3–0.4) (0.1–300 MPa)
Yogurt (nonfat)	4.25	3.61	−0.58 (0.1–300 MPa)

constant as no significant differences were observed for pressures higher than 588 MPa. Nevertheless, the initial pH of all buffers was restored after depressurization indicating that the acidity increase was pressure driven. The values observed for organic buffers (Eq. 21.3) were compared to the pH predictions obtained with Eq. (21.2). Differences were more evident as the pressure level increased and the authors attributed the discrepancies to the methodology approach for pH measurement (optical methods vs. pH sensor) used on each study (Samaranayake and Sastry 2010). The pH changes of distilled water and several commercial food products measured by Samaranayake and Sastry (2013) are shown in Table 21.1. Distilled water experienced the highest pH decrease (–0.69 ± 0.07 units). Milk and fruit juices exhibited a slight pH change (<0.3 units) up to 500 MPa as previously described for buffer solutions (Samaranayake and Sastry 2010). The pH evolution of pressurized chicken broth and semiliquid foods was similar, showing a higher acidification at low pressure range (100–300 MPa) before stabilizing at 0.30–0.58 pH units below their initial value. The authors suggested that the stability of milk and fruit juices reflected the presence of weak organic acids in food which act as buffer agents reducing the impact of pH change. In the case of chicken broth and semiliquid foods, Samaranayake and Sastry (2013) proposed that the high compressibility of fats and enclosed air bubbles favored hydrogen bond formation that resulted in a steep pH fall below 300 MPa, whereas the breakdown of hydrogen bonds and the exposure of hydrophobic moieties of denaturalized proteins led to the subsequent pH stabilization at 300–800 MPa. Although the measurements performed by the pH sensor for both buffers and food products are certainly promising, Samaranayake and Sastry (2010, 2013) concluded that further experimentation is needed to reach an agreement with previous research and to elucidate ionization mechanisms under high pressure.

21.3 Applications to Dairy Flavor Volatiles

Changes in the rate of chemical reactions during food processing are more important than the equilibrium point shift predicted by the Le Chatelier principle because the processing time is too short to reach it. A primary kinetics model describing the changes with time (t) in concentration (c (t)) as affected by pressure (p) and temperature (T) for chemical reactions of any order n can be expressed as follows:

$$\frac{\mathrm{d}\,c(t)}{\mathrm{d}\,t} = k(p,T)\; c(t)^n \tag{21.4}$$

where k is the reaction rate constant at a given pressure and temperature. Under isobaric and isothermal conditions, integration of Eq. (21.4) yields the following expressions:

$$\text{Zero order}: \quad c(t) - c_0 = k(p,T)t \tag{21.5}$$

$$\text{First order}: \quad \log c(t) - \log c_0 = k(p,T)t \tag{21.6}$$

$$\text{Second order}: \quad \frac{1}{c(t)} - \frac{1}{c_0} = k(p,T)t \tag{21.7}$$

The expression with the best correlation coefficient (R^2) for experimental determinations of $c(t)$ conducted at several "constant" p and T levels is then used to determine pressure and temperature effects on the rate constant k. A major challenge is to obtain experimental data at constant p and T levels starting from food initially at atmospheric pressure and at refrigeration or room temperature considering that the pressure vessel, pressurizing fluid and the food will need to be preheated to reach lethal temperatures to the microorganism of concern. Differences in thermophysical properties among food components and the pressurizing fluid, combined with heat transfer to the pressure vessel and the environment, result in significant food temperature changes with time and placement in the vessel. If the temperature is not constant and not uniform, this will impact the observed rate of the chemical reactions and microbial or enzymatic inactivation. These rate expressions must be taken into account in process design and optimization (Grauwet et al. 2010a, b, c, 2011, 2012; Khurana and Karwe 2009; Rauh et al. 2009; Torres et al. 2009b). This also means that the c_o value in Eqs. (21.5)–(21.7) is at best a "pseudo-initial concentration." If c_o values at each test temperature do not change with pressure regardless of the treatment time, it will confirm that sample handling effects and heat losses have been minimized.

The theoretical frame developed originally for gas reactions (Serment et al., 2014) postulates that reactants must reach first an activated state (Fig. 21.2a) characterized by the temperature-independent Arrhenius activation energy (E_a) value estimated at constant pressures using Eq. (21.8). The slope of this curve is $-E_a/R$

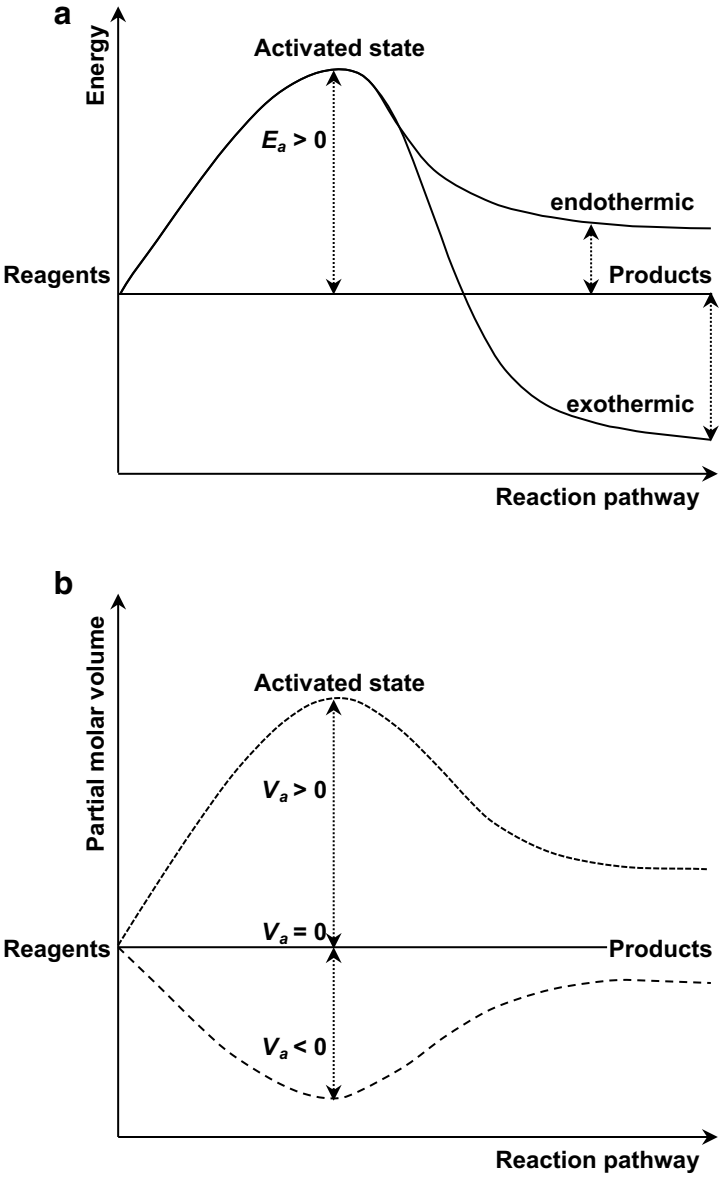

Fig. 21.2 Definition of the activation energy (E_a) for endothermic and exothermic reactions (**a**) and activation volume (V_a) for reactions inhibited ($V_a > 0$), inhibited ($V_a < 0$), or not affected by pressure ($V_a = 0$) (**b**)

(R = universal gas constant, 8.314×10^{-3} kJ mol^{-1} K^{-1}) and the intercept is ln k_o (k_o = pre-exponential constant):

$$\ln(k) = \ln(k_0) - \frac{E_a}{RT} \tag{21.8}$$

In the case of PATP-treated foods, the pressure effect on E_a values is analyzed by introducing the *partial activation molar volume* (V_a) concept (Fig. 21.2b), defined as the difference of the partial molar volume between the activated state and the unreacted molecule at the same pressure and temperature (Eq. 21.9, McNaught and Wilkinson 1997). Equation (21.9) can be integrated to obtain Eq. (21.10) where ln A is the integration constant. Values for V_a at constant temperature are obtained by linear regression of ln(k) versus pressure p:

$$V_a = -RT\left(\frac{\partial \ln k}{\partial p}\right)_T \tag{21.9}$$

$$\ln k = \ln A - \frac{(V_a)\ p}{R\ T} \tag{21.10}$$

E_a values required to reach the activated state are always positive while V_a values may be negative, positive, or zero (Torres et al. 2009b). The sensitivity of a chemical reaction rate to pressure depends on the absolute value of V_a (positive or negative). Large-magnitude V_a values (positive or negative) mean that the chemical reaction is highly sensitive to pressure while reactions with $V_a = 0$ are pressure independent. The activation energy E_a will decrease, remain unchanged, or increase with pressure if V_a is negative, zero, or positive, respectively. At constant temperature, the rate of a reaction will increase with pressure if V_a is negative while the opposite effect will be observed if V_a is positive. When V_a values are negative, the reaction kinetic constant k at constant temperature will increase with pressure. The opposite behavior will be observed for reactions with E_a values increasing with pressure, while no pressure effects on E_a values will correspond to reactions with $V_a = 0$. Chemical reactions with $V_a \gg 0$ will be so slow at high pressure that no changes will be observed during the PATP-treatment time needed to inactivate enzymes and microorganisms. This will result in a much enhanced quality of PATP-treated foods. On the other hand, in reactions with a very large negative V_a, the concern will be a significant loss of quality (e.g., loss of nutrients) and a higher formation of undesirable compounds.

The approach previously described was used to analyze the formation of 27 volatile compounds associated with cooked flavor in PATP-treated milk (Vazquez et al. 2007). In the case of straight-chain aldehydes, first-order kinetic constants fitted well the Arrhenius model with activation energy (E_a) values decreasing significantly with pressure. For example, E_a values for hexanal formation decreased nearly 40 times from 35.2 kJ mol^{-1} at 482 MPa to 0.9 kJ mol^{-1} at 655 MPa. In reactions following zero-order kinetics, E_a values increased with pressure for 2-methylpropanal and 2,3-butanedione but remained practically unchanged for hydrogen sulfide regardless of the pressure level. Most significantly, the concentration of the remaining 18 volatiles analyzed in PATP milk did not change under high pressure, suggesting that their formation reactions had $V_a \gg 0$. These findings provided an explanation to a previous principal component analysis (PCA) indicating that the volatile profiles

of PATP- and conventionally pasteurized milk are significantly different (Vazquez 2006; Vazquez et al. 2006). PCA showed that PATP milk treated at moderate temperature and pressure levels with a refrigerated shelf life longer than 7 weeks had a volatile profile similar to thermally pasteurized milk with a shelf life of only 2–3 weeks. A further observation was that for severe PATP processes, i.e., applying temperature conditions approaching those needed for commercial milk sterilization in combination with pressure, the PCA showed a smaller and different-direction volatile profile shift to the one observed for commercial UHT milk. Both findings are consistent with the observation that pressure had a different effect on the formation of the 27 volatile compounds analyzed in PATP milk. For example, aldehyde formation was accelerated while the reactions leading to the formation of 18 compounds were inhibited to negligible rates. Another important conclusion from the PATP milk study is that the increase, decrease, or lack of change caused by pressure and temperature on the formation of volatiles in PATP milk can be described with no need to assume alternative reaction pathways.

21.4 Assessment of a Potential Chemical Toxic Risk in PATP-Treated Foods

The benefits of heat treatments as a preservation method and to improve desirable food qualities such as palatability and nutritional value are well established. However, the formation of undesirable compounds is also possible, and subsequently, chemical toxic risks are receiving considerably more attention even in conventionally processed foods, particularly in Europe (e.g., Eisenbrand et al. 2007). The assessment of high hydrostatic pressure processing of foods at high temperature must consider potential chemical toxicology risks in addition to ensuring microbial safety, product stabilization, and the retention of flavor compounds, nutrients, and functional ingredients. These risks depend greatly on the presence of molecules with known influence in the potential formation of toxic compounds (e.g., reducing sugars and free amino acids via the Maillard reaction). The health hazards of Maillard reaction compounds such as acrylamide and of many other chemical compounds formed during thermal processing (e.g., heterocyclic aromatic amines, furans, and monochloropropanediol) should be evaluated in foods subjected to PATP treatments. Other reactions occurring during the heating of foods, such as the oxidation of unsaturated fatty acids and the advanced glycation between amino acids and sugars, have undesirable nutritional and health effects, and they must be considered also when treating high-fat products with PATP (Kanekanian 2010).

The next sections will cover examples of chemical reactions responsible for toxic risks in foods and the very few studies analyzing them under PATP conditions (Escobedo-Avellaneda et al. 2011; Segovia-Bravo et al. 2012). Unfortunately, the kinetics of formation of acrylamide, polycyclic aromatic hydrocarbons, heterocyclic amines, N-nitroso compounds, and other chemicals known for their toxicological risks in thermal processing is mostly unknown under PATP conditions.

Although theoretical considerations indicate that chemical reactions involving positive V_a values will be inhibited by high pressure and lead to a decreased formation of undesired compounds and/or significantly lower nutrient losses than under conventional thermal processing, it is not yet possible to predict whether the *Va* value will be positive or negative. Therefore, demonstrating the absence of undesirable chemical changes in PATP-treated products requires experimental evidence. On the other hand, chemical changes examined so far in PATP-treated foods and model systems have followed known reaction mechanisms making it unnecessary to postulate new reaction mechanisms. Finally, it is important to highlight that at present there is *no experimental* evidence questioning the safety of PATP and HPP foods. Moreover, some studies have shown a toxicity reduction such as a decrease in the formation of biogenic amines in ripened meat products treated by HPP (Ruiz-Capillas et al. 2007; Ruiz-Capillas and Jiménez Colmenero 2004) and an inhibition of acrylamide formation in model systems subjected to PATP treatments (de Vleeschouwer et al. 2011).

21.4.1 Acrylamide Risk

The Maillard reaction between amines and carbonyl compounds, which generates pleasant flavor and color compounds in cooked foods, can lead to acrylamide formation during the heating of foods containing the amino acid asparagine. In the first step of the reaction, asparagine reacts with a reducing sugar forming a Schiff's base which follows a complex reaction pathway including decarboxylation and a multistage elimination reaction (Zyzak et al. 2003). Acrylamide formation investigated in model systems has shown that free asparagine is a limiting factor since asparaginase treatment can prevent its formation (Weisshaar 2004). The formation of minor amounts of acrylamide in the presence of glutamine and methionine has also been reported (Stadler et al. 2002). In 2002, the Swedish National Food Authority and the University of Stockholm reported finding considerable acrylamide levels in starch-based foods (Anonymous 2002). Since acrylamide is neurotoxic, induces germ cell mutagenicity, and is classified as probably carcinogenic to humans, finding it in foods had a major international impact. Later research efforts have focused on confirmations of initial findings (Matthäus 2004; Zyzak et al. 2003), mechanisms of formation (Zhang and Zhang 2007; Zyzak et al. 2003), improvements to analytical methods (Kim et al. 2007), and efforts to reduce its level in processed food (Haase 2004). The concerns about acrylamide presence in foods reflect several evidences of safety risks. Glycidamide, an acrylamide metabolite that binds to DNA causing genetic damage, has been found in studies with mice, rats, and humans exposed to acrylamide. In vitro and in vivo studies have shown that acrylamide induces gene mutations in cell cultures and in animal studies (Gamboa da Costa et al. 2003). Neurological damage was observed when rats were given acrylamide in their drinking water and also in humans exposed to high acrylamide doses (Fullerton and Barnes 1966). Finally, decreased fertility has been observed in rats exposed to

5–10 mg acrylamide/kg body weight per day (Anonymous 2002). Under conventional thermal processing, significant acrylamide formation has been demonstrated to require temperatures higher than 120 °C (Pedrenski 2007). If the acrylamide reaction pathway were characterized by a large negative V_a value, pressure would accelerate its formation and significant levels could be formed even in foods processed at lower temperature but high pressure. The pressure-induced pH shift during PATP treatments also could affect the amount of acrylamide formed.

The acrylamide reaction has been under investigation for some time (Claeys et al. 2005a, b; de Vleeschouwer et al. 2006, 2008a, b; Anonymous 1994; Weisshaar and Gutsche 2002). The Maillard reaction is affected by high pressure and different effects have been reported, i.e., increasing or decreasing the formation of intermediate or final products depending on the reaction stage evaluated (Moreno et al. 2003; Isaacs and Coulson 1996; Schwarzenbolz et al. 2000, 2002) which increased concerns about the lack of studies on the acrylamide formation in PATP-treated foods. In model systems, de Vleeschouwer et al. (2011) showed that the amount of acrylamide formed at 115 °C and 600 MPa is much lower than the one observed for conventional thermal treatments (1700 ppb vs. 6500 ppb). The inhibition of acrylamide by pressure suggesting a positive V_a value for the reaction needs to be confirmed in food systems.

21.4.2 Polycyclic Aromatic Hydrocarbons, Heterocyclic Amines, N-Nitroso Compound Risk, and Hormone-Like Peptides

The formation in foods of polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds with two or more fused aromatic rings, depends on the cooking method. Grilling meat, fish, or other foods with intense heat over direct flame is one of the cooking procedures increasing the formation of PAHs. At temperatures in the 400–1000 °C range, organic compounds are fragmented into smaller compounds yielding relatively stable PAHs. Benzo[α]pyrene, a five-ring polycyclic aromatic hydrocarbon, is the PAH most commonly studied because of its mutagenic activity being the most carcinogenic compound of all PAHs (Anonymous 1983; Lee et al. 1981). Benzo[α]pyrene, benz[α]anthracene, and dibenz[α,β]anthracene have been reported to be carcinogenic in animals by oral intake and considered as *probably* carcinogenic to humans. It remains to be determined if the lower temperatures to be used in the production of PATP-treated foods (typically under 120 °C) will result in significant formation of PAHs.

Another group of toxic compounds formed during food heating is heterocyclic amines grouped into amino-carbolines, imidazoquinolines, imidazoquinoxalines, and imidazopyridines and characterized by two or three rings with an exocyclic amino group. They are produced via the Maillard reaction from creatine or creatinine, certain free amino acids, and sugars when cooking or heating meat or fish (Jägerstad and Skog 2005; Jagerstad et al. 1998). If these three precursor groups are

present in a PATP-treated food, it should be analyzed for the presence of heterocyclic amines. In addition, foods treated by PATP containing nitrites, typically added to cured meat and fish products for *Clostridium botulinum* control, should be tested for the presence of genotoxic N-nitroso compounds. As there is no evidence that PATP affects their formation rate, PATP treatment of packaged foods opens the possibility of eliminating this additive.

Research has been focused also on other undesirable reactions that can take place under high pressure such as the formation of peptides with hormone-like effects. Chemical cyclization reactions could take place under high pressure and change the relative concentration of short-chain peptides in foods with unpredictable biological effects. For instance, Fernández García et al. (2003) reported that high pressure induces the formation of hormone-like substances by cyclization of glutamine at the N-terminus of certain peptides. The same authors have reported that application of pressure accelerates the formation of diketopiperazines, a peptide with biological activity formed from aspartame (Butz et al. 1997, 2002).

21.5 Screening the Formation of Mutagenic Agents in PATP-Treated Food

The identification of genotoxic substances is an important procedure in the assessment of processed food safety including novel foods. The Ames *Salmonella*/microsome mutagenicity assay is a rapid bacterial reverse mutation assay designed to detect a wide range of chemical substances that can produce genetic damage leading to gene mutations (Mortelmans and Errol 2000). In the test, several histidine-dependent *Salmonella* strains with different mutation genes of the histidine operon are used. These mutations are locations responding to the food compound to be tested. Some carcinogenic substances, such as aromatic amines and polycyclic aromatic hydrocarbons, are biologically inactive if they are not metabolized to active forms. In humans, these compounds are metabolized by the cytochrome-based P450 oxidation system in the liver; however, as bacteria do not have this metabolic system, a rat liver extract is added to the mixture of bacteria to activate the potential carcinogenetic chemical (Ames et al. 1973). In the test, *Salmonella* strains are grown in a media with very low levels of histidine, so only bacteria able to revert to an histidine-independent mutant can grow in that media and form colonies. The test is positive if the number of colonies that grow in the media without histidine is higher than the number of spontaneous revertants in media without the test substance. The difference is proportional to the dose effect of the test substance.

The single cell gel electrophoresis assay, also known as the comet assay, is a sensitive technique for detecting DNA damage at the level of the individual eukaryotic cell. This technique was first developed to detect DNA damage at the single cell level (Östling and Johanson 1984) and later the technique was modified with an alkaline electrophoresis step (Singh et al. 1988). The standard alkaline comet assay and its various modifications provide a relatively simple, sensitive, and rapid

method of analyzing DNA damage and repair. It is economical, simple, and fast, so its use has been rapidly expanding in recent years. It involves the encapsulation of cells in a low-melting-point agarose suspension. A sample of cells derived from an in vitro cell culture is dispersed into individual cells and suspended in molten low-melting-point agarose at 37 °C. This mono-suspension is cast on a microscope slide of cells lysed in neutral or alkaline (pH > 13) conditions followed by electrophoresis of the suspended lysed cells. The migrating DNA is quantified by staining with ethidium bromide and by measuring the intensity of fluorescence at two fixed positions within the migration pattern using a microscope photometer equipped with imaging software.

21.6 Conclusion

Although PATP products are not yet on the market, a near-future commercialization of this technology is expected since commercial prototypes are being now used to evaluate the quality and safety of PATP-treated foods at the laboratory and pilot plant level. Among the challenges to overcome, the unpredictable effect of pressure on the kinetics of chemical reactions at the high temperatures required to produce shelf-stable foods remains as one of the most important. This effort must ensure the absence of toxicological risks in PATP-treated foods through reaction studies like the ones here described, to determine whether the formation of these known unwanted compounds is accelerated or decelerated by PATP treatments. It may be also necessary to consider the possibility of new toxicological risks since the formation of compounds that are not present after conventional thermal processing at a detectable rate could occur in PATP-treated foods, reflecting chemical reactions with a large positive activation volume.

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Chapter 22
Effect of High-Pressure Processing on Bioactive Compounds

Swetha Mahadevan and Mukund V. Karwe

Abstract High pressure processing (HPP) has varying effects on bioactive compounds in food matrices. Pressure treatment at moderate temperatures can retain bioactivity and other food quality attributes of flavor, color and nutritional properties due to its minimal effects on covalent bonds of bioactive compounds. Pressure treatment at elevated temperatures, however, has been shown to induce biochemical reactions that could reduce the nutritional value of food products. The following chapter discusses effects of HPP on stability of different bioactive compounds in model systems and food matrices. As a direct consequence of the stability/instability of these bioactive compounds during HPP, their bioactivity (e.g., antioxidant capacity, bioavailability, antimutagenicity) and sensorial properties of food products are affected. Additionally, degradation mechanisms/pathways of some of these bioactive compounds are discussed in this chapter.

Keywords High-pressure processing • Bioactivity • Sensory • Fruits and vegetable • Meat

22.1 Introduction

High-pressure processing (HPP) has been shown to inhibit and inactivate microorganisms, rendering foods safe to consume. This minimal processing technique has exhibited extensive industrial applications in retaining food quality attributes of color, flavor, and nutritional characteristics. The limited effects of HPP on non-covalent bonds at moderate temperatures, thought to be a reason for minimal changes to nutritional properties of foods, are a unique feature of this technology (Hogan et al. 2005). Numerous studies on vitamins and polyphenol stability have demonstrated that high-pressure processing at moderate temperatures does not significantly affect their levels in food products. Much of their bioactivity is also

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retained, making this technology a promising alternative for thermal processing in selected applications (Rastogi et al. 2007; Oey et al. 2008a, b). Recently, the US FDA has accepted a petition for commercial use of pressure-assisted thermal sterilization process (PATS) that uses high temperatures along with high pressures to achieve commercial sterility of low-acid food products (Food Processing 2009). Such treatment, although successful in reducing food safety risks, can cause degradation of thermolabile bioactive compounds. In light of these new developments, the effect of HPP on bioactive compounds, particularly on their stability, bioactivity, and overall influence on sensorial quality of food products, will be reviewed in this chapter.

22.1.1 Bioactive Compounds: Short Description

As per the consensus of researchers at the 23rd Hohenheim Consensus Meeting at Stuttgart, the definition of bioactive compounds include essential and non-essential compounds, such as vitamins and polyphenols, that occur in nature, are part of the food chain, and can be shown to have an effect on human health (Biesalski et al. 2009). This definition, albeit different from the definition adopted at present by the US Department of Health and Human Services of National Institutes of Health (NIH), is more comprehensive and complete from a regulatory standpoint (Grocery Manufacturers of America 2005).

Bioactive compounds are classified as nutraceuticals and toxicants. Nutraceuticals have disease-preventing and human health-promoting effects, whereas toxicants have adverse health effects in humans. These compounds are commonly found in many food products, such as fruits, vegetables, medicinal plants, herbal products, nuts, grains, marine products, and meat products.

The scope of this chapter will be limited to the study of effects of HPP on nutraceuticals and will not discuss toxicants.

22.1.2 High-Pressure Processing: Short Description

High-pressure processing (HPP) has received much focus as a non-thermal process due to its different applications. In this process, food materials are subjected to isostatic pressures ranging from 40 MPa (5 kpsi) to 1000 MPa (145 kpsi) for a period of 1–20 min (Rastogi et al. 2007). As compared to thermal processing at atmospheric pressure, HPP has a great advantage of instantaneously transmitting uniform pressure from all sides on the food material, irrespective of the size and shape of the product (Hogan et al. 2005).

When pressure is applied to foods, many food-dwelling microorganisms are inhibited or inactivated. Bioactive and sensorial compounds, such as vitamins, flavor compounds, and color pigments, however, are minimally affected. Foods can thus

be pasteurized and preserved to retain fresher and healthier characteristics desired by consumers (Rastogi et al. 2007). One possible reason for higher stability of bioactive compounds under high pressure compared to heat treatment is that high pressure affects only non-covalent bonds (hydrogen, ionic, and hydrophobic bonds). This effect on non-covalent bonds causes macro-molecules such as protein chains to unfold under high pressure but has little effect on chemical constituents associated with desirable food quality components such as flavor, color, and nutritional compounds (Hogan et al. 2005).

HPP is accompanied by increase in temperature of food materials. The increase in temperature of food materials during pressurization can be explained by compression heating. Heat of compression is a thermodynamic outcome of high pressure processing. Although many food and non-food materials are considered as incompressible at atmospheric and low pressures, they do get compressed substantially under very high pressures (water can be compressed by 4 % at 100 MPa and 15 % at 600 MPa and 22 °C) (Tauscher 1995). Moreover, to pasteurize and achieve commercial sterility in foods, HPP is commonly used in conjuction with moderate to high temperatures. Effects of pressure and temperature on food components are governed by activation volume and activation energy. Difference in sensitivity of substances to pressure (activation volume) and temperature (activation energy) defines the possibility of retention or destruction of food quality attributes such as vitamins, polyphenols, enzymes, color pigments, and flavor compounds (Rastogi et al. 2007).

In the following sections, the effects of HPP on stability of bioactive compounds (nutraceuitcals) in foods will be discussed in details, including vitamins, carotenoids, flavonoids, mono- and polyunsaturated fatty acids, and sulfur-containing bioactive compounds. As a direct consequence of the stability/instability of these bioactive compounds during HPP, different functional properties of the food products are affected. These properties include antioxidant activity, bioavailability, antimutagenic activity, and sensorial characteristics, which will also be discussed in this chapter. The understanding of mechanisms responsible for degradation, elevation or retention of bioactive compounds under pressure is minimal.

22.2 Effect of HPP on Stability of Bioactive Compounds

22.2.1 B Vitamins

22.2.1.1 Thiamin and Pyridoxal

Most studies testing the stability of B vitamins under high pressure have reported no significant loss in their concentration and activity at room temperature with some losses at elevated temperatures. Model multivitamin systems containing varied levels of thiamin (vitamin B1), pyridoxal (vitamin B6), and ascorbic acid (vitamin C) were subjected to pressures ranging from 200 to 600 MPa for 30 min at 20 °C to

determine the stability of these vitamins. Thiamin and pyridoxal in the model system were unaffected by high-pressure processing (Oey et al. 2008a). Even unusually long processing times of 18 h at 600 MPa and 25 °C, Butz et al. (2007) reported no loss of thiamin and thiamin monophosphate in a model acetate buffer system (pH 5.5). However, at temperatures above 40 °C, thiamin and thiamin monophosphate showed accelerated degradation following first-order degradation kinetics; thiamin monophosphate was more temperature sensitive (40 °C and 60 °C) than thiamin. Decay of thiamin and thiamin monophosphate was further accelerated in the presence of L-ascorbic acid. This decay was caused by oxidation of ascorbic acid to dehydroascorbic acid and consequent oxidation of thiamin and thiamin monophosphate by dehydroascorbic acid. The same vitamins (thiamin and thiamin monophosphate) present in food products, such as rehydrated pork lyophilizate and minced pork fillet, were found to be substantially more stable after HPP than observed in the model system (Butz et al. 2007).

22.2.1.2 Riboflavin, Niacin, and Pantothenic Acid

Riboflavin (vitamin B2), abundantly found in meats, poultry, fish, and dairy products, was found to be very stable under pressure in the model buffer solution (pH 5.5) and in minced pork meat up to 600 MPa for a treatment time of 15 min and processing temperature ranging between 20 °C and 100 °C. Riboflavin content even increased under pressure (600 MPa/25, 60 and 100 °C/45 min) in fresh minced pork. The increase in riboflavin content was attributed to release of chemically or physically bound vitamins in the food matrix (Butz et al. 2007). Riboflavin was found to be a pressure- and temperature-resistant B vitamin. Other food systems tested for B vitamin stability included (1) red-orange juice, which showed no change in content of vitamins B2, B6, and niacin for treatment conditions of 200–500 MPa, 30 °C for 1 min (Donsi et al. 1996), and (2) sprouted alfalfa sprouts in citric acid pickle, which showed no change in riboflavin, niacin, and pantothenic acid after pressure treatment at 500 MPa for 10 min at room temperature. However, during storage at 4 °C, levels of niacin and pantothenic acid in pressure-treated sprouts decreased by almost 60 % after the third day. Riboflavin levels did not change significantly during storage up to 21 days (Gabrovska et al. 2005).

22.2.1.3 Folic Acid

Another extensively studied B vitamin is folic acid, which is found in fruits and vegetables. Folic acid deficiency has been of major concern in underdeveloped countries, and understanding its stability during processing is therefore of interest to many researchers. In plant tissues, B vitamins are commonly present as formyltetrahydrofolate and methyltetrahydrofolate conjugated to a chain of two to eight glutamate moieties, known as folate polyglutamate (Melse-Boonstra et al. 2002). Detailed kinetic studies show that (a) folate stability under high-pressure, high-temperature treatment

depends on the type of folate form and its source (Melse-Boonstra et al. 2002; Indrawati et al. 2004), (b) folate degradation increases with increasing pressure (above 200 MPa) at constant temperature and with increasing temperature (above 40 °C) at constant pressure (Nguyen et al. 2003, 2005; Verlinde et al. 2008), and (c) ascorbic acid enhanced thermo- and baro-stabilities of 5-methyltetrahydrofolic acid. The minimum concentration of ascorbic acid required to prevent oxidation of folate was at least two times as high as the initial oxygen concentration present in the system when ascorbic acid is the only antioxidant in the system (Oey et al. 2006).

Folate poly-γ-glutamate in model buffer solutions (pH 4–9) showed significant loss of 10–90 % after combined pressure-temperature treatment at 100–600 MPa and 40 °C for 25 min. Extent of loss was dependent on pH and pressure-temperature conditions (Verlinde et al. 2008). Degradation of 5-methyltetrahydrofolate was shown to follow first-order kinetics in model buffer solutions after pressure-temperature treatments (Indrawati et al. 2004). Folate degradation during HPP treatment is primarily caused by oxidation and cleavage of covalent bonds at high temperatures due to pressure-enhanced oxidative reactions. However, in the presence of ascorbic acid, oxidative degradation of 5-methyltetrahydrofolic acid and 5-formyltetrahydrofolic acid was retarded (Indrawati et al. 2004; Verlinde et al. 2008). Ascorbic acid acts as an antioxidant preventing oxidation of folic acid. This effect of ascorbic acid in a folate system is unlike its behavior in a system containing thiamin (vitamin B1) where ascorbic acid promotes vitamin degradation. In addition, non-oxidative chemical conversion is also induced during HPP, especially at elevated temperatures. For example, in the presence of ascorbic acid, oxidative decay of folate was decreased during HPP, but reduction of 5-formyltetrahydrofolic acid to 5,10-methynyltetrahydrofolic acid was significantly higher (Oey et al. 2008a). This conversion involves volume reduction, such as formation of charged molecules and cyclization reactions, which are favored under high pressure following Le Chatelier's principle (Butz et al. 2004).

Detailed folate stability studies have been conducted in food matrices. Folate polyglutamates were more stable in food products during different temperature-pressure treatments than in model buffer solutions (Indrawati et al. 2004). Depending on the food matrix, folates showed a different extent of sensitivity to pressure-temperature treatment. 5-Methylhydrofolic acid in orange juice and kiwi puree was more pressure stable (up to 500 MPa/60 °C/60 min) than in carrot juice and asparagus. Different stabilities in food products could be due to the presence of different levels of endogenous ascorbic acid inhibiting degradation of folates (Indrawati et al. 2004). Similar to the studies using model buffer solutions, the presence of ascorbate in food matrices retarded degradation of folates under pressure, as shown in Fig. 22.1 (Oey et al. 2006). Broccoli, green beans, cauliflower, and leeks showed significant loss (48–80 %) in total folate concentration after high-pressure treatment (100–600 MPa/25–45 °C/5–30 min) (Melse-Boonstra et al. 2002; Verlinde et al. 2008). These studies also reported formation of significant amounts of folate monoglutamates from folate polyglutamate forms found in vegetables. This result was seen as an increase in free folate concentration in leeks and orange juice at pressure treatments of ≥200 MPa at 25 °C (Melse-Boonstra et al. 2002; Indrawati et al. 2004).

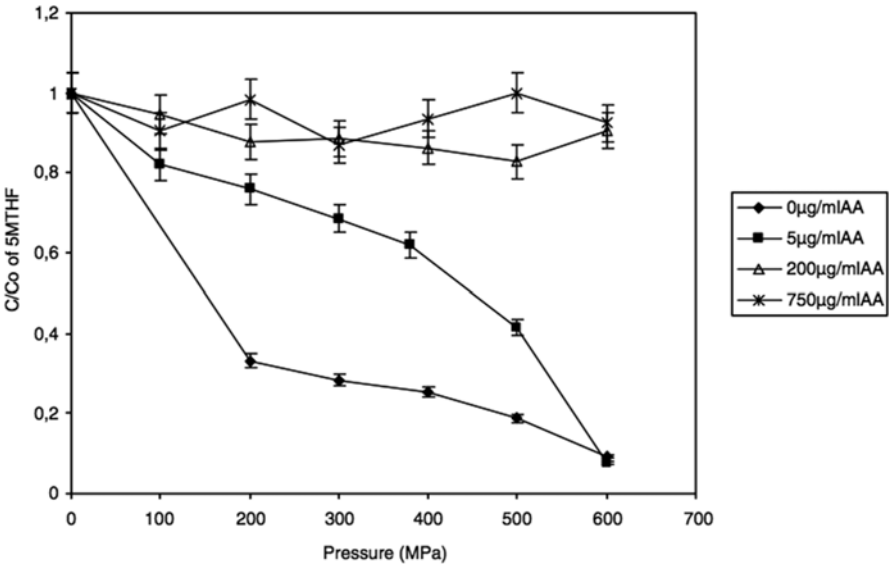

Fig. 22.1 Effect of L-ascorbic acid concentrations (L-AA) on pressure stability of 5-methyltetrahydrofolate (0.2 µg/ml) in model phosphate buffer solution (0.1 M; pH 7) with 8.11 ppm initial oxygen concentration. Sample treatment was carried out at 40 °C for 15 min. Plot shows L-AA concentration above 200 µg/ml had no additional protective effect on folate stability. (Reprinted from Oey et al. 2006 with permission from publisher)

22.2.1.4 Vitamin C or L-Ascorbic Acid

Vitamin C or L-ascorbic acid is the most researched vitamin for its post-processing stability due to its ubiquitous presence in plant- and animal-based products. Numerous reports have been published on vitamin C stability under pressure in controlled environment buffer systems and food products and during subsequent storage. Most studies have shown that L-ascorbic acid is pressure resistant and survives pressure treatments below 50 °C. However, there is significant loss during high-pressure, high-temperature combination treatments (Van Den Broeck et al. 1998). Oxygen has been shown to play an important role in ascorbic acid degradation, since this degradation is a rapid oxygen-dependent reaction that proceeds until oxygen is depleted, followed by anaerobic degradation. Therefore, limiting oxygen exposure can control ascorbic acid degradation. Similar to thermal degradation kinetics of L-ascorbic acid, combination pressure-temperature treatment also tends to follow first-order kinetics (Van Den Broeck et al. 1998).

In buffer solutions, a major part of ascorbic acid oxidation occurred below 100 MPa. Increasing pressure beyond 100 MPa up to 600 MPa did not show significant change in residual ascorbic acid levels, as shown in Fig. 22.2 (Oey et al. 2006). These observations suggest that aerobic oxidation of ascorbic acid was enhanced at relatively low pressures (<100 MPa) when all oxygen was consumed. Little or no

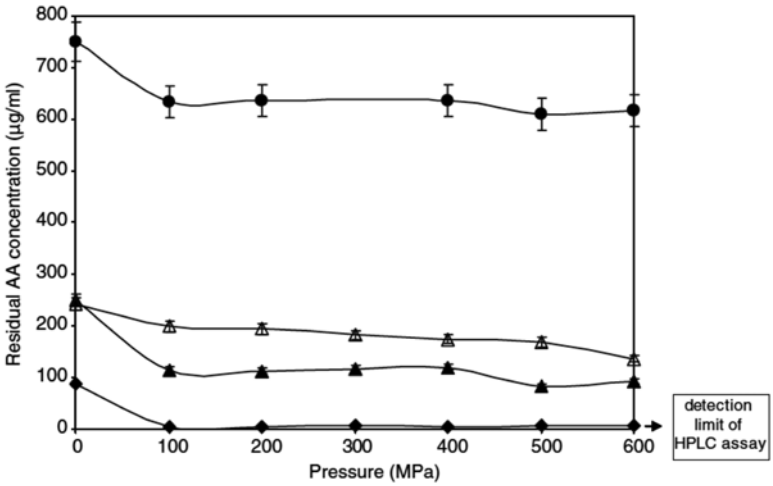

Fig. 22.2 Effect of applied pressure at 50 °C on stability of L-ascorbic acid in model phosphate buffer solution in the presence of 8.11 ppm (*closed symbols*) and 2.11 ppm (*open symbols*) of oxygen. Concentrations of L-AA tested were 100 µg/ml (*filled diamond*), 250 µg/ml (*filled triangle*, *open triangle*), and 750 µg/ml (*filled circle*). Plot shows that pressure stability of L-AA is dependent on its concentration and oxygen levels. At pressures <100 MPa, all oxygen is consumed resulting in aerobic oxidation of L-AA, and higher pressures (>100 MPa) did not initiate anaerobic degradation of L-AA. (Reprinted from Oey et al. 2006 with permission from publisher)

effect on ascorbic acid stability with further increase in pressure indicates that higher pressures (>100 MPa) did not initiate anaerobic oxidation under the tested conditions (Van Den Broeck et al. 1998). Sancho et al. (1999) observed similar results when ascorbic acid in phosphate buffer (pH 7) was pressurized at 200, 400, and 600 MPa each for 30 min at 20 °C. Percent degradation of ascorbic acid was not more than 13 % and was not significant compared to untreated samples.

However, when high temperatures and longer processing time were used in combination with high pressure (850 MPa, 65–80 °C, up to 6 h), considerable degradation of ascorbic acid occurred (Van Den Broeck et al. 1998). Figure 22.3 shows that pressure stability is dependent on concentrations of both ascorbic acid and oxygen. When the initial oxygen concentration in 250 µg/ml of ascorbic acid solution was dropped from 8.11 ppm (0.253 mM) to 2.11 ppm (0.066 mM), the percent decrease in residual ascorbic acid concentration changed from 40 % to 10 % (Oey et al. 2006).

Among food products, ascorbic acid stability has been extensively studied in fruit and vegetable products. In strawberry puree and strawberry coulis, pressure treatment (400–600 MPa/20 °C/15–30 min) resulted in ~10–12 % decrease in vitamin C levels (Sancho et al. 1999; Patras et al. 2009b). Fruits such as oranges, apples, apple purees, mixed citrus juices, carrots, tomatoes, and frozen raspberries showed no significant difference after HPP treatment (400–800 MPa/25–44 °C/6 min) compared to unprocessed samples (Butz et al. 2003; Landl et al. 2010). In orange juice (Navel and Valencia), total vitamin C loss was <9 % (Bull et al. 2004;

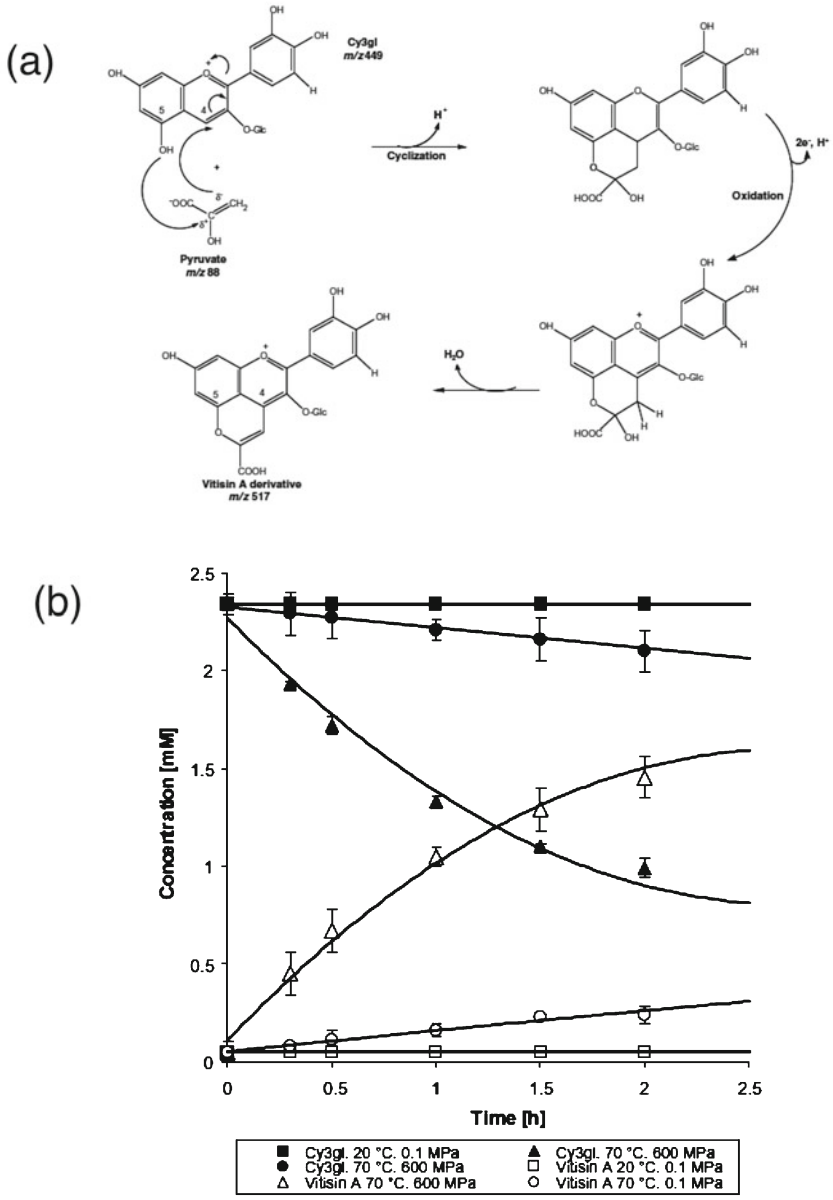

Fig. 22.3 Illustration of degradation of cyanidin-3-glucoside and formation of vitisin A derivative in acetate buffer showing reaction mechanism (**a**) and rate of change of concentrations of cyanidin-3-glucoside and vitisin A derivative (**b**). *Plot* shows that rate of degradation of cyanidin-3-glucoside and formation of vitisin A derivative is much higher at 600 MPa/70 °C than in thermally treated or unpressurized samples. (Reprinted from Corrales et al. 2008 with permission from publisher)

Sanchez-Moreno et al. 2005). In melons (*Cucumis melo* L.), treatment at 600 MPa/room temperature/10 min showed 1.3- to tenfold decrease in L-ascorbic acid levels. These losses were found to be cultivar dependent (Wolbang et al. 2008).

Vegetable-based products showed higher degradation of ascorbic acid compared to fruit-based products. In green peas, pressure treatment between 400 and 700 MPa/33–40 °C/5–10 min showed only 50–80 % degradation of L-ascorbic acid (Quaglia et al. 1996). Alfalfa sprouts pickled in citric acid showed 77 % loss of L-ascorbic acid after pressure treatment at 500 MPa/room temperature/10 min (Gabrovska et al. 2005). Cowpeas germinated for 4–6 days showed 10–69 % decrease after HPP treatment (300–500 MPa/room temperature/15 min) (Doblado et al. 2007). Only one study on sweet red bell peppers reported an increase in L-ascorbic acid by 10–20 % after pressure treatment (100 and 200 MPa/25 °C/10 and 20 min). This increase in ascorbic acid level in red peppers could not be explained (Castro et al. 2008).

Ascorbic acid retention increased with increase in pressure and decrease in processing time in vegetable products. Green peas processed at 900 MPa/43 °C/5–10 min retained 82 % of ascorbic acid compared to 50 % retained after processing at 400 MPa/33 °C/5–10 min (Quaglia et al. 1996). In green beans, pressure treatment to achieve pasteurization (500 MPa/room temperature/60 s) and sterilization (two pulses of 1000 MPa/75 °C/80 s) resulted in 92 % and 76 % retention of L-ascorbic acid, respectively (Krebbers et al. 2002). Higher retention of ascorbic acid with increase in pressure was correlated to reduction in peroxidase activity (Quaglia et al. 1996). HPP at room temperature did not have a significant effect on ascorbic acid levels in egg yolks and human milk (Sancho et al. 1999; Molto-Puigmarti et al. 2011).

Stability of vitamin C during storage in high-pressure-processed food products is related to enzyme activity and availability of oxidants that catalyze oxidation of ascorbic acid. Studies have reported that degradation of vitamin C can occur during storage and that its degradation can be eliminated by storage at low temperatures. In HPP-treated green beans (500 MPa/20 °C/1 min), ambient storage for 1 month resulted in ascorbic acid levels dropping to negligible amounts. Frozen storage, however, resulted in retention of ascorbic acid (Krebbers et al. 2002). Orange juice pressure treated (600 MPa/40 °C/4 min) and stored for 1 month at 5 °C retained 84 % ascorbic acid (Polydera et al. 2005a). Sprouted alfalfa seeds pickled in citric acid showed about 10–20 % loss of L-ascorbic acid during refrigerated storage for 21 days after HPP treatment (500 MPa/room temperature/10 min) (Gabrovska et al. 2005). In general, it is concluded that pressure neither accelerates nor slows degradation rate. Only the presence of prooxidants such as light, air, and heat increased degradation rate. Here, the major loss in ascorbic acid is associated with chemical degradation, i.e., aerobic oxidation to L-dehydroascorbic acid (Sancho et al. 1999). Another hypothesis is that ascorbic acid could be degraded due to enzymatic reactions, i.e., peroxidase catalyzed oxidation of ascorbic acid (Krebbers et al. 2002).

In summary, L-ascorbic acid or vitamin C is stable under high-pressure treatment carried out at mild temperatures (<60 °C). Vitamin C is unstable at high-pressure, high-temperature combination treatments. The major pathway of degradation during

high-pressure processing is observed as aerobic oxidation. Anaerobic oxidation plays a role in degradation of ascorbic acid when processed for longer times at high pressures combined with high temperatures. Contradictory results on stability of vitamin C in HPP-treated food products could possibly be due to variation in experimental conditions, i.e., the amount of prooxidants and antioxidants present in the system. Further investigation is required to understand the exact mechanisms of degradation during processing and storage.

22.2.1.5 Vitamins A, E, and K

Literature on the effect of high-pressure treatment on fat-soluble vitamins is less extensive compared to water-soluble vitamins. Pressure stability of retinol and vitamin A has been studied in model systems and some food products. Vitamin A (retinol) stability has been more commonly studied in its provitamin form (β-carotene), which is discussed in a later section on carotenoids. In model systems, high-pressure treatment has been shown to degrade vitamin A. For example, high-pressure treatment at elevated temperatures (600 MPa/40, 60, and 75 °C/5 min) resulted in a decrease in retinol levels by 45 % (Tauscher 1999). When the treatment time was increased to 40 min, the retinol levels dropped by 70 % (Butz and Tauscher 2000). This degradation of retinol in solution followed second-order kinetics. Another study with retinol acetate in 100 % ethanol solution showed a decrease in concentration with increasing pressure and temperature. Processing at 650 MPa/70 °C/15 min and 600 MPa/25 °C/40 min showed 50 % retention of retinol acetate. High-pressure sterilization (600 MPa/90 °C/2–16 min) of retinol acetate resulted in its complete degradation. During retinol degradation, retinol may be converted to its kitol form through the Diels-Alder reaction mechanism, which is highly favored under pressure (Butz and Tauscher 2000). Oxygen does not influence retinol and retinol acetate decay (Tauscher 1999; Indrawati et al. 2002). The mechanism of pressure degradation of vitamin A is still not completely understood.

In food products, vitamin A has been shown to be considerably more stable under pressure compared to in model systems. Vitamin A levels in orange juice was retained after high-pressure treatment at room temperature (Bignon 1996). Stability of retinol in light cream and milk was also not affected between 0.1 and 600 MPa at 75 °C up to 40 min. Stability of vitamin A in food matrices suggests that other reaction pathways for degradation of vitamin A may occur than in monomolecular solutions of vitamin A that may not be favored under pressure (Butz and Tauscher 2000). Longer time and higher temperatures combined with pressure treatment increased vitamin A content of orange juice. For example, vitamin A level increased by 45–52 % after pressure treatment of 100–350 MPa/30–60 °C/2.5–15 min (de Ancos et al. 2002). Another study by Sanchez-Moreno et al. (2005) showed that vitamin A level increased by 39 % for treatment of 400 MPa/40 °C/1 min. This increase in vitamin A levels could be explained by an increase in extractability after HPP. Storage of high-pressure-processed (50–100 MPa/30 °C/5 min) orange juice at 4 °C showed 24–42 % loss in vitamin A after 30 days. These levels, however,

were retained in orange juice processed at higher pressures between 200–350 MPa and 30–60 °C after 30 days. The differences observed could be due to partial/reversible inactivation of peroxidase and pectin methyl esterase at lower pressures resulting in vitamin A degradation (de Ancos et al. 2002).

Limited information is available on vitamin E stability in food products after high-pressure processing. Bignon (1996) showed that vitamin E was retained after pressure treatment at room temperature in orange juice. Another study showed that egg white and egg yolk processed between 392 and 980 MPa for 30 min at 25 °C showed no loss in α-tocopherol content (Hayashi et al. 1989). Human milk that was high-pressure treated (400–600 MPa/22–27 °C/5 min) also retained delta-, gamma-, and alpha-tocopherols post-processing (Molto-Puigmarti et al. 2011).

In literature, information on vitamin K stability is limited. Its stability has been tested only in buffer solutions. Tauscher (1999) showed that pressure processing at 650 MPa/70 °C/3 h caused vitamin K1 degradation forming *meta*- and *para*-isomeric Diels-Alder reaction products. This degradation reaction pathway is similar to what was observed in vitamin A model solutions. It is clear that Diels-Alder reactions are highly favored under pressure due to associated volume contractions (Tauscher 1995).

In summary, fat-soluble vitamins (A, E, and K) are less stable in model systems compared to food products. Vitamins A and E in food products were relatively stable to high-pressure processing at room temperature. Longer times and high temperatures combined with high-pressure treatment even increased the level of vitamin A due to an increase in extraction. Information available on stability of fat-soluble vitamins during HPP and subsequent storage is limited to drawing any conclusions on the general behavior of these vitamins in food systems.

22.2.2 Carotenoids

Carotenoids are yellow-orange pigments commonly found in fruits and vegetables. These compounds have been found to be somewhat pressure stable in food matrices. Several studies have also tested the stability of carotenoids in model systems. During HPP treatment, isomerization of all-*trans*-lycopene to 13-cis-isomers has been observed in hexane and tributyrin solutions after HPP (Qiu et al. 2006; Varma et al. 2010). Lycopene solution in hexane showed a significant decrease in *trans*-lycopene content. There was 21 % loss in *trans*-lycopene during treatment at 500 MPa/20 °C/12 min and 56 % loss for treatment of 600 MPa/20 °C/12 min. The same treatments also caused an increase in 13-cis-isomers of lycopene. Lower pressures (100–400 MPa) showed no significant change in lycopene contents and isomerization between treated and untreated samples (Qiu et al. 2006). In tributyrin solutions, *trans*-lycopene reduced by ~70 %, but total *cis*-isomers increased by only ~40 % after pressure treatment (320–620 MPa/room temperature/3 min). Besides isomerization, some *trans-lycopene* was also degraded. Such isomerization was also observed in tomato pulp. This pressure-induced conversion of *trans*-lycopene to its

cis form by HPP has been claimed to improve the bioavailability of lycopene; *cis*-lycopene is more bioavailable in the human body (Varma et al. 2010).

Among fruit- and vegetable-based products, pressure effects on carotenoid stability were widely different from model systems. HPP has been shown to have minor or no significant effect on carotenoid stability within food matrices. HPP treatment has also been shown to increase extraction of carotenoids from their plant matrix. For example, the lycopene content of tomato and tomato puree, high-pressure processed (300–700 MPa/20–90 °C/30–120 s), was almost unchanged after processing (Krebbers et al. 2003; Butz et al. 2003). In 2008, Hsu, Tan, and Chi showed that there was a 56–62 % increase in lycopene and total carotenoid levels of tomato juice processed at 300 MPa at 4 and 25 °C for 10 min. This increase in lycopene concentration could explain the increased redness in HPP-processed tomato juice compared to untreated juice. In watermelon juice, lycopene level was unchanged after moderate-pressure treatment (300–600 MPa/room temperature/5 min) and increased only after higher-pressure treatment between 600 and 900 MPa at room temperature for 20–50 min (Zhang et al. 2011).

The effect of HPP on carotenoid yield is influenced by fruit cultivar. Pressure treatment of 50–400 MPa at 25 °C for 15 min showed 9 % increase in carotenoids in Rojo Brillante cultivar of persimmon puree and 27 % increase in Sharon cultivar (de Ancos et al. 2000). Applied pressure is another factor influencing extraction yield of carotenoids after HPP. Pressure hold time and temperature have a much smaller influence on carotenoid yield. Orange juice carotenoids have been found to be relatively stable to combination treatments of pressure/temperature/time. Pressure treatment below 350 MPa had no significant effect on carotenoid concentration (lutein, zeaxanthin, β-cryptoxanthin, α-cryptoxanthin, α-carotene, and β-carotene) in orange juice. However, for pressure treatment of 350 MPa/30 °C/5 min, their concentration significantly increased. This increase was attributed to better extraction of carotenoid compounds from the fruit matrix. Longer treatment time (up to 15 min) did not change carotenoid extractability for pressure treatment between 50 and 300 MPa (de Ancos et al. 2002). Carotenoid level changes in carrots (α-carotene and β-carotene), green beans (lutein), and broccoli (lutein and β-carotene) after pressure treatments in the range of 400–600 MPa for 2 min at room temperature were not significant compared to unpressurized carrots (McInerney et al. 2007). High-pressure pasteurization of carrot pieces at 500–600 MPa at elevated temperatures of 25–45 °C for 16–20 min showed no degradation of β-carotene. No or limited isomerization of β-carotene was observed after high-pressure pasteurization (500 MPa/25 °C/16 min and 600 MPa/45 °C/20 min) and high-pressure sterilization (600 MPa/117 °C/9.6 min) (Knockaert et al. 2011). In carrots, β-carotene is present in a protective matrix that could explain its stability. Another study testing carotenoid stability in three cultivars of melons (*Cucumis melo* L.) found an increase in β-carotene concentration at much higher-pressure treatment of 600 MPa/10 min/25 °C (Wolbang et al. 2008).

During shelf-life studies of pressure-treated fruit juices, carotenoids were found to be stable at low temperatures (4–10 °C). For example, no change was observed in carotenoid levels of Valencia and Navel orange juices pressure treated (600 MPa/20

°C/60 s) and stored at 4 and 10 °C for 12 weeks (Bull et al. 2004). Significant differences were observed between pressurized tomato puree (100–400 MPa/20 °C/12 min) stored at 4 °C and 24 °C for 16 days each; the lower the storage temperature, the lesser was the loss in lycopene (Qiu et al. 2006). Pressure-processed orange juice (400 MPa/36 °C/1 min) stored at 4 °C for 40 days showed a marginal decrease of 11 % in total carotenoids (Plaza et al. 2011). In a food preparation, such as Mediterranean vegetable soup (*gazpacho*), which was pressurized (150–350 MPa/60 °C/15 min), total carotenoid loss of up to 46 % was observed over a storage period of 40 days at 4 °C (Plaza et al. 2005). This degradation of carotenoids could be due to the presence of prooxidants in the soup (additives).

Among fish products, high-pressure-treated smoked salmon (600–700 MPa/20 °C/1–3 min) exhibited a breakdown of astaxanthin pigment. During HPP, astaxanthin oxidized and its degradation is coupled with reduction of metmyoglobin. The exact mechanism of this redox-based degradation is still unknown (Tintchev et al. 2010).

22.2.3 Flavonoids

Flavonoids have received considerable attention in the scientific community due to their high nutraceutical value and health-promoting effects. The flavonoid family consists of a variety of bioactive compounds. Here, we will discuss the effects of HPP on stability of anthocyanins, flavonols, isoflavones, and flavanones.

22.2.3.1 Anthocyanins

Extensive research has been conducted on anthocyanin stability and the understanding of their degradation mechanisms during combined pressure/temperature treatments. Long holding times (>1 h) and high temperatures combined with pressure are critical parameters influencing anthocyanin degradation. The effect of increasing pressure was smaller than the effect of increasing temperature on anthocyanin degradation. In a model acetate buffer containing excess pyruvate, Corrales et al. (2008) showed no significant loss of cyanidin-3-O-glucoside (Cy3gl) for pressure treatments in the range of 200–600 MPa at 25 °C. Increasing temperature to 70 °C at 200 MPa also did not show significant loss in Cy3gl. At 600 MPa and 70 °C, however, Cy3gl loss was significant (up to 25 % for 30 min and 53 % for 6-h treatment time) and was a function of treatment time. Combined pressure-/temperature-assisted degradation of Cy3gl was reported as a result of condensation reactions of anthocyanins with pyruvate forming vitisin A-type derivative (cycloadduct), as shown in Fig. 22.3. Such cycloaddition products formed due to condensation reactions will result in a hypsochromic shift of UV-visible spectra causing discoloration of a food product (Corrales et al. 2008). Similar condensation reactions are possible when anthocyanins covalently associate with other flavonols present in fruit juices that can lead to formation of a new pyran ring (Tiwari et al. 2009).

Among food products tested for anthocyanin stability, strawberry juice pressurized between 200 and 800 MPa for 15 min at ambient temperatures showed unchanged levels of anthocyanins compared to fresh juice (Zabetakis et al. 2000). Raspberries (200–800 MPa/22 °C/15 min) also showed no loss in levels of cyanidin-3-glucoside and cyanidin-3-sophoroside (Suthanthangjai et al. 2005). Wine processed at 600 MPa/70 °C/1 h showed a decrease in anthocyanin monomer concentrations. Under commercial HPP pasteurization conditions for wine (400–700 MPa/25–50 °C/10–30 min), no chemical or organoleptic changes were observed in the samples (Corrales et al. 2008). Patras et al. (2009b) found no significant changes in the anthocyanin levels of raspberry and blackberry purees between high-pressure-treated (400–600 MPa/10–30 °C/15 min) and untreated samples. Pressure treatment of pomegranate juice (400–600 MPa for 5–10 min), however, showed a decrease in anthocyanin concentrations at room temperature and an increase in concentration at temperatures above 45 °C. These observations suggest that there could be loss in enzyme activity which could catalyze anthocyanin degradation and improve extractability, resulting in increased anthocyanin concentrations (Ferrari et al. 2010).

During high-pressure sterilization (PATS), at elevated pressures and temperatures, owing to faster heating times (compression heating) and thus shorter treatment times, more phytochemicals can be retained in foods. For example, pressure sterilization at 600 MPa/110 °C/3 min led to 80–90 % retention of total anthocyanins in strawberry paste (Verbeyst et al. 2010).

Anthocyanins in pressure-treated fruits and vegetables are not stable during subsequent storage. Various mechanisms have been implicated in anthocyanin instability during storage of pressurized food products. The first hypothesized mechanism is due to incomplete inactivation of enzymes in fruits that catalyze anthocyanin degradation reactions. Most common enzymes found to be involved are polyphenol oxidase (PPO), peroxidase (POD), and β-glucosidase. Suthanthangjai et al. (2005) found that cyanidin-3-glucoside and cyanidin-3-sophoroside, the two major anthocyanin pigments in raspberry, were most stable at refrigeration temperature. Shelf-life study at 4 °C for 9 days showed the highest retention of these two anthocyanin pigments in raspberries processed at 200 and 800 MPa (22 °C/15 min). Samples from pressure treatment at 400 and 600 MPa showed greater loss of both pigments, probably due to incomplete inactivation of PPO, POD, and β-glucosidase. Partial inactivation of these enzymes will result in catalysis of anthocyanin degradation reactions. Higher losses of anthocyanins (average loss ~ 65 %) were observed during storage at 20 °C and 30 °C. Garcia-Palazon et al. (2004) found that PPO in red raspberry and strawberry was completely inactivated after pressure treatment at 800 MPa/22 °C/15 min and 800 MPa/22 °C/10 min, respectively. At 600 MPa/22 °C/15 min, PPO in red raspberry was in fact activated, which explains Suthanthangjai et al.'s (2005) observation of increased anthocyanin degradation at similar treatment conditions. POD in strawberries was partially inactivated at 600 and 800 MPa/22 °C/15 min. β-Glucosidase in red raspberries and strawberries was marginally inactivated (Garcia-Palazon et al. 2004).

The second mechanism implicated in anthocyanin instability during storage is based on specificity of β-glucosidase responsible for the selective degradation of

anthocyanins. Zabetakis et al. (2000) showed that for the same level of residual β-glucosidase activity in HPP-treated strawberries, pelargonidin-3-glucoside was degraded to a greater extent than pelargonidin-3-rutinoside. The highest levels of anthocyanins were retained in strawberries treated at 800 MPa/22 °C/15 min and stored at 4 °C for 9 days.

The third mechanism postulated concerns on influence of ascorbic acid on the stability of anthocyanins during storage. Kouniaki et al. (2004) found a strong correlation between ascorbic acid and anthocyanin content in blackcurrants. Ascorbic acid, apart from being an antioxidant, accelerates the degradation of anthocyanins. In this study, higher loss of ascorbic acid was accompanied by smaller loss of anthocyanins during storage at 5 °C and vice versa during storage at 20 °C and 30 °C. A strong influence of ascorbic acid on anthocyanin degradation was also observed in blood orange juice (Tiwari et al. 2011).

The fourth possible mechanism involved in the degradation of anthocyanins in fruit products could be due to condensation reactions between anthocyanins and flavanols or organic acids through covalent association resulting in the formation of pyran ring compounds (Tiwari et al. 2009). Such condensation reactions have also been observed in model buffer solutions of cyanidin-3-glucoside containing pyruvic acid (Corrales et al. 2008).

22.2.3.2 Other Flavonoids: Flavonone, Isoflavones, and Flavonols

Isoflavones, flavonols, and flavonones exhibit diverse effects upon processing. For example, isoflavones in pressurized soy milk (400–750 MPa/25 °C/10 min) showed no difference in total concentration compared to soy milk thermally processed to 95 °C (control). The isoflavone profile of soy milk, however, was altered during pressure treatment (400–750 MPa/75 °C/10 min) with glycitin-, daidzin-, and genistin-malonyl-β-glucosides being converted to their corresponding β-glucosides. This conversion was attributed to adiabatic heating that promotes conversion of malonyl- to β-glucoside isoflavones. In the case of soy milk prepared from pressurized soybeans, total isoflavone content and isoflavone profile of soy milk were influenced by pressure (100–700 MPa), being highest in soy milk extracted from beans processed at 700 MPa and lowest in beans processed at 300 MPa. The decrease in total isoflavone concentration at 200 and 300 MPa was reflected in the increase in isoflavone-β-glucoside compounds. The isoflavone profile changes were attributed to pressure-induced changes in enzyme activity, disruption of bean cell walls, and modification in isoflavone-protein interactions (Jung et al. 2008).

Among flavonols, quercetin, quercetin-3-glucoside, and quercetin-3,4-diglucoside present in onions were reported to increase in concentration after pressurization between 100 and 400 MPa/5–50 °C/5 min compared to untreated onions. The percentage increase in individual flavonol concentrations and total flavonol content was lower at 50 °C than at 5 °C for 100 MPa treatment. Onions treated at 100 MPa/5 °C/5 min showed a 26 % increase in total quercetin level and 18 % increase at 100 MPa/50 °C/5 min compared to untreated onions. Increase in flavonol

concentration after pressurization was attributed to better extractability from the plant matrix, and use of higher temperatures (>30 °C) in combination with pressure caused detrimental effects on the flavonols (Roldan-Marin et al. 2009).

Flavonones such as naringenin and hesperitin found in orange juice increased in concentration by 20 % and 40 %, respectively, after pressure treatment (400 MPa/40 °C/1 min) (Sanchez-Moreno et al. 2005). With storage of HPP-treated orange juice at 4 °C, naringenin and hesperitin levels decreased by almost 50 % after 20 days. This degradation of flavonones during storage could be attributed to incomplete inactivation of polyphenol oxidase and peroxidase (Plaza et al. 2011).

22.2.4 Monounsaturated and Polyunsaturated Fatty Acids

Higher intake of mono- and polyunsaturated fatty acids and lower consumption of saturated fatty acids in the diet are associated with reduced cardiovascular disease risk. Changes in the fatty acid profile of food can also influence flavor characteristics. Recent studies have focused on HPP-induced nutritional and sensorial changes in food products due to change in fatty acid composition. The nutritional indicators measured in these studies were levels of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and ratios of PUFA/SFA and omega-6/omega-3 (n-6/n-3) fatty acids.

Tofu subjected to high-pressure treatment at 100–350 MPa/22 °C/15 min showed no significant difference in fatty acid profile and in ratios of PUFA/SFA and n-6/n-3 between untreated control and pressurized tofu (Prestamo and Fontecha 2007). These results were consistent with earlier work which showed that polyunsaturated fatty acids are relatively pressure resistant for treatment time of less than 1 h (Butz and Tauscher 2000). Combined pressure/temperature treatment, however, resulted in autoxidation of unsaturated C_{18} fatty acids. Autoxidation of linoleic acid ($C_{18:2}$ n-6 fatty acid) was promoted by pressure processing between 350 and 600 MPa at 40 °C for 1–20 h. The primary oxidation products formed significantly increased only after 2 h of treatment (Butz and Tauscher 2000). Autoxidation of α-linolenic acid ($C_{18:3}$ n-3 fatty acid) under high pressure is not clearly understood. At 350 MPa (up to 15-h pressure hold), more primary oxidation products (conjugated dienes) were formed than at atmospheric pressure. However, when the pressure was raised to 600 MPa, pressure had a protective effect on linolenic acid (Tauscher 1995). It should be noted that, in these studies, the test time was unusually long and is not relevant to a practical scenario. Typical pressure treatment time ranges between 5 min and 60 min for most food products. Limited or no primary oxidation of fatty acids is observed at treatment time of less than 1 h. In salmon, there was an increase in peroxide value and TBARS value due to autoxidation of the lipids. The oxidation of salmon lipids was, however, suspected to be the result of metal ions present in food rather than high pressure, since purified lipids were stable under high pressure (Lakshmanan et al. 2003).

22.2.5 Sulfur-Containing Bioactive Compounds

Various sulfur-containing compounds such as glucosinolates and their hydrolysis products, mainly found in the Brassicaceae family of vegetables (e.g., cabbage, broccoli, Brussels sprout, etc.), are bioactive as anticarcinogenic compounds. High pressure has been shown to induce glucosinolate hydrolysis to isothiocyanate and other oligomers. Van Eylen et al. (2009) found 20–63 % loss in glucosinolates after 15 and 35 min treatments at 100–500 MPa and 40 °C with a larger proportion of glucosinolate hydrolysis at higher pressures. At 20 °C, very small levels of glucosinolate hydrolysis products were observed (100–500 MPa for 15 min). During combined high-pressure and high-temperature processing, permeabilization of plant tissue occurs, decompartmentalizing myrosinase enzyme, which hydrolyzes glucosinolates. At 40 °C, myrosinase is inactivated more slowly at 300 MPa compared to a faster rate at 500 MPa. This may explain why a lower level of hydrolysis is observed at 500 MPa than at 300 MPa.

Isothiocyanates (glucosinolate derivatives), particularly sulforaphane, have been shown to induce better anticarcinogenic effects in humans compared to intact glucosinolates. Sulforaphane is formed by hydrolysis of glucoraphanin, a major aliphatic glucosinolate found in broccoli. After pressure treatments at 200–500 MPa/20–40 °C/15–35 min, reasonable amounts of sulforaphane were formed in the broccoli heads. The greatest amounts of sulforaphane were formed after treatment at 300 MPa/40 °C/35 min, and the least amounts formed after treatment at 500 MPa/40 °C/35 min. Increase in sulforaphane levels corresponds to decrease in glucoraphanin level (Van Eylen et al. 2009). Butz and Tauscher (2000) showed that sulforaphane in water is not destroyed after high-pressure pasteurization at 600 MPa/25 °C/40 min; however, pressure combined with higher temperatures (75 °C) showed reasonable loss of sulforaphane in water. In minced broccoli, pressure processing at 0.1–600 MPa/25 and 75 °C/20 and 40 min resulted in increased levels of sulforaphane due to improved extractability from broccoli (Butz and Tauscher 2000). However, in broccoli juice, Houska et al. (2006) found loss of sulforaphane during high-pressure pasteurization (500 MPa/25 °C/10 min) that was comparable to levels found after freezing of broccoli juice.

Sulforaphane nitrile is another derivative formed in large quantities (85–90 %) along with sulforaphane (10–15 %) during hydrolysis of glucoraphanin. When processing conditions are more intensive, more glucoraphanin is converted to sulforaphane and less sulforaphane nitrile is formed, indicating that the enzyme required for sulforaphane nitrile formation is less baro resistant than myrosinase. This is beneficial because sulforaphane is a more potent anticarcinogen than sulforaphane nitrile (Van Eylen et al. 2009).

Another isothiocyanate in broccoli, iberin, derived from hydrolysis of glucoiberin, showed that increasing pressure up to 300 MPa (35 min) increased iberin formation. Unlike sulforaphane, iberin was not detected after autolysis of samples treated at 500 MPa/20 °C/35 min. This could be due to iberin instability or reduced activity of myrosinase (Van Eylen et al. 2009).

In addition to aliphatic glucosinolates, broccoli also contains reasonable levels of less studied indole glucosinolates. Indol-3-ylmethylglucosinolate is the main indole glucosinolate in broccoli. In pressure-treated (100–500 MPa) broccoli at 20 °C, very small levels of indole-glucosinolate hydrolysis products were observed. At 40 °C and elevated pressures (>100 MPa), tri-indole hydrolysis compound levels increased. Some hydrolysis products of indole glucosinolates have also shown potential anticarcinogen effects (Van Eylen et al. 2009). In summary, due to the diverse properties of glucosinolate and its derivatives, it is first important to determine the type of products formed upon hydrolysis of glucosinolates and then study their processing stability.

22.3 Influence of HPP on Bioactivity and Sensorial Properties of Foods

As a direct consequence of the stability/instability of bioactive compounds during HPP, different functional properties of foods may be altered. Commonly affected properties are antioxidant activity, bioavailability, and sensorial quality (color, texture, flavor) of food products. The following sections discuss in detail the effect of HPP on these properties.

22.3.1 Influence on Antioxidant Activity

Many bioactive compounds, e.g., vitamins, flavonoids, and carotenoids, are strong antioxidants. It is also clear that HPP affects stability and extraction yields of these bioactive compounds. As a consequence, antioxidant activity of these bioactive compounds will also be affected.

The effect of high pressure on antioxidant activity of bioactive compounds in different food matrices is diverse. Orange juice processed at 5–350 MPa at 30 °C for 5 min showed lower radical scavenging activity (DPPH assay)[1] compared to untreated juice. The lowest antiradical activity was observed at 350 MPa/30 °C/5 min. This treatment, however, showed the highest carotenoids extracted and highest vitamin A value. These results suggest that carotenoids are not the primary contributors to antiradical activity. Vitamin C and phenols present in orange juice may contribute to its antioxidant activity (de Ancos et al. 2002). On comparison of antiradical activity (DPPH radical scavenging activity) of two cultivars of persimmon puree (Rojo Brillante and Sharon), Rojo Brillante was 8.5 times more efficient in scavenging radicals compared to Sharon. However, after pressurization (150–300 MPa/25 °C/15 min), their antiradical activities

[1] DPPH assay is an antiradical assay that is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$). In the presence of a hydrogen donor (antioxidant), $DPPH^{\cdot}$ is converted to DPPH and is quantitatively monitored using UV-visible spectrophotometer.

were comparable (de Ancos et al. 2000). Antiradical activity of blackberry puree was not significantly different between pressure-treated (400 and 500 MPa/20 °C/15 min) and non-treated samples. At 600 MPa, however, antiradical activity was significantly higher (67 %) compared to the unprocessed sample. The increase in antiradical power is attributed to improved extractability of anthocyanins after HPP. In strawberry puree, antiradical power was considerably lower after pressurization at 400 MPa compared to the untreated control (Patras et al. 2009b).

Hydrophilic antioxidant activity of vegetables, such as broccoli, green beans, and carrots, was differentially affected by HPP. FRAP[2] values of broccoli were unchanged after pressure treatment. In the case of carrots and green beans, FRAP values were slightly reduced at 400 MPa for carrots and increased at 400 and 600 MPa for green beans. The increase in antioxidant activity in green beans could be correlated to the increase in lutein concentrations (McInerney et al. 2007). Roldan-Marin et al. (2009) observed no difference in the DPPH radical scavenging activity of pressure-treated (100–400 MPa/5–50 °C/5 min) and unpressurized onions. The authors also observed a significant positive correlation between the phenolic content and antiradical power. These results indicate that phenols (flavonols) present in onions greatly influence their antioxidant activity.

Antioxidant capacity ($DPPH^{\cdot}$ radical scavenging activity) of tomato and carrot puree was significantly higher after pressurization (500–600 MPa/21 °C/15 min) compared to untreated samples. Phenolic content of carrot puree also showed significantly higher values compared to unprocessed samples. Phenolic content of tomato puree was not significantly affected (Patras et al. 2009a). In contrast, TEAC values[3] ($ABTS^{+\cdot}$ cation radical scavenging activity) and deoxyribose test assay[4] ($^{\cdot}OH$ radical scavenging activity) showed no significant changes in tomato pulp post-pressurization (600 and 800 MPa/25 °C/5 min) (Butz et al. 2002).

The presence of additives (such as citric acid, NaCl) influenced the radical scavenging activity of tomato puree at varying pressure levels as reported by Sanchez-Moreno et al. (2004b). In the absence of additives, pressure treatment (50–400 MPa/25 °C/15 min) increased the $DPPH^{\cdot}$ radical scavenging activity of the aqueous fraction of tomato puree. With the addition of citric acid (1.2–2 %), antioxidant activity (aqueous portion) reduced between 300 and 400 MPa. Similarly, addition of NaCl (0–0.8 %) also reduced antioxidant activity (aqueous portion) immediately after pressure treatment. However, antioxidant activity of organic extract of tomato puree was highest in the presence of NaCl during treatment at 400 MPa (Sanchez-Moreno et al. 2004b).

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[2] FRAP (Ferric Reducing/Antioxidant Power) assay is based on extent of Fe^{+3}-tripyridyltriazine complex reduction.

[3] TEAC (Trolox equivalent antioxidant capacity) assay is an antiradical assay based on extent of 2,2′-Azinobis (3-ethylbenzothiazoline-6 sulfonic acid)/$ABTS^{+\cdot}$ radical reduction. The assay uses change in radical chromophore absorption to measure antiradical activity.

[4] Deoxyribose assay is an antiradical assay based on extent of $OH^{\cdot}$ radical reduction in the presence of an antioxidant. The assay uses change in radical chromophore absorption to measure antiradical activity.

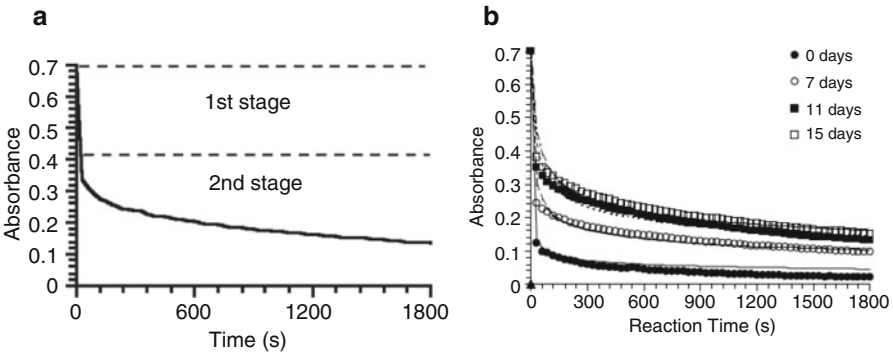

Fig. 22.4 $ABTS^{+\cdot}$ discolorization curve after addition of high pressure processed orange juice as a source of antioxidants as a function of storage period of pressurized orange juice at 30 °C. Instantaneous decrease in the first stage shown in *plot* (**a**) was attributed to antioxidant activity contributed by ascorbic acid. Change in absorbance in the second stage was attributed to antioxidant activity contributed by components other than ascorbic acid (flavonoids and other phenolic compounds). *Plot* (**b**) shows that antioxidant activity during storage progressively decreased with storage time; lower absorbance indicates higher antioxidant activity. (Reprinted from Polydera et al. 2004, 2005b with permission from publisher)

Antioxidant capacity of other food products, such as gazpacho vegetable soup and germinated cowpeas, has also been tested immediately after HPP. Antiradical effectiveness (DPPH assay) of gazpacho soup was unchanged before and after HPP (150 and 350 MPa/60 °C/15 min) (Plaza et al. 2005). In raw and germinated cowpeas, there was a slight decrease in TEAC values after HPP (300–500 MPa/room temperature/15 min) (Doblado et al. 2007).

During subsequent storage after HPP, reconstituted orange juice showed a decrease in antioxidant activity ($ABTS^{+\cdot}$ decolorization) on storage at 0, 5, 10, 15, and 30 °C for up to 111 days. However, the rate of loss in antioxidant activity in HPP-treated orange juice was lower than in untreated juice. Much of this loss is attributed to reduction in ascorbic acid content shown as an instantaneous decrease in the ABTS decolorization curve (Fig. 22.4). Other simultaneous or consecutive effects may also be responsible for loss in antioxidant activity. These effects could include (1) loss of other naturally occurring antioxidants (flavonoids) besides ascorbic acid, (2) formation of pro-oxidant compounds such as reactive radicals formed during Maillard reaction, and/or (3) change of oxidation state of polyphenols and consequently that of Maillard reaction products that can reduce antioxidant capacity (Polydera et al. 2004, 2005b).

22.3.2 Influence on Bioavailability

High-pressure processing of fruits and vegetables has been shown to cause some changes in bioavailability of bioactive compounds. For example, the formation of more bioavailable form of folate, i.e. folate monoglutamate, is increased in broccoli, green

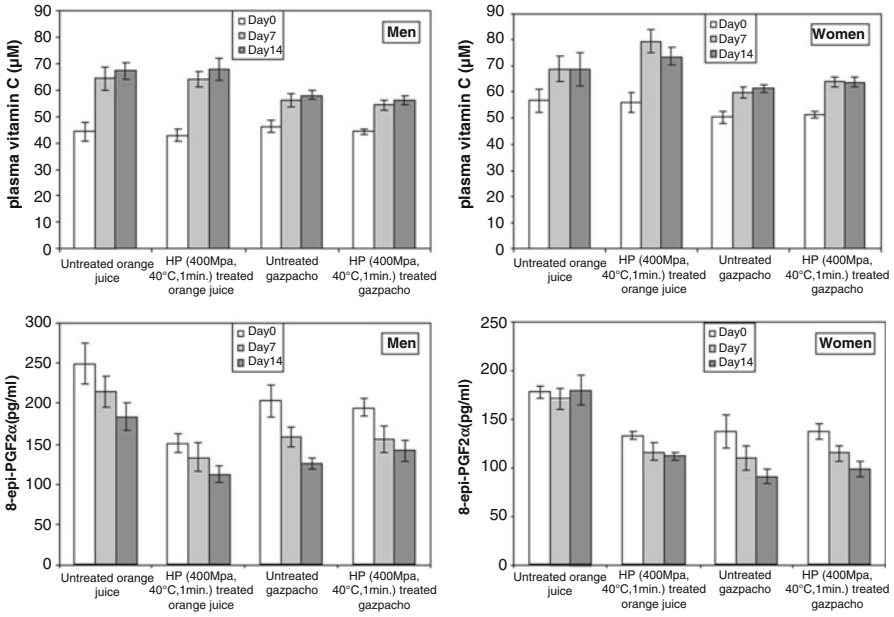

Fig. 22.5 Effects of consumption of high pressure processed (400 MPa/40 °C/1 min) orange juice and gazpacho soup on plasma levels of vitamin C and oxidative stress marker (8-epi-PGF_{2a}) in humans. *Plot* shows an increase in plasma vitamin C levels after consumption of pressure-processed samples compared to untreated samples suggesting improved bioavailability. There is an inverse correlation between vitamin C levels and 8-*epi*PGF_{2a}. (Reprinted from Oey et al. 2008a with permission from publisher)

beans, cauliflower, and leeks after pressure treatment (200 MPa/room temperature/5 min) (Melse-Boonstra et al. 2002; Verlinde et al. 2008). Hence, HPP can result in improving bioaccessibility of natural folates found in fruits and vegetables.

The influence of HPP on bioavailability of nutraceuticals in foods has been tested using in vitro and in vivo methods. In a study testing vitamin C levels in plasma after consumption of pressure-processed orange juice (400 MPa/40 °C/1 min), researchers observed an increase in plasma vitamin C levels. There was an inverse correlation between vitamin C levels and F_2 isoprostanes (8-*epi*$PGF_{2\alpha}$, an oxidative stress biomarker) and vitamin C and prostaglandin E_2 (PGE_2, an inflammatory biomarker), as shown in Fig. 22.5. These results suggest that pressure-processed orange juice contains vitamin C that is more bioavailable, which may help reduce the risk of chronic diseases (Sanchez-Moreno et al. 2003). Similar results were reported in pressure-processed gazpacho soup. Consumption of gazpacho soup showed higher vitamin C levels in plasma and lower levels of oxidative stress and inflammatory biomarkers than in untreated soup, as shown in Fig. 22.5 (Sanchez-Moreno et al. 2004a).

Disruption of plant cellular tissue on exposure to high pressure has been hypothesized to influence carotenoid bioavailability (McInerney et al. 2007; Knockaert et al. 2011). Simulated gastric digestion of pressure-processed coarse carrot homogenate (600 MPa/25°C/10 min) showed lower extractability of carotene compared to

unpressurized samples (Butz et al. 2002). In contrast, simulated gastric and pancreatic digestion of carrots, broccoli, and green beans pressure treated for lesser time at similar conditions (400–600 MPa/room temperature/2 min) demonstrated an increase in carotenoid bioavailability after pressure treatment (McInerney et al. 2007; Knockaert et al. 2011). Approximately 26 %, <6 %, and 30 % increase in carotenoids was released by in vitro digestion of carrots, broccoli, and green beans, respectively, in those that were pressure processed (400–600 MPa/room temperature/2 min) (McInerney et al. 2007).

22.3.3 Influence on Antimutagenic Activity

Dietary fruits and vegetables contain antimutagens or inhibitors of carcinogenesis such as flavonoids, coumarins, and other unidentified bioactive compounds. The stability of these antimutagens in foods to high-pressure processing will determine the antimutagenic potency of the food product.

The Ames test or SOS chromo test is generally used to measure antimutagenic activity in food products. In these methods, antimutagenic effectiveness is measured against carcinogen (heterocyclic aromatic amines or aflatoxin)-induced mutagenicity in *Salmonella typhimurium* TA 98 or *Escherichia coli* K12 PQ 37 (Butz et al. 1997).

Among food products, antimutagenic activity has been found to be stronger in vegetables than in fruits. Butz et al. (2007) tested different fruits and vegetables for their antimutagenic potency after HPP. Strawberries and grapefruit showed moderate antimutagenic activity and no change in activity after pressure treatment (400–800 MPa/25–50 °C/10 min). Vegetables such as beets, carrots, cauliflower, kohlrabi, leeks, and spinach also did not show any change in their antimutagenic activity. High-pressure, high-temperature treatment of 600 MPa/50 °C/10 min and 800 MPa/35 °C/10 min in tomatoes and 800 MPa/35 °C/10 min in beets caused significant decrease in their antimutagenic activity. Using the SOS chromo test, Houska et al. (2006) showed that high-pressure-treated broccoli juice showed strong positive antimutagenic activity. The exact reasons for the observed antimutagenic activity in fruits and vegetables are not clear. Some research shows good correlation between antimutagenic activity of fruit juices with their peroxidase activity (Houska et al. 2006). This result suggests that active components in fruit juices may act as bioantimutagen while interacting with enzymes and prevent mutation in cellular processes. However, Butz et al. (1997) have also shown that higher pressure (>600 MPa) can almost completely inactivate peroxidases and not significantly affect the antimutagenic potency of fruits and vegetables. This suggests that other modes of action, e.g., where bioactive compounds act as desmutagens that directly interact with the active mutagen and form inactive complexes or make it unsuitable for enzymatic action, may be responsible for antimutagenicity of fruit- and vegetable-based products (Kada and Shimoi 1987).

22.3.4 *Influence on Sensorial Properties: Color, Texture, and Flavor*

Bioactive compounds, such as carotenoids and anthocyanins, are pigments responsible for color in fruits and vegetables. It is clear that HPP (at moderate temperatures < 60 °C) has a limited effect on their stability. Pressure treatment at higher temperatures, however, can cause bioactive compound degradation. As a consequence, the color and overall appearance of these pressure-processed fruit- and vegetable-based food products will also be affected.

In fruit-based food products, minimal or no visual color differences (based on CIE L^*, a^*, b^*) have been observed immediately after HPP treatment. For example, mango pulp pressurized (100–400 MPa/room temperature/15–30 min) showed no significant change in color parameters, (a/b), C (chroma), and h (hue). The ΔE (total color difference) values decreased with an increase in pressure intensity. Changes in ΔE observed at lower pressures (100–200 MPa) were attributed to changes in b values (carotenoids) (Ahmed et al. 2005). Another study showed that strawberry puree had higher Hunter a^* values (redness) compared to thermally treated samples but lower than untreated samples. Treatment at 600 MPa/10–30 °C/15 min showed the highest retention of redness. In blackberry puree, redness values after pressure treatment were significantly lower than those of fresh samples. The red color of both fruits is attributed to the presence of anthocyanins. The levels of anthocyanins (pelargonidin-3-glucoside and cyanidin-3-glucoside) directly correlated to redness values (a^*). However, loss of anthocyanins is not the only cause for loss in color of fruit-based products. Increased enzyme activity, e.g., peroxidase, polyphenol oxidase, and glucosidase, could also cause loss in color due to formation of brown pigments on phenolic oxidation. The ΔE (total color difference) values for strawberry and blackberry puree were lower after high-pressure treatment than after thermal treatment, except at 400 MPa for blackberry puree. HPP had a smaller effect on color change compared to thermal processing (Patras et al. 2009b).

In vegetable-based products, such as carrot and tomato puree, there was significant difference in the color parameters after HPP. Tomato puree showed significant increase in Hunter a^* (lycopene) and chroma values. L^* values also slightly changed; however, there was no major contributor to change in chroma values. Color intensity of high-pressure-processed samples (400 and 500 MPa/room temperature/15 min) was higher than that of untreated samples. These changes were attributed to the compacting and homogenizing effect of HPP. For carrot purees, there was a significant increase in color intensity and L^* values after HPP compared to fresh samples. Treatment at 500 MPa, however, had a different hue angle compared to other HPP and fresh samples. This effect could not be explained and warrants further investigation (Patras et al. 2009a).

In smoked salmon, color changes were observed for pressure treatment >500 MPa (20 °C and 60 s). A slight whitening effect, increase in L^* value and decrease in a^*

and b^* values, was observed. These color changes were attributed to changes in astaxanthin pigment, a carotenoid found in salmon. Salmon color is more stable compared to the color of turkey, pork, and chicken processed under the same pressure/temperature/time conditions. Higher color stability in salmon compared to turkey, pork, and chicken could be explained by differences in molecular structures of color pigments—astaxanthin, the color pigment in salmon and myoglobin, the color pigment in meat (Tintchev et al. 2010).

During storage of pressurized food products, discoloration mainly occurs due to enzymatic browning. In pressurized (500 MPa/35 °C/5 min) reconstituted orange juice, discoloration of juice was observed during storage at 0, 5, 10, and 15 °C. These color changes recorded in chroma values (C) did not correlate to processing conditions and storage temperature (Polydera et al. 2003). The same authors found that navel orange juice pressurized at 600 MPa/40 °C/4 min showed positive linear correlation between color changes and loss in ascorbic acid (Polydera et al. 2005a). Vitamin C behaves as an antioxidant. Loss of vitamin C can promote enzymatic browning reactions in orange juice.

Color changes in HPP-treated fruits and vegetables can be related to textural changes. For example, tomato puree processed at 400 MPa/25 °C/15 min showed a higher L^* value compared to untreated puree, indicating lightening of surface color (Sanchez-Moreno et al. 2006). This change in CIElab parameter could be due to gel-like formation of tomato puree at pressures < 400 MPa (Oey et al. 2008b; Patras et al. 2009a).

Flavor, a sensory perception of taste and smell, is indirectly related to the behavior of bioactive compounds in foods. In general, flavor compounds in fruits and vegetables consist of small molecular structure, volatile or nonvolatile compounds, such as aldehydes, esters, acids, lactones, ketones, alcohols, sulfur compounds, etc. Their structure is not directly affected by high pressure because they are covalently bonded molecules. These compounds are not nutraceuticals as such (bioactive compounds), and therefore their discussion is beyond the scope of this chapter. However, high pressure has been shown to indirectly influence the activity of some enzymes that catalyze reactions involving bioactive compounds resulting in off-flavor compounds formation. For example, linoleic and linolenic acids, found in tomatoes, are oxidized to n-hexanal. When present in high concentrations, n-hexanal is responsible for rancid taste in tomatoes. Reaction forming n-hexanal is catalyzed by the lipoxygenase and hydroperoxide lyase inherently present in tomatoes. In tomato juice, lipoxygenase and hydroperoxide lyase were not completely inactivated at 650 MPa/20 °C/12 min (Rodrigo et al. 2007). In diced tomato, lipoxygenase was reduced to 50 % after processing at 400 MPa/25 and 45 °C/1–5 min and was almost negligible after HPP at 800 MPa (Shook et al. 2001). In strawberry pulp, no changes in hexanal levels were observed up to 200 MPa. Above 200 MPa, hexanal levels increased with increasing pressure with more pronounced effects at 25 °C than at 40 °C. Pressure treatment above 60 °C caused no changes in hexanal levels due to lipoxygenase inactivation (Butz and Tauscher 2000). Other bioactive compounds, such as flavonoids, can also influence the flavor of foods; however, they have not been explored and need further investigation.

22.4 Conclusions

Based on current knowledge, HPP at moderate temperatures can retain bioactive compound stability in fruit- and vegetable-based products. HPP carried out at elevated temperatures (e.g., PATS applications), however, can cause decay of these bioactive compounds. Bioactive compound stability is highly influenced by chemical reactions that are favored under high pressure. For example, chemical reactions accompanied by a decrease in volume, such as *trans*- to *cis*-isomerization in carotenoids or anthocyanin condensation reactions forming pyran rings, are enhanced under pressure. At elevated temperatures, effects on pressure-enhanced chemical reactions have been shown to be different from effects during pressure treatment at moderate temperatures. Thus, the combined contribution of temperature and pressure on bioactive compound stability at elevated temperatures is still not clearly understood. Enzyme activity (activation/inactivation) is another major factor implicated in bioactive compound stability/instability. Peroxidase (POD), polyphenol oxidase (PPO), and β-glucosidases are the most commonly found enzymes in fruits and vegetables that are shown to play a role in catalyzing reactions that degrade bioactive compounds, especially flavonoids and vitamins. Furthermore, the presence of vitamin C or L-ascorbic acid in food products has also been shown to influence bioactive compound stability under pressure. Vitamin C behaves both as a prooxidant and antioxidant. For example, folate oxidation in the presence of ascorbic acid is reduced; however, ascorbic acid greatly enhanced oxidation of thiamin and thiamin monophosphate. HPP effects on bioactivity and sensorial (color, texture, flavor) properties of fruit- and vegetable-based products correlate to bioactive compound stability/instability. However, the effect of HPP on functional properties of bioactive compounds cannot be generalized since study on basic scientific insights is still limited in this subject and these properties are product composition dependent.

Although the extent of bioactive compound degradation was found to be considerably higher in model systems, more studies using model systems simulating conditions in a food product are required to elucidate degradation reaction mechanisms of bioactive compounds under high pressure. Further, studies interconnecting mechanisms and kinetics of degradation are required. Such information will be indispensable for the future to develop design guidelines for the food industry to produce food products with high nutraceutical and health value.

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Chapter 23
Structural Changes in Foods Caused by High-Pressure Processing

Tomas Bolumar, Dana Middendorf, Stefan Toepfl, and Volker Heinz

Abstract High-pressure processing (HPP) has been mainly applied in the food industry as a post-packaging pasteurization method in order to ensure food safety. In more recent years, HPP has also been extensively considered in relation to the structural changes that HPP treatments induce in food systems. These structural changes are based on the effect of high pressure on the cell structure and on the biopolymers present in food. These changes can lead to diverse food applications such as creation of novel textures, improvement of the water binding, or mediation of gelation processes. This research area has been intensified in the last 15 years and is currently evolving at a rapid pace. Different applications have already described in the literature for different foodstuffs such as processed fruits and vegetables, meat, and dairy products. The present chapter explains the main mechanisms underlying these modifications and summarizes the research carried out in this novel field. HPP provides an ideal tool for structure modifications by means of physical nonthermal processing and can play an important role in future product development and in the production of food ingredients with enhanced functionality.

Keywords High-pressure processing • HPP • Structure modification • Texture • Functionality • Novel food processing

23.1 High-Pressure Processing (HPP)

In the last two decades, the application of high pressure to food systems, particularly ultrahigh pressures (>100 MPa), has received much attention by the scientific community, and as a consequence, the vision of commercial installations has come to pass (Bajovic et al. 2012). The application of high-pressure processing (HPP) to food systems offers possibilities that cannot be achieved by conventional

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procedures and thus has high potential in the development of novel-optimized food applications.

HPP is an alternative to traditional preservation by thermal processing. HPP results in an effective reduction of microbial counts with minimal effect on sensorial quality and nutritional content. Given these advantages, HPP becomes very useful as a post-packaged preservation technology for ready-to-eat (RTE) whole or sliced meat products, seafood, fresh-cut fruits and juices, as well as deli salads, condiments, dips, dressings, soups, salsas, and sauces (Hendrickx and Knorr 2002). Moreover, since processing time and energy consumptions of HPP are considerably lower compared to conventional thermal methods, this makes it an interesting processing alternative of increasing importance in today's world, aiming at a sustainable food supply.

In addition, HPP has also been considered in relation to the structural changes that HPP treatments induce in food systems. Whereas thermal treatment has high influence in the breakage of covalent bonds and is transmitted by a gradient, pressure, in contrast, has minimum effect on the breakage of covalent bonds and is transmitted instantaneously, and thus no gradients are formed. Thereby, the application of pressure is very useful to preserve food freshness as it inactivates microorganisms with minimal effect on the destruction of molecules. Moreover, HPP also represents an ideal tool to tune non-covalent interactions like hydrogen bonds and destabilize the hydrophobic effect. The tertiary and quaternary spatial structure of macromolecules like proteins and starch can be modified with negligible effect on their secondary structure, and as a result, unique modifications can be obtained. In brief, HPP represents an alternative to thermal processing that can be employed as a method to ensure microbial safety and to modify the structure of foodstuffs with a concomitant preservation of nutritional content.

23.2 Structure Modification by HPP

The application of high pressure (100–1000 MPa) can be employed by the food industry to modify the structure of food systems. Structural changes induced in food systems by HPP rely on the effect of pressure on food constituents. Macromolecules or biopolymers that have a spatial distribution stabilized by molecular interactions such as hydrophobic interactions and electrostatic bonds are susceptible to being modified by HPP. In this sense, the effect of pressure on proteins and starch has been extensively investigated. The effects of high pressure on proteins such as unfolding, disassociation, denaturation, and aggregation have been reviewed by Boonyaratanakornkit et al. (2002) and Balny et al. (2002) and the gelling mechanisms of starch under pressure by Pei-Ling et al. (2010) and Kim et al. (2012). The molecular mechanisms regarding these modifications are described and discussed in detail in the next section. Novel and diverse applications of HPP as a tool for structure modification of different foodstuffs such as processed fruits and vegetables, meat, and dairy products are also described in this chapter (Oey et al. 2008; Sun and Holley 2010; Devi et al. 2013). The development of natural food ingredients with

enhanced functionality such as emulsifiers, stabilizers, texturizers, and water retention-intended ingredients by HPP-induced modifications in protein and starch matrixes is also the focus of numerous investigations where biopolymers are submitted to static (i.e., high-pressure processing, HPP) or dynamic (i.e., high-pressure homogenization, HPH) high-pressure conditions. These HPP-obtained natural food ingredients can contribute to clean labeling by substitution of artificial additives.

23.2.1 Pressure and Temperature

According to the principle of *Le Chatelier*, pressure affects molecular systems toward a reduced volume. Any change in a biochemical reaction involves a change in free energy. This change in Gibbs energy is a function of pressure (P) and temperature (T) and is governed by the change in volume (ΔV) and the change in entropy (ΔS). Since during pressure processing the temperature is constant, the pressure dependence of Gibbs energy is given by the volume change (ΔV) (Eq. 23.1). The equilibrium constant (K) is defined by the law of mass with the volume difference between products and reactants (ΔV). Accordingly, if the reaction is promoted by pressure conditions, K increases and ΔV becomes negative and the final state occupies less volume than the initial state:

$$(\partial \ln K / \partial P)_T = -(\Delta V / RT) \tag{23.1}$$

where K is the equilibrium constant, P the pressure, ΔV the volume change, R the universal gas constant, and T the temperature.

In the case of a nonequilibrium process, pressure dependence of the reaction rate (k) is given similarly to that depicted in Eq. (23.1) but by the volume of activation ($\Delta V^{\#}$) according to Eq. (23.2). The reaction rate constant is increased or decreased depending on whether the activation volume ($\Delta V^{\#}$), which is the difference between the volume of the transition state and the initial state, is negative or positive. The volume of activation ($\Delta V^{\#}$) is a useful parameter to compare the rate of different reactions under high-pressure conditions (Bolumar et al. 2012). The more negative the volume of activation, the more favored is the reaction under pressure:

$$(\partial \ln k / \partial P)_T = -(\Delta V^{\#} / RT) \tag{23.2}$$

where k is the reaction rate constant, P the pressure, $\Delta V^{\#}$ the volume of activation, R the universal gas constant, and T the temperature.

The effect of pressure on covalent bonds can be almost neglected under regular pressures ranging from 0.1 to 1500 MPa and low temperatures (0–40 °C) (Aertsen et al. 2009) because the total energy input into the system corresponds to only a few kJ mol^{-1}. In contrast, in heat processing, the energy input into the system is much higher and in the magnitude of a few hundred kJ mol^{-1} which can easily lead to the breakage of covalent bonds. Whereas temperature can only affect the reaction rate, pressure can in fact direct the reaction mechanism because it will favor the formation of species with the smallest volume (Aertsen et al. 2009).

Moreover, when a solution is compressed, its temperature will increase due to adiabatic heating (heat of compression). Conversely, the temperature will drop during decompression. In the case of water, the pressure increase leads to a temperature increase around 2–3 °C/100 MPa. This inevitable temperature change might also have an effect on structure formation.

As described, the effects of pressure and temperature are related to each other, as both variables are thermodynamically linked. Both pressure and temperature have an effect on the structure of macromolecules, although by different mechanisms. Temperature-induced changes often unfold the molecular structure irreversibly due to covalent bond breakages and/or intense aggregation. In contrast, high pressure can leave parts of the molecule unchanged (Knorr et al. 2006). Therefore, both pressure and temperature are important for a target modification of the structure and have to be taken into account simultaneously. In the case that cooling or heating is applied simultaneously with pressure, an additional variable with significant effect will be introduced in the system. It must be noted that if the sample is submitted to intense heating under pressure conditions, this will also result in partial destruction of the nutrients.

23.2.2 Operative Pressure and Temperature Range for Structure Modification

HPP as a preservation method is applied at pressure levels around 500 MPa and up to 1000 MPa (Patterson 2005), while HPP aiming at food structure modification can be applied at much lower pressures (50 MPa) or at the highest pressure (1000 MPa), depending on the final goal. Current standard industrial high-pressure equipment operates at maximum pressures of 600 MPa, although experimental high-pressure equipment can reach higher pressures. In addition, temperature during HPP can also be adjusted in a range from 5 °C to 100 °C. Industrial equipment for food applications with an in situ system allowing the regulation of the temperature within the pressure chamber is not available for industrial applications so far, although it exists for experimental purposes. Alternatively, heating or cooling of the pressure transmitted fluid can be used to influence temperature during pressurization. These mentioned points impose certain restrictions and establish a framework of pressure and temperature conditions which can be further transferred to currently available industrial equipment.

23.2.3 Product and Process Parameters Influencing Structure Modification

During HP processing (100–1000 MPa/20 °C to 100 °C), (1) cell wall and membrane disruption, (2) enzyme-catalyzed conversion processes, (3) chemical reactions, and (4) modification of biopolymers including enzyme inactivation, protein denaturation, and gel formation can occur at the same time (Oey et al. 2008). HPP can lead to denaturation and to different states of aggregation, surface

Table 23.1 Product and process parameters influencing structural modification of macromolecules by HPP

Product parameters	Process parameters
Type of protein/carbohydrate	Pressure level
Concentration	Pressure gradient
pH	Holding time
Ionic strength	Product initial temperature
Solubility	Temperature
Intramolecular interactions	
Intermolecular interactions	

hydrophobicity, and gelation, depending on the type of constituents present in the food, treatment temperature, chemical conditions of the solution, and magnitude and duration of the applied pressure. Biopolymer stability or coagulation zones can be explained in pressure/temperature denaturation/gelatinization graphs where separation of native and coagulated forms is possible. The most important product and process parameters influencing structure modification are shown in Table 23.1. The physicochemical properties of a solution, which defines the extent of the solubility and their interactions, are decisive in the occurring structural modifications by pressure. Regarding process parameters, the higher the pressure, the temperature, and the treatment time, the higher will be the modification. Examples of the effects of the different parameters are further described in Sections 23.3 and 23.4. Researchers and product developers must bear these variables in mind for designing and analyzing trials and the results. The number of combinations is extremely high, as it raises the complexity of the system and the resulting intermolecular interactions. In practice, it becomes difficult to predict the behavior of a complex system like a food matrix unless previous specific studies tackling the particular application exist. Optimization of treatment conditions on a case-by-case basis is required in order to obtain the desired functionality.

23.3 Modification of Biopolymers

Biopolymers, such as proteins and starches, show changes of their native structure by application of high hydrostatic pressure or temperature, although the mechanisms involved under pressure or temperature are different. These changes are in some cases analogous to the changes occurring at high temperatures (Knorr et al. 2006). The effect of pressure on proteins and starch is related to reversible or irreversible changes of their native structure (Balny et al. 2002).

23.3.1 Proteins

Proteins are polymers of amino acids that are linked to each other in a chain by peptide bonds. This amino acid sequence determines chain structure, size, and electric charge and thus the folding (structure) and functionality of the protein.

So, the primary structure only allows particular secondary structures to form. For a protein to be functional, its polypeptide chain needs to be properly folded into its native conformation. The development of energetically favored structures and the simultaneous action of a complex biological process are responsible for delivering the correct native conformation. No more than one or possibly a few native conformations exist for any given naturally occurring protein (Hedin 2010). In an aqueous environment, the hydrophobic effect will cause hydrophilic residues and the polar peptide backbone to be hydrated by water molecules, while hydrophobic parts are hidden in the protein interior. The native conformation is maintained by intra- and intermolecular forces.

Four levels of organization can be defined in the protein structure:

1. Primary structure, which is defined by the sequence of amino acids.
2. Secondary structure, which corresponds to the local folding of the chain. The most widely occurring secondary structures are α-helices and β-sheets.
3. Tertiary structure, which is the final folding of the chain and its spatial arrangements and interactions between the elements of secondary structure.
4. Quaternary structure, which comprises the interactions between and arrangement of two or more folded chains, or subunits, into a larger functional protein complex.

In contrast to temperature, which destabilizes the protein molecule by transferring nonpolar hydrocarbons from the hydrophobic core toward water, pressure denaturation by pressure is initiated by forcing water into the interior of the protein matrix (Knorr et al. 2006). The loss of contact between groups in the nonpolar domains results in the unfolding of parts of the molecule. As a result of water penetration into the protein interior, pressure is likely to lead to conformational transitions resulting in unfolding (Knorr et al. 2006).

The application of high pressure to proteins leads to different degrees of protein structure modification. As a general mechanism, unfolding of protein and subsequent refolding after pressure release is accepted. This folding/unfolding process leads, depending on the specific protein and conditions applied, to partial or total denaturation and tuning of electrostatic interactions. Application of high pressure has a disruptive effect on intramolecular hydrophobic and electrostatic interactions with minimum impact on covalent bonds, which makes pressure an ideal tool to tune non-covalent interactions and destabilize the hydrophobic effect. Therefore, HPP treatments induce modifications on the quaternary and tertiary structure of the proteins with negligible effect on their secondary and primary structure. Covalent bonds are rarely affected by high pressure, and even α-helix or β-sheet structures appear to be almost incompressible (Heremans and Smeller 1998). HPP can also dissociate protein subunits (Aertsen et al. 2009). The causes for pressure-induced dissociation of oligomeric proteins are imperfect van der Waals contacts between monomers, solvent electrostriction at the level of salt linkages at the interfaces of monomer subunits, and solvation of the nonpolar groups at the boundaries of contact in the oligomers (Balny 2002). A general scheme of the changes taking place on proteins under pressure is presented in Fig. 23.1.

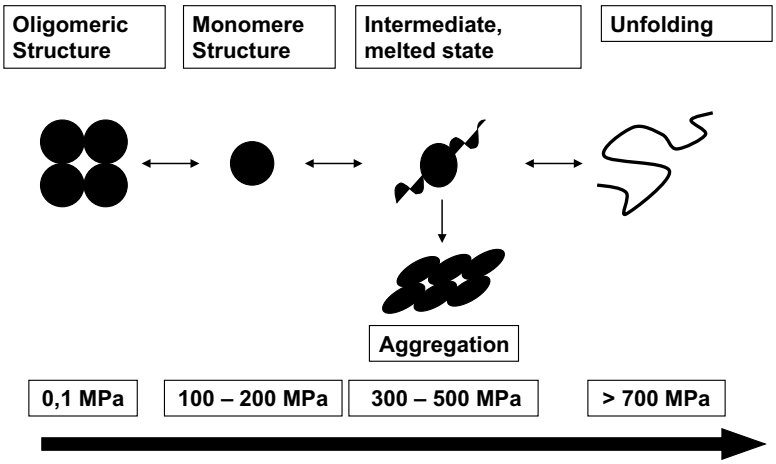

Fig. 23.1 Scheme of protein structure modification by high-pressure processing (HPP)

Protein denaturation is one of the key mechanisms for microbial inactivation; irreversible changes in proteins start at a comparative level to that required for the inactivation of microorganisms (400–600 MPa). HPP induces the breakdown of salt bonds, due to electrostriction and some hydrophobic interactions (Boonyaratanakornkit et al. 2002). In contrast, hydrogen bonds appear to be slightly strengthened under pressure (Cheftel and Culioli 1997). Quaternary structure is mainly held by hydrophobic interactions and thus it is very sensitive to pressure (Rastogi et al. 2007). Major changes in the tertiary structure are observed beyond 200 MPa and changes in secondary structure will only take place at very high pressure above 700 MPa (Rastogi et al. 2007). Generally speaking, the application of pressure >400 MPa easily leads to denaturation of proteins, whereas exposure to mild HP conditions around 200 MPa often affects only their quaternary structure, leading to the dissociation of oligomeric proteins (Aertsen et al. 2009; Balny 2002). Protein refolding becomes the key element for desired structure modification.

An application based on the refolding of proteins under pressure is the in vitro dissociation of protein aggregates, or inclusion bodies, which are often formed during the expression of recombinant proteins and which consist of inactive and improperly folded polypeptides (Jungbauer and Kaar 2007). HP-assisted dissociation of these aggregates allows for a subsequent accurate refolding of the constituent proteins, and it is unique in permitting simultaneous solubilization and refolding (i.e., without prior denaturation) in a relatively concentration-independent manner. As a result, refolding is possible at higher protein concentrations and with better yields compared to other refolding methods (Jungbauer and Kaar 2007). Recently, the technology of HP refolding is also being commercially utilized in large-scale production of protein therapeutics (see http://www.barofold.com). Relying on the same principle of reversible association–disassociation at moderate pressure levels,

HP treatment has raised significant interest as an alternative method for inactivation of viruses and development of vaccines (Aertsen et al. 2009).

Pressure-induced changes are greatest at higher pressures and extended holding times (Tedford et al. 1999). Changes in protein structure at high pressure can be minimized at low-temperature treatments (Kolakowski et al. 2001; Kunugi and Tanaka 2002). Exposition of sulfhydryl groups (SH) by application of HPP and subsequent oxidation and formation of disulfide bonds can lead to stabilized protein aggregates of importance to structure modification (Van der Plancken et al. 2005).

Overall, HPP affects the disruption of non-covalent interactions (hydrophobic and electrostatic) which are followed by rearrangement and reformation of intra- and intermolecular bonds at the interfaces of proteins leading to different degrees of denaturation, disassociation, aggregation, and gelation (Fig. 23.1). Unspecific protein–protein interactions can result in the formation of large complexes with high molecular weight. One limitation to unveil structural modifications by HPP is that protein–protein interactions in studies conducted on individual proteins can be completely different from those occurring in real systems and, thus, HPP-induced changes are difficult to envisage. Many of the effects governing pressure-/temperature-induced protein structure modifications are quite complex and not fully understood.

23.3.2 Carbohydrate Biopolymers

Carbohydrate biopolymers have a defined spatial arrangement and therefore are susceptible to structure modification by heat or pressure treatments. One of the most common carbohydrate biopolymers is starch. Starch is a common storage carbohydrate in plants and also a component of a large number of different food products (Belitz et al. 2008). Starch consists of D-glucose entities appearing in two different forms; 10–30 % of starch consists of linear glucose chains having a helical structure where single glucose molecules are linked by a α-1,4-glycosidic bond. This mainly amorphous part of starch is named amylose. The second part, 70–90 % of amylopectin, consists of a highly branched crystalline structure with α-1,4- and α-1,6-glycosidic linked glucose molecules (Buléon et al. 1998). Amylopectin and amylose, as crystalline and amorphous lamellae, alternate in a distance ranging from 120 to 400 nm forming crystalline and amorphous growth rings of starch granules. The crystallinity is classified as A type or B type, dependent on the configuration of the unit cell of the double helix of the polysaccharides (Tester et al. 2004). Thus, stability and functionality of starch are determined by highly ordered structural components.

Disruption of this native starch structure by heat is called gelatinization (Morris 1990). Besides heating, gelatinization can also be initiated by high hydrostatic pressure. Effects can be characterized by measurement of gelatinization enthalpy. Experiments carried out on starch isolated from commercially available

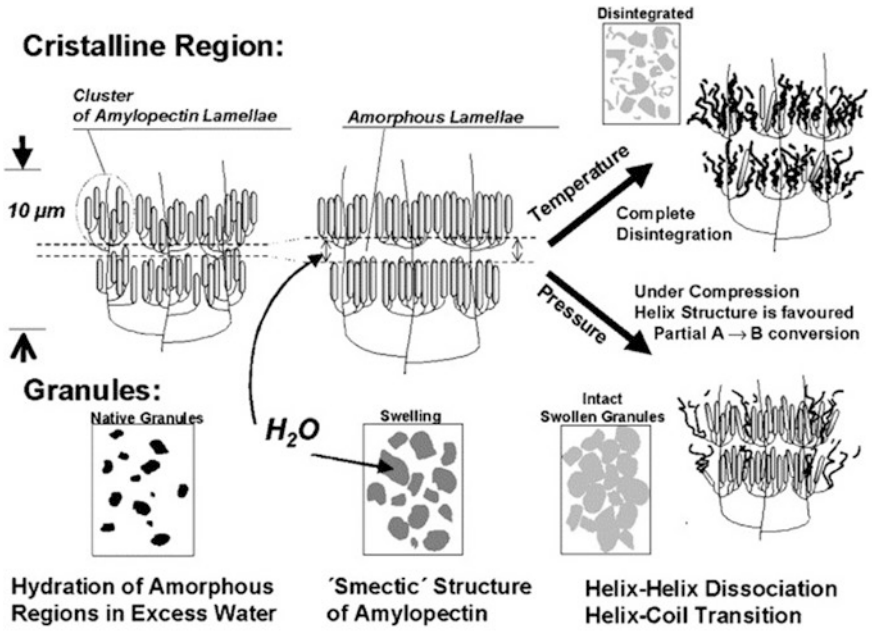

Fig. 23.2 Scheme of starch gelatinization. Reprinted from Knorr et al. (2006) with permission from Elsevier

wheat flour type 405 are described in this chapter. The amount of energy increases from 8.8 J/g (untreated) to 9.7 J/g (200 MPa, 40 °C initial temperature) due to HP treatment (Lampe et al. 2013). According to Knorr et al. (2006), the pressure range in which gelatinization occurs partly depends on the starch crystalline structure. In excess water, the amorphous growth ring regions are hydrated, and in the crystalline domain, amorphous lamellae are formed. The granule swells, and the crystalline structure is decomposed by helix–helix dissociation followed by helix coil transition when the gelatinization temperature is exceeded. It is suggested that under pressure the disintegration of the macromolecule is incomplete since the pressure stabilization of hydrogen bonds favors the helix conformation. Even crystalline conversion from A to B isomorph under pressure has been reported (Fig. 23.2) (Knorr et al. 2006).

HP treatment of wheat flour–water suspensions leads to higher amounts of bond water. Moreover, temperature also has significant influence. At the same pressure and initial temperatures of 10 and 40 °C, extensively higher amounts of water are bound. The value increases from 33.8 to 37.6 g/100 g. This result can be related to modifications of ternary and quaternary protein structures of the proteins covering the starch granule surfaces. In addition to temperature, the accessibility of starch to water is also influenced by the protein coverage of starch granules. As the temperature increases adiabatically during HP treatment, swelling processes often occur with high initial temperatures. Consequently, the amount of bond water to starch is

increased. At 600 MPa, the granular starch structure is no longer present, and water binding is again dominated by the proteins (Lampe et al. 2013).

Rumpold (2005) also confirmed that the degree of gelatinization and water adsorption of a starch suspension increases with increasing pressure and increasing pressure holding time. This result was likewise applied to the conversion of crystalline starch structures (Rumpold 2005). Hence, it can be concluded that interactions between macromolecules and water are influenced by hydrostatic pressure. The fraction of bond water is increased by functional groups of macromolecules which potentially form hydrogen bonds by making them available to the water molecules (Lampe et al. 2013).

These results can also be supported by atomic force microscopy (AFM). This technique is based on the measurement of interactions between a sharp tip and the surface being characterized (Binnig et al. 1986). The tip is moved across the surface for certain distances or directly in contact to the surface depending on the desired surface properties to be determined. In this way, for example, images of surface topographies up to several tenths of a nanometer can be obtained. The topographies of starch granules show that the surface consists of a multitude of similar structures with a size of ca. 30–50 nm in diameter (Fig. 23.3). In order to understand these structures, the molecular structure of starch itself must be considered (Pérez et al. 2009). The average diameter of amylopectin molecules is of 200–400 nm. These molecules consist of 20–40 amylopectin side chain clusters with a diameter of about 10 nm. Therefore, the smallest structures visible through AFM can be dedicated to one single amylopectin side chain cluster (Fig. 23.3). The larger structures of 20–50 nm consist of 2–5 or more amylopectin side chain clusters (Fig. 23.3).

After high-pressure treatment at different conditions, the starch surfaces show topography changes (Fig. 23.3b–d) (Lampe et al. 2013). These changes are due to a modification in structure of single amylose side chain by application of hydrostatic pressure. With pressure, water molecules are squeezed into the cavity in an irreversible way. It can be assumed that these cavities are free spaces between the crystalline and amorphous lamellae, as depicted in Fig. 23.4. Accordingly, the intrusion of water molecules into the starch granule leads to an increase of the single amylopectin side chain cluster. In turn, this leads to a rough-textured particle surface. Besides surface topography, adhesion forces between the AFM tip and the granule surface were already estimated (Fig. 23.5). Compared to untreated starch, adhesion forces are increased by hydrostatic pressure. This implies that water molecules are integrated into naturally existing protein surfaces covering the starch granule surface, leading to a change in conformation of the tertiary and quaternary protein structure and increased contact area between the AFM tip and sample surface. This increased contact area results in larger tip–sample interactions, resulting in higher adhesion forces. Basically, it can be said that non-covalent bonds of proteins are modified by high-pressure treatment. This result leads to changes in morphology and an increase of surface reactivity (influenced by the higher amount of bond water).

In order to examine the molecular structure of pressure-treated starch granules, X-ray powder diffraction pattern can be captured (Lampe et al. 2013). In this pattern, a

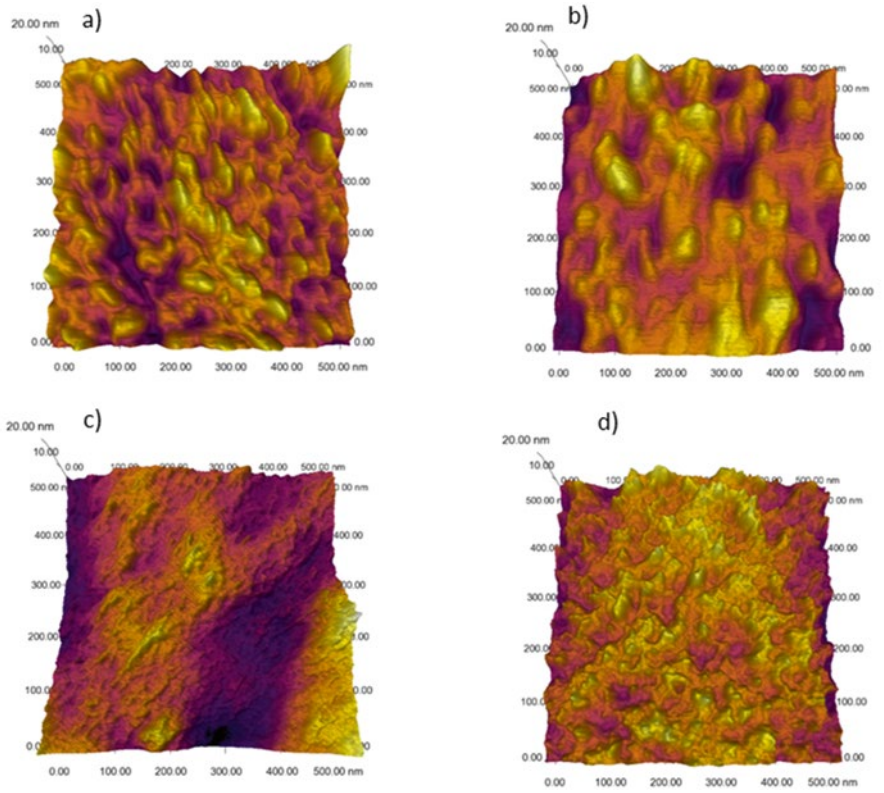

Fig. 23.3 Granular structure of starch and atomic force microscopy (AFM) height images (500×500×20 nm) of wheat starch surfaces isolated from wheat flour type 405 treated under different pressure conditions. (**a**) Reference, (**b**) 200 MPa 10 °C initial temperature (IT), (**c**) 200 MPa 40 °C IT, (**d**) 600 MPa 20 °C IT. Reprinted from Lampe et al. (2013) with permission from Elsevier

peak describes the distance between the atoms in a crystal structure; peak width is determined by size and quality of the crystal or the elementary cell, respectively. The signal intensity is equivalent to the position of the atoms in the elementary cell. This means that the more crystalline a structure is, the sharper the obtained peak. Thus, amorphous phases cannot be detected using this method. Figure 23.6 shows the X-ray diffraction patterns of wheat starch isolated from wheat flour type 405. There are no significant differences between the structures of the 200 MPa treated sample in comparison to the untreated one. But there are severe structural changes after the HP treatment with 600 MPa. These changes are comparable to those occurring during thermal treatment of starch, but not identical. Sharp peaks in the range above 25 2 θ arise from crystalline sodium chloride caused by sample preparation.

In contrast to X-ray powder diffraction patterns, radial distribution reveals information on the local arrangement of atoms in amorphous phases. Radial distribution

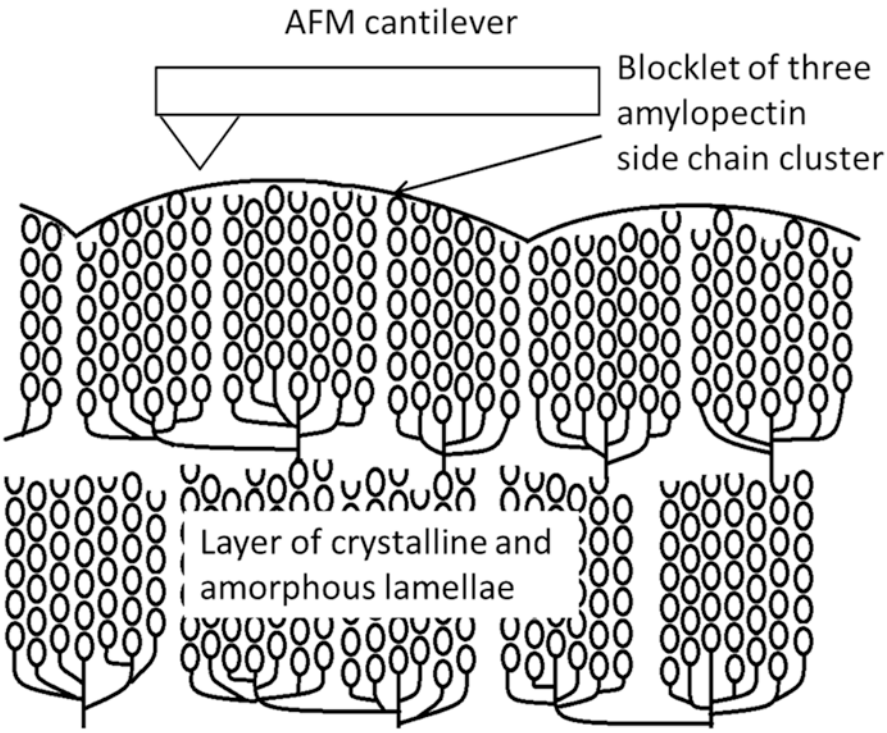

Fig. 23.4 Schematic structure of starch. Reprinted from Lampe et al. (2013) with permission from Elsevier

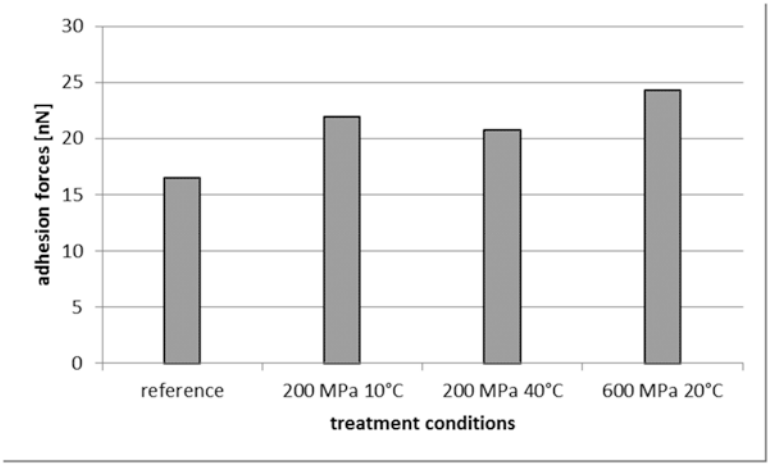

Fig. 23.5 Adhesion forces and surface potential differences of wheat starch granules isolated from wheat flour type 405

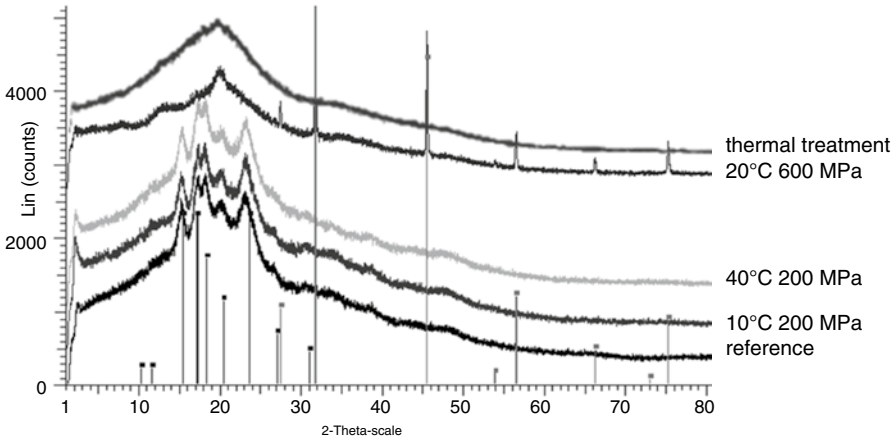

Fig. 23.6 X-ray powder diffraction pattern of treated and untreated wheat starch granules isolated from wheat flour type 405 compared to thermally treated starch

depends on separation between two residences of atoms, so that the particle-number density of the mean separation to the reference atom is detectable. There are some differences between the reference and the samples treated with 200 MPa. However, the treated samples themselves are so similar to each other that no differentiation can be made using this method (data not shown). Thus, all samples have a basically similar local structure (Lampe et al. 2013).

Overall, the influence of hydrostatic pressure on starch is due to water intrusion, granule volume, and rearrangement of amorphous and crystalline regions. HPP treatment has a significant influence on material properties such as water binding and gelatinization enthalpy. Water binding is highest at moderate pressures (200 MPa) and higher initial temperatures (40 °C). Obviously, in this case, the degree of unfolding of proteins and starch equals the state of optimal interaction with water molecules. At lower temperatures, water binding on starch is very low, and at excessive pressure the contact between macromolecules and water is again decreased. Compaction and phase separation occur. Gelatinization enthalpy decreased due to application of hydrostatic pressure. At high pressure (600 MPa), it is no longer detectable. The results of X-ray diffraction revealed that the structure of the granules treated with 200 MPa basically remain unaffected. At 600 MPa, structural changes comparable to thermal treatment take place.

23.4 Applications

The application of high-pressure conditions to food systems can be delivered by HPP (high-pressure processing) or HPH (high-pressure homogenization). Basically, all types of solid or liquid products can be submitted to static high-pressure

conditions (HPP). But only solutions or dispersions, which are sufficiently liquid and thus pumpable, can be submitted to dynamic pressures (HPH). With both systems, HPP and HPH, structure modification can be obtained. Besides pressure itself, in HPH, other phenomena such as shear forces, cavitation, and local temperature increase are important factors determining the structure modification (for further details, see Chapter High-Pressure Homogenization). In addition, HPP operates in a batch mode, whereas HPH is a continuous system. Irrespective of the mentioned reasons, HPP is a more mature technology than HPH. HPH at higher pressures above 200 MPa still remains at the level of semi-industrial pilot hall facilities with incipient industrial applications.

The majority of applications concerning structure modification of food systems by HPP are described in processed fruits and vegetables, meat, and dairy products and are presented in this chapter. These modifications serve as a reference and inspiration for modifications occurring in other foodstuffs. The production of different food ingredients through the processing of biopolymers by HPP or HPH is also presented.

23.4.1 Processed Fruits and Vegetables

Since HPP shows minimal effects on color and flavor components of fruits and vegetables, it is very promising to keep freshness and extend the shelf life of such products. Changes in texture experienced by HP-processed fruits and vegetables are related to transformations in cell wall polymers due to enzymatic and nonenzymatic reactions (Sila et al. 2008). High pressure changes cell permeability and enables movement of water, enzymes, and metabolites from inside to outside the cell (Prestamo and Arroyo 1998). The degree of cell disruption depends on the pressure applied and on the type of plant cell. Plant cells disintegrate and intercellular spaces are no longer filled with gas (e.g., in spinach leaf) (Prestamo and Arroyo 1998). After HP processing, cavity formation occurs and a firm texture and a soaked appearance (e.g., cauliflower) are noticed (Prestamo and Arroyo 1998; Oey et al. 2008). In addition, pressure can modulate the enzymatic action of pectin methylesterase (PME) and polygalacturonase (PG) whose sequenced action causes the breakdown of pectin. Pressure treatment can enhance the desired action of pectin methylesterase (PME) and lower the undesired activity of polygalacturonase (PG) (Verlent et al. 2006). In this way, a substantial modification of texture and viscosity of the processed vegetable and fruit juices, which cannot be formed by thermal processing, takes place (Oey et al. 2008).

HPP of fruits and vegetables can result in increased firmness or tissue softening (Basak and Ramaswamy 1998). Cell wall disintegration and PME and PG activities have been suggested as the main reasons behind this result. The pressure effects on the cell walls of carrot tissue are shown in Fig. 23.7. Temperature induces tissue softening, which is evidenced by pectin degradation and increased loosening of the cells (Fig. 23.7b versus a). In contrast, the application of pressure results in a firmer structure (Fig. 23.7c), due to the action of the endogenous PME whose activity is

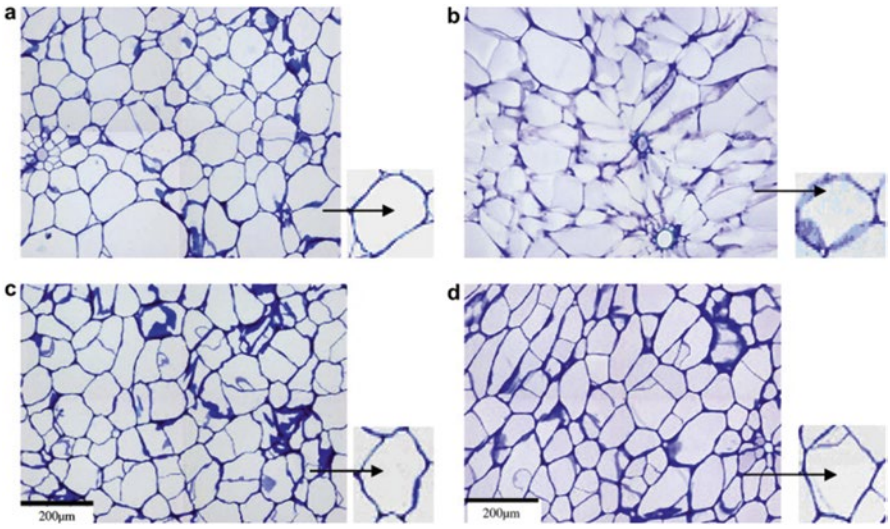

Fig. 23.7 Pressure effects on the cell walls of carrot tissue. Microscopy images of carrot tissue from (**a**) untreated (raw) carrots, (**b**) carrots cooked at 100 °C for 120 min, (**c**) carrots pretreated with HP (400 MPa, 15 min, 60 °C) and non-cooked, and (**d**) carrots pretreated with HP (400 MPa, 15 min, 60 °C) before being cooked at 100 °C for 120 min. Cell wall material was colored with toluidine blue, and each micrograph represents four fused snapshots taken at ×40 magnification. Reprinted from Sila et al. (2008) with permission from Elsevier

thermodynamically favored under HP (because of the negative volume change associated with the conversion). PME catalyzes the demethoxylation of pectin, resulting in the formation of carboxylated pectin that now has increased ability to cross-link with divalent ions (such as calcium) and form rigid supramolecular assemblies. HP pretreatment (400 MPa, 15 min, 60 °C), which allows PME-mediated pectin changes, is useful to counteract this tissue softening due to thermal processing (Fig. 23.7d) (Sila et al. 2008). In addition, the use of HP processing combined with pretreatments such as infusion of exogenous pectinases (Duvetter et al. 2005) and/or soaking in calcium chloride solutions (Sila et al. 2006) provides alternatives to reinforce the prevention of tissue softening of processed fruits and vegetables.

HPP treatments carried out in combination with elevated temperatures result in texture preservation in comparison to thermal treatments (De Roeck et al. 2008, 2009). These results are very promising in the context of the texture preservation of high-pressure-sterilized fruits and vegetables, as β-elimination, which is accepted as one of the main causes of thermal softening, is retarded, whereas low methoxylated pectin can enhance tissue strength by forming cross-links with present calcium ions (De Roeck et al. 2009).

Again, the modulation of activities, i.e., PME (a desire enzymatic activity) and PG (depolymerization of pectin), by HPP can lead to considerable changes in the viscosity of juices and purees (Oey et al. 2008). For instance, orange juice treated by HPP (600 MPa/40 °C/4 min) resulted in higher viscosity and more limited cloud loss than for thermal treatment (80 °C/60 s) (Polydera et al. 2005).

23.4.2 Meat Products

HPP has been much adopted by the meat sector. This is likely due to the fact that meat products have a high commercial value and can afford HPP investment and processing costs. Currently, the use of HPP in the meat industry represents around 25–30 % of the total HPP equipment installed in the food industry (Tonello 2010). In addition to microbial inactivation at low temperatures, which was the primary aim of the technology, pressure can affect the processes of meat protein denaturation, dissociation, solubilization, aggregation, and gelation, and thus HPP has been used for meat tenderization and to induce structure formation in different meat products.

23.4.2.1 Structuring Effect of HPP on Muscle Proteins

Muscle proteins are divided into water-soluble extractable proteins or sarcoplasmic proteins, salt-soluble extractable proteins or myofibrillar proteins, and non-soluble proteins and connective tissue (collagen). These proteins have distinct roles regarding meat quality, and it is of interest to evaluate these fractions separately.

Sarcoplasmic proteins account for 25–35 % of the total cell proteins and are mainly enzymes and heme pigments. Most of them are globular proteins, and thus they are very susceptible to denaturation. Marcos et al. (2010) reported a relationship between changes induced by HPP on sarcoplasmic proteins and meat quality traits such as water holding capacity and color, particularly at pressure level above 200 MPa. Myofibrillar proteins account for 50–55 % of total muscle proteins and are related to the meat structure. The most abundant myofibrillar proteins are fibrous myosin and globular actin. Myofibrillar proteins are unfolded up to a pressure of 300 MPa, and higher pressures result in increased denaturation, agglomeration, and gel formation. These structural modifications of meat proteins under pressure can be employed in product development to influence structure formation and/or water binding (Sun and Holley 2010). One of the most remarkable effects of high pressure on meat proteins is the modification of the actin–myosin complex. Pressure conditions induce an increase of soluble materials from myofibrils, which is markedly promoted by pressurization above 150 MPa (Nishiwaki et al. 1996). Suzuki et al. (1990) investigated the effect of high pressure (100–300 MPa) on post-rigor beef muscle and observed that maximum disruption was achieved at 300 MPa for 5 min. Additionally, the Z-line in myofibrils was not apparent in pressurized muscle (Suzuki et al. 1990). In agreement, Iwasaki and co-workers also described the disruption of the M-line and Z-line and the dissociation of thick and thin filaments in the pressurized myofibrils. This disposition of thin and thick filaments within myofibrils is mainly responsible for the water holding capacity in muscle cells. Therefore, any spatial rearrangement or disruption between them affects the ability to retain water. This might explain changes in drip loss during storage observed by Iwasaki et al. (2006), who concluded that pressure-induced global structural changes of myosin subfragment 1 (S-1) begin to occur about 150 MPa and that local structural

changes in ATPase and actin binding sites followed with elevating pressure to 250/300 MPa. Chapleau et al. (2003) investigated modifications of myofibrillar proteins by HPP (from 50 to 600 MPa for 10 min at 20 °C) and showed that no changes in the secondary structure of proteins took place. The same authors described that aggregation accompanied by an increase in hydrophobic bonding starts at pressures higher than 300 MPa. Modification of tertiary and quaternary structures of proteins may induce a molten globule state. In contrast, limited effect after HPP treatment is visible on connective tissue at ambient temperature, and little effect is seen at higher temperatures (Beilken et al. 1990). As collagen is primarily stabilized by hydrogen bonds, it is little affected by pressure, whereas changes in the structure of myofibrillar proteins are thought to be responsible for the textural modification induced by HPP.

23.4.2.2 Tenderness Improvement by HPP

Based on the HPP-induced dissociation of myofibrillar proteins, HPP has raised interest as a method for tenderness improvement. The application of HPP to pre-rigor meats results in intense contraction (a length reduction of 35–50 %) and severe meat disruption of muscle structure (Sun and Holley 2010). Macfarlane (1973) was the first to tenderize pre-rigor meat by HPP treatment (103 MPa, 30–35 °C, 1–4 min). However, the application of HPP at pre-rigor state requires the development of hot boning processing at slaughterhouses, which is not a common practice. Thus, the same group continued their investigation on post-rigor meats and found no beneficial effect of high pressure at low temperatures (<30 °C) (Bouton et al. 1977). The influence of HPP on meat tenderness depends on the rigor stage, pressure and temperature level applied, and their combination (Sun and Holley 2010). During post-rigor HPP treatment, extensive modifications in sarcomere structure but no contraction of bands are observed (Sun and Holley 2010). Meat tenderization by HPP treatment of postmortem meat only occurs combined with high temperature (60 °C) and is likely caused by lysosome breakdown and subsequent release to the medium of proteolytic activity (Hugas et al. 2002; Grossi et al., 2012). In a recent study, Sikes et al. (2010) stated that meat was tenderized after treatment (200 MPa, 60 °C, 20 min) and ascribed the effect to an enzymatic occurring activity, suggesting that cathepsins are responsible. Briefly, low pressures (<200 MPa) can tenderize pre-rigor meat (Souza et al. 2011), whereas tenderization post-rigor by HPP can only be achieved when combined with high temperatures (Sikes at al. 2010). Tenderization of meat by HPP treatment has found limited applicability so far.

23.4.2.3 Structure Modification of Meat Products by HPP

HPP can be used in meat product development as a texturizing method (Grossi et al., 2011), for instance, in the production of liver sausage, a traditional German cooked spreadable sausage, which requires two individual thermal treatments. Due to the high time and temperature requirements, valuable macro- and micronutrients are

Fig. 23.8 Comparison of traditional (**a**) and high-pressure-processed (**b**) liver sausage

lost (Heinz et al. 2009). These twin thermal treatments can be replaced by HPP at 600 MPa for 2–5 min at room temperature. The first pressure treatment of raw material is designed to denature myofibrillar proteins and to create correct product characteristics of consistency and texture, while the second pressure treatment is carried out after the pressurized raw material is emulsified using raw liver in a bowl chopper in order to increase shelf life and to ensure final product characteristics. A comparison of traditional liver sausage and the novel HPP liver sausage is shown in Fig. 23.8. Replacement of the two thermal steps results in a significantly smoother and homogenous product with an increased liver taste, as well as significant improvements in time and energy consumptions and nutritional value (Heinz et al. 2009). Other meat products that can benefit from this application or similar HPP processes, with increased quality and improved energy efficiency, are cooked cured products such as pork loins and/or spreadable fermented sausages (Lickert et al. 2010).

Pressure treatment at low pressure prior to heating can improve the rheological properties of cooked meat products. In this sense, meat functionality of turkey meat with low pH or PSE meat and pork was improved by HPP treatment at 100 and 200 MPa, respectively (Chan et al. 2011; Iwasaki et al. 2006). The mechanisms behind this improvement are related to higher protein surface hydrophobicity, greater exposure of sulfhydryl groups, and disassociation of thin and thick filaments. Phosphates are believed to act on muscle proteins by increasing pH and ionic strength and, specifically, by complexing protein-bound Mg and Ca, thus leading to increased solubilization of myosin and actin (actomyosin dissociation and depolymerization of thick and thin filaments) (Offer and Knight 1988). High-pressure application has been shown to act on myofibrillar proteins in a similar manner to salts; therefore, both sodium chloride and phosphates could be reduced by this method (Fernandez-Martin et al. 2002). Pressurization of raw meat before the cooking step was employed in the production of bologna-type cooked sausage as a method to functionalize meat. Figure 23.9 shows the effect of HPP treatment of raw meat (100 and 400 MPa, 20 °C for 5 min) on the structure formation of cooked sausage. The application of HPP treatment (400 MPa, 20 °C for 5 min) on raw meat was detrimental to structure formation (Fig. 23.9, sausages 4 and 5). Meanwhile, an HPP treatment (100 MPa, 20 °C for 5 min) improved the functionality of meat proteins, allowing the formation of a proper structure (no phase separation) and therefore the production of cooked sausage without the addition of phosphates (Fig. 23.9, sausages 2 and 3).

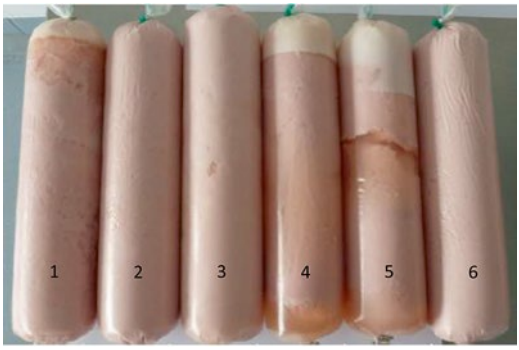

Fig. 23.9 Functionalization of raw meat by HPP treatment applied to production of cooked sausages. HPP treatment for 5 min at specified pressure was applied to raw meat with or without salt addition. (*1*) Control cooked sausages without added phosphates, (*2*) cooked sausages treated by HPP at 100 MPa with salt addition before HPP treatment, (*3*) cooked sausages treated by HPP at 100 MPa with salt addition after HPP treatment, (*4*) cooked sausages treated by HPP at 400 MPa with salt addition before HPP treatment, (*5*) cooked sausages treated by HPP at 400 MPa with salt addition after HPP treatment, (*6*) cooked sausages with added phosphates (0.06 %)

In addition, pressure induces texture modifications by affecting myofibrillar protein structure and its gel-forming properties. Pressure-induced gels are generally smoother, more glossy, less firm, more elastic, and with improved water holding capacity in comparison to thermally induced gels (Cheftel and Culioli 1997; Jimenez Colmenero 2002). Sikes et al. (2009) made use of HPP to reduce cooking loss and to improve the texture of low-salt beef sausage batters. Pressure treatment contributed to enhanced binding through protein solubilization and gelation through partial protein unfolding. The effect of combined application of high pressure with temperature on a traditional and popular German product, Frankfurters, was investigated by Tintchev and co-workers (Tintchev et al. 2013). Frankfurter sausage batter is a polydispersed system consisting of a liquid continuous phase (water, soluble proteins, ions), a dispersed liquid phase (fat droplets), and a dispersed solid phase (non-solvated muscle fiber particles, connective tissue, spices). Standard transformation from batter to sausage is produced by heating to a core temperature of approx. 72 °C where simultaneous microbial inactivation also takes place. This thermal treatment can be replaced by the application of high pressure in combination (or not) with temperature. The degree of solubilization of meat proteins due to high-pressure conditions, particularly the structural protein myosin, was a key factor with significant effect on batter structure (Tintchev et al. 2013). Maximal solubilization level took place at 200 MPa/40 °C and depended on treatment time. Pressurization gradient (PG), which is analogous to the temperature gradient applied in cooking processes, had significant impact on the structure. Protein network and functional properties of sausage were improved at low PG (2.5 MPa/s) (Tintchev et al. 2013). According to analysis of protein bands by SDS-PAGE, the major role in solubilization, aggregation, and gelation processes occurring in the aqueous phase was due to

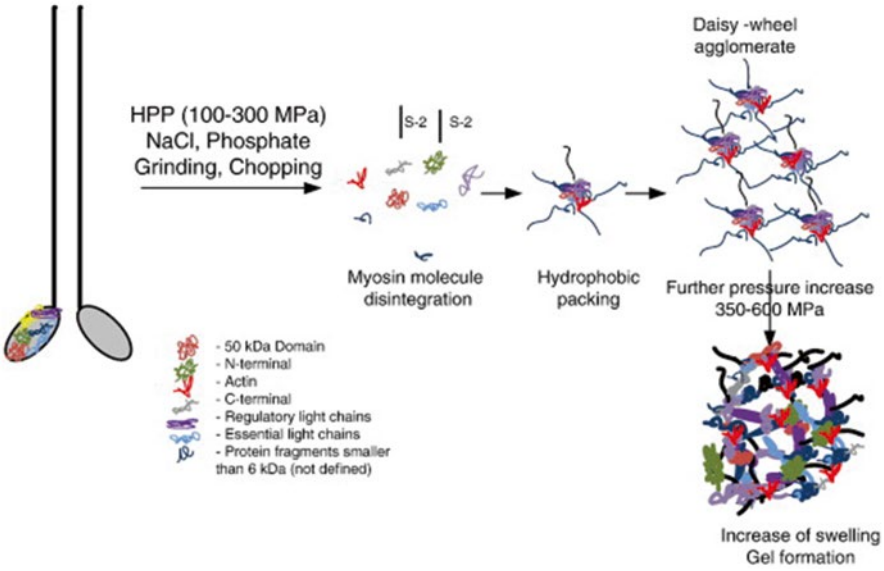

Fig. 23.10 Hypothetical mechanism of secondary structure formation during high-pressure temperature treatment. Reprinted from Tintchev et al. (2013) with permission from Elsevier

myosin S-1 and S-2, N terminal, C-terminals, MLC, and actin during high-pressure/high-temperature treatment (Tintchev et al. 2013).

Figure 23.10 shows the hypothetical mechanisms of secondary structure formation during high-pressure and high-temperature treatment based on Yamamoto's mechanism of hydrophobic packing and formation of daisy-wheel conformations (Yamamoto et al. 1993). The meat industry conventionally uses chemical, mechanical, and temperature treatments to alter myosin solubilization and further denaturation and gelation. Analogous effects can be obtained by HPP treatments. Maximum solubilization of the three S-1 myosin domains (N-terminal and C-terminal—50 kDa and 20 kDa) and regulatory and essential light chains, as well as actin, takes place in the range of 150–250 MPa (Tintchev et al. 2013). Then, due to the high hydrophobic character of myosin head domains, agglomeration begins to occur (Iwasaki and Yamamoto 2003). Transformation of protein subfragments to a more stable energy level, known as hydrophobic packing, occurs because of the pressure-induced volume decrease in this pressure range (Fig. 23.10). Next, aggregation of different hydrophobic packs leads to the first stage of secondary matrix network formation. The exact configuration of these aggregates and the detailed protein–protein interactions, head to head and tail to tail, are unknown. Pressures above 400 MPa lead to more unfolded structures due to breaking of hydrophobic interactions (Chapleau et al. 2003). With increasing pressure, the agglomerations developed themselves (induced by swelling) in a protein network, which under certain conditions form gel structures (Fig. 23.10).

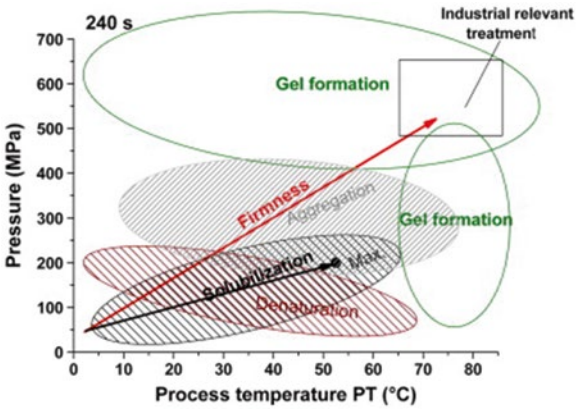

Fig. 23.11 Hypothetical *P–T* ranges of myosin solubilization, aggregation, and gelation after HPP treatment of 240 s. Reprinted from Tintchev et al. (2013) with permission from Elsevier

The processes of batter protein solubilization, aggregation, denaturation, and gel formation after 240 s (industrial relevant preservation treatment time) were also summarized in a *P–T* diagram by Tintchev and co-workers (Tintchev et al. 2013) (Fig. 23.11). Myosin solubilization (or molecule disruption) occurs in the range of 0–300 MPa with maximum solubilization at 200 MPa/40 °C. This solubilization range corresponds to the myosin denaturation area of 0–200 MPa. Aggregation proceeds at pressures higher than 300 MPa (Iwasaki and Yamamoto 2002). The aggregation process continues up to higher pressure levels, where gelation begins (above 400 MPa). Gel formation is also possible at lower pressures with higher temperatures (above 60 °C). Meat protein denaturation increases linearly with increasing pressure and temperature, resulting in an increase in firmness. Tintchev and coworkers concluded that pressure/temperature formation of Frankfurter sausages after 240 s of holding time with improved functional properties and with microorganism inactivation takes place at pressure above 500 MPa and initial temperatures above 40 °C. Analogous to the effect of low gradients of temperature increase, low gradient of pressure increase also leads to better gel formation.

23.4.3 Dairy Systems

Dairy systems have been the object of extensive studies concerning structure modification by HPP since it offers the possibility of developing dairy products with specific textures which at the same time retain high nutritional value. These structural modifications by HPP can be used in the production of different dairy products such as cheese or yogurt or in the creation of novel products such as HPP-induced milky gel (Cheftel and Dumay 1996; Lopez-Fandino 2006; Devi et al. 2013). Moreover, the addition of hydrocolloids offers the possibility for further tailoring texture (Orlien et al. 2006a, b; Devi et al. 2013).

23.4.3.1 Structuring Effect of HPP on Dairy Proteins

Heat and pressure treatments induce similar effects on milk: denaturing and aggregating whey proteins and diminishing the number of viable microorganisms. However, there are significant differences between the effects of these two treatments on protein unfolding and the subsequent thiol-catalyzed disulfide-bond interchanges that lead to different structures and product characteristics (Considine et al. 2007). HPP-induced structural changes to milk proteins can be grouped according to the effect of HPP on the two most abundant dairy proteins, casein and whey milk proteins.

Casein micelles disaggregate upon pressures >200 MPa. The rate and extent of micellar disruption increases with pressure and is probably due to the increased solubility of calcium phosphate with increasing pressure, which also leads to substantial modification of the mineral balance in milk (Huppertz et al. 2006; Lopez-Fandino 2006). On prolonged treatment at 250–300 MPa, reassociation of micellar fragments occurs through hydrophobic interactions (Orlien et al. 2006b). This process does not occur at pressures >300 MPa, leading to considerably smaller micelles (Huppertz et al. 2006). As a result of disassociation size, the number and hydration of casein micelles in HP-treated milk significantly differ from those in untreated milk.

The two most abundant whey proteins, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), denature at pressures >400 or >100 MPa, respectively (Huppertz et al. 2006). The majority of denatured β-LG in HP-treated milk is associated with casein micelles, although some denatured β-LG remains in the serum phase or is attached to the milk fat globule membrane. HP-denatured α-LA is also associated with milk fat globules (Huppertz et al. 2006).

23.4.3.2 Structure Modification of Dairy Products by HPP

Milk pressurization accelerates subsequent casein coagulation due to micelle disassociation, and whey protein denaturation hinders the aggregation of renneted micelles (Lopez-Fandino 2006). These modifications have implications on the processes leading to structure formation (e.g., gelation) and structure destabilization (syneresis, proteolysis) in cheese and yogurt manufacture. Denaturation of whey protein and its incorporation to the curd can enhance cheese yield (Lopez-Fandino 2006). Due to HPP-induced changes on dairy proteins, HPP can be applied in the production of cheese, aiming at a modification of its structure by improvement of rennet coagulation, assisting in curd formation, enhancing ripening, and improvement of microstructure/texture (Devi et al. 2013). HPP treatment of milk favors acid coagulation and produces acid gels whose structure is greatly determined by different micellar sizes attainable and degree of whey protein denaturation (Lopez-Fandino 2006). Due to HP-induced changes in buffering capacity, when HP-treated reconstituted milk is acidified, onset of gelation occurs at a higher pH than in the case of unpressurized milk (Desobry-Banon et al. 1994; Famelart et al. 1997). Interesting attributes such as increased water retention or reduced syneresis by pretreatment of milk by HPP have also been reported in yogurts (Penna et al. 2007; Capellas and Needs 2003). Mechanistic studies to unveil structure formation and

destabilization of dairy systems after high-pressure treatment and the behavior and interactions of different milk proteins are essential to better understand the product and process parameters governing structure formation.

According to Orlien and co-workers, high-pressure technology offers new opportunities for the development of nutritional and healthy milk products. Based on skim milk and added whey protein and/or hydrocolloids, high pressure makes it possible to produce milk products ranging from yogurt-like to pudding-like but without the sour taste and with less sugar (Orlien et al. 2006a, b, Orlien 2010). Such processing will comprise the following stages: mixing of milk powder and hydrocolloid; addition of water; addition of colorants, flavors, and other ingredients (for instance, fruit juices), along with flexible packaging; and finally HPP treatment (Orlien 2010). HP processing is rather simple in comparison to fermentation processes in the case of yogurt or gelation by thermal treatments in the case of pudding. Moreover, HPP will render significant energy savings compared to traditional processing. Final texture of the product can be modulated mostly by the concentration of protein or hydrocolloid, pressure level, and holding time. The effect of concentration of whey protein, pressure level, holding time, and storage time on elasticity, water retention, and whiteness are summarized in Table 23.2. In general, an increase in protein concentration, pressure level, and holding time induces a higher elastic modulus (G'). The non-incorporated liquid (NIL) becomes higher with increasing protein concentration and with pressure processing within the range 200–400 MPa, whereas upon pressure > 400 MPa, water retention is higher. Normally, increased protein concentration results in increased gel strength without affecting water retention. However, at some critical protein concentrations, water holding capacity begins to decrease (Table 23.2). This result may be caused by the formation of a denser network structure due to the increased amount of protein molecules capable of forming the network, thereby expelling water during pressure treatment. In other words, there is no space for water molecules in the gel network, and they are expelled (Orlien et al. 2006a, b). In addition, lightness or whiteness of the product is promoted by protein concentration, pressure level, and holding time. Storage time of 5 days did not affect

Table 23.2 Influence of increasing whey protein isolate (WPI) concentration in skim milk, pressure level, pressure holding time, and storage on the physical characteristics of pressure-induced gels

	G' (elastic modulus)	NIL (non-incorporated liquid)	L^* (white color)
WPI concentration (5 %, 10 %, or 15 % w/w)	↑[a]	↑	↑
$200 < P$ (MPa) < 400	↑	↑	↑
$400 < P$ (MPa) < 600	↑↑	↓	↑
Pressure holding time (15 or 30 min)	↑	↑	↑
Storage (1–5 days)	ns	ns	ns

[a]Arrow ↑ denotes an increase and arrow, ↓ denotes a decrease, ↑↑ denotes extreme increase, and ns denotes no significant changes
Adapted from Orlien (2010)

Fig. 23.12 Pictures of milk gels obtained by high-pressure processing. Courtesy of Vibeke Orlien

elasticity or water retention (Table 23.2). Gels made of whole milk powder (10 % w/w in tap water) with (around 10 %) whey protein isolate (WPI) and processing by HPP at 600 MPa for 10 min at 20 °C can be seen in Fig. 23.12. The white product is milk without colorant. These products represent an example of a gel type with two upward arrows in the table, which indicates an extreme increase in G' (Table 23.2). The main drawback of these dairy products is their lack of mouth feel and creaminess. These quality attributes must be further investigated and developed to achieve consumer acceptance and therefore industrial practicability. The addition of hydrocolloids such as iota- and kappa-carrageenan has synergistic effects on the texture formation and hence allows the reduction of the protein concentration (Orlien et al. 2006a, b; Devi et al. 2013). Addition of fruit juices to milk solution prior pressurization also results in an attractive formula to produce novel foods with improved nutritional profile (i.e., increased content of vitamins and antioxidants due to minimized destruction during HPP). Hypocaloric foods based on skim milk and HPP-induced gelation can be produced. Overall, HPP-induced dairy gels represent an innovative method to produce dairy products with customized texture and improved nutritional profile.

23.4.4 Food Ingredients

The modification of protein and carbohydrate biopolymers by high pressure offers the possibility of creating novel enhanced functional ingredients such as emulsifiers, stabilizers, texturizers, and water retention-intended ingredients. Protein

solutions can also be submitted to HPP or HPH aiming at an improving functionality (Galazka et al. 2000; Dumay et al. 2006, 2012). The resulting ingredients must be subsequently tested in model or real food systems to assess the improved functionality or stability of the food structure.

The application of HPP appears to be effective in improving meat (Bajovic et al. 2012; Sun and Holley 2010), egg (Ngarize et al. 2005; Miyoshi and Koseki 2010), and soy (Roesch and Corredig 2003; Wang et al. 2008) protein gelation properties, as well as the coagulating properties of milk (Bouaouinaa et al. 2006; Considine et al. 2007; Lim et al. 2008; Venir et al. 2010). This finding can be transferred to products with improved texture using fewer additives such as emulsifiers (Bader et al. 2011; Roesch and Corredig 2003; San Martin-Gonzalez et al. 2009; Wang et al. 2008), stabilizers (Bouaouinaa et al. 2006; Lim et al. 2008; Roesch and Corredig 2003; San Martin-Gonzalez et al. 2009), texturizers (Bader et al. 2011; Bajovic et al. 2012; Ciron et al. 2010; Considine et al. 2007; Ngarize et al. 2005; Sun and Holley 2010; Venir et al. 2010), and water retention-intended additives like phosphates or gums (Bajovic et al. 2012; Ciron et al. 2010; Sun and Holley 2010). Dairy proteins can also be submitted to HPP to induce gel formation (Ngarize et al. 2005; Venir et al. 2010) or to HPH to improve protein functionality such as foaming or stabilizing properties (Bouaouinaa et al. 2006; Lim et al. 2008).

23.5 Conclusions and Future Perspectives

Considerable progress on quantifying and understanding HP-induced changes in proteins and starch has been made in the past 15 years. However, HP modifications still remain elusive and the complex interrelationships between phenomena occurring during HPP treatment and their resultant structural rearrangements are not fully understood. A better understanding of product and process parameters required for optimal modification is needed in order to unveil the full potential of the use of HPP as a structuring method. It seems feasible that certain applications of structural modification of foodstuffs by HPP treatment reach the market in the near future; most likely these changes will occur within the meat sector. Structural modification of food by HPP at high temperatures still requires the development of industrial equipment. Production of food ingredients with improved functionality is very promising to provide the industry with alternatives for clean labeling. Different ingredients with enhanced functionality obtained through HPP already exist in the market and their number will increase in the future. The use of HPP as a physical method for structure formation has promising perspectives of being accepted by consumers in order to produce novel foods with customized texture and enhanced nutritional profile.

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Part VI
High Pressure Applications in Various Food Industry Sectors

Chapter 24
High-Pressure Effects on Fruits and Vegetables

Ariette Matser and Rian Timmermans

Abstract The chapter provides an overview on different high pressure based treatments (high pressure pasteurization, blanching, pressure-assisted thermal processing, pressure-shift freezing and thawing) available for the preservation of fruits and vegetable products and extending their shelf life. Pressure treatment can be used for product modification through pressure gelatinization of starch and pressure denaturation of proteins. Key pressure–thermal treatment effects on vitamin, enzymes, flavor, color, and texture of fruits and vegetable are discussed.

Keywords High pressure • Fruits and vegetable • Quality • Pasteurization • Shelf-stable

24.1 Introduction

In overviews of high-pressure (HP) processing for food products, the positive effects of this technology on the quality and shelf life of fruits and vegetables are often highlighted. This is also shown in the many scientific publications on effects on and mechanism of high pressure on specific fruits and vegetables and the large number of fruit and vegetable products on the market that are treated with high pressure. Examples are fruit juices, jams, vegetable mixes, and ready-to-eat meals that are pasteurized by high-pressure processing to give them an extended shelf life of 1–2 months when stored refrigerated. Some good reviews are available that give an overview of the effects of high pressure on fruits and vegetables (Oey et al. 2008a; Sila et al. 2008; Sánchez-Moreno et al. 2009).

A classic example of the positive effects of high pressure on fruits and vegetables is guacamole or avocado puree. This is one of the oldest high-pressure products on the market and is a good illustration of the possibilities of high pressure as a preservation method. A freshly made avocado puree has a light green color and a fresh

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taste. However, due to the enzymes in the product (mainly polyphenoloxidase), the color of the puree changes in a dark olive-green color within a few hours after preparation. Lowering the pH by addition of lemon juice slightly slows down this process, but is insufficient to create a product with a shelf life of several days. A heat treatment inactivates the enzymes and therefore preserves the green color of the product. Moreover, it inactivates spoilage microorganisms and pathogens. However, thermal treatment or air-drying of avocado and products made thereof induces the development of an unpleasant off-taste: a pungent mouthfeel and lingering bitter aftertaste (Bates 1970). Thermal pasteurization is therefore no option for preservation of guacamole. In contrast, high-pressure processing works well; it inactivates enzymes and microorganisms at room temperature and therefore no color change occurs, the fresh taste is preserved, and the shelf life is extended to 4–6 weeks when stored refrigerated. For this product, high pressure has clearly an added value compared to conventional processing. This resulted in introduction of avocado-based high-pressure products on the market in, e.g., the United States, Europe, and South America.

24.2 Application Areas of High Pressure in Relation to Fruits and Vegetables

Depending on the pressure, time, and temperature conditions chosen, different applications can be defined of high pressure in relation to fruits and vegetables (see also Table 24.1). Details of the processing conditions and other important aspects of these applications can be found in other chapters of this book.

Table 24.1 Possible application areas of high pressure for fruits and vegetables

	Typical conditions	Application	Examples
High-pressure pasteurization	500–600 MPa, room temperature	Pasteurization of products, shelf life up to 4–6 weeks at 4 °C	Fruit juices, smoothies, vegetable mixes, ready-to-eat meals
Pressure-assisted thermal sterilization	700–900 MPa, elevated temperature	Commercial sterilization of products, shelf life up to 1 year at ambient conditions	Vegetables, ready-to-eat meals
Pressure shift freezing and thawing	0–200 MPa, freezing temperature	Freezing and thawing with preservation of texture	Individual fruit pieces, vegetables
High-pressure cell permeabilization	100–200 MPa, room temperature	Extraction of components at mild conditions	Extraction of vitamins
High-pressure texture modification	Depending on application	Forming of gels	Preparation of fruit jam, texture modification of vegetables

24.2.1 High-Pressure Pasteurization

At ambient temperatures, pressures in the range of 400–600 MPa reduce the number of vegetative microorganisms and inactivate enzymes involved in product spoilage while retaining the products' fresh characteristics. The main characteristics of high pressure for pasteurization are:

- Treatment times: 1–10 min high-pressure time at ambient temperature.
- Throughput: 1–2 tonnes per hour.
- Shelf life: >1 month in refrigerated chains.

As HP pasteurization has limited effects on microbial spores, refrigerated storage or additional preservation is necessary for ambient storage. Industrial equipment for HP pasteurization can be delivered by several suppliers and this pasteurization technique is widely implemented in the food industry.

An overview of industrial applications is presented in Fig. 24.1, showing that the majority of the high-pressure pasteurized products are vegetable products and fruit juices, such as guacamole, vegetable based meals, fruit salads, juices, salsa, and smoothies (Tonello 2011; Oey et al. 2008a).

24.2.2 Pressure-Assisted Thermal Sterilization (PATS)

During pressure-assisted thermal sterilization (PATS), products are pre-heated to 70–90 °C and thereafter pressurized to 800 MPa. Due to adiabatic heating, the temperature can rise to 120 °C. More technical description about the PATS technology is provided elsewhere in this book.

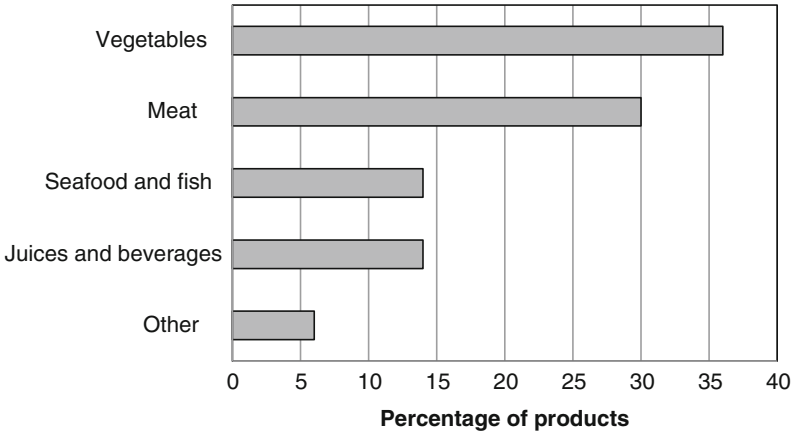

Fig. 24.1 High-pressure applications based on product categories (Source: Tonello 2011)

The main characteristics of high pressure for sterilization are:

- Treatment times: after preheating, <3 min at elevated temperature and pressure.
- Throughput: 80 L/h at the largest pilot-scale equipment currently available.
- Shelf lives up to months for ambient stable products.

At these conditions, enzymes and microorganisms including spores are inactivated, resulting in an ambient stable product. Until now, products have not been introduced onto the market as equipment for production is not available yet. However, high-pressure sterilized ambient stable products are considered as highly attractive (Wilson et al. 2008; Matser et al. 2004; Van der Plancken et al. 2012). Although not available on the market, FDA issued no objection to an industrial petition for pressure-assisted thermal sterilization of a potato puree (Sommerville and Balasubramaniam 2009). As high-pressure sterilization is relatively expensive, added value is important to make the process economically feasible. Products that are not attractive with classical thermal sterilization are good candidates, such as avocado puree for high-pressure pasteurization. Vervoort et al. (2012a) showed that high-pressure sterilization of carrots clearly has an advantage over conventional sterilization. Effects of high-pressure sterilization on the quality of basil (Krebbers et al. 2002a) and tomato puree (Krebbers et al. 2003) have been documented. It is expected that more studies will follow comparing the effects of thermal treatment and high-pressure treatment of fruits and vegetables.

24.2.3 High-Pressure Shift Freezing and Thawing

High-pressure shift freezing is a way to induce extensive super cooling of a product, by formation of small ice crystals after pressure release (0–200 MPa) at a temperature close to the freezing point. It induces instant, uniform ice nucleation simultaneously throughout the product, which has advantages over conventional freezing. Pressure shift freezing results in longer and uniform ice crystals. The main characteristics of this process are:

- Cooling of a product under pressure close to the freezing point, but products remain unfrozen.
- At uniform temperature: pressure release (rapidly or slowly) and ice formation starts at atmospheric pressure and under pressure.
- Small ice crystals resulting in better texture of food products compared to conventional freezing technologies.

The process has been extensively studied, but up to now no industrial applications are implemented (Otero and Sanz 2006). Effects on fruits and vegetables could be very interesting, as texture and color are usually better retained compared to conventional freezing processes. Applications to several fruits and vegetables have been investigated: potato cubes (Koch et al. 1996) carrots (Fuchigami et al. 1997), eggplant (Otero et al. 1998), peach and mango (Otero et al. 2000), and broccoli (Fernandez et al. 2006), showing all less cell damage, lower drip losses, and better texture than conventional freezing.

24.2.4 High Pressure for Cell Permeabilization

Relatively low pressures (100–200 MPa) cause crystallization of phospholipids in cell membranes, and as a result the permeability of the cell membrane increases and extraction of components is possible. The main characteristics of high pressure for cell permeabilization are:

– Relatively low pressures: 100–200 MPa at room temperature.
– High throughputs possible.
– Extraction of components.

Especially for fruits and vegetables, this can be an interesting application of high pressure. Extraction of, e.g., phenols from onion can be enhanced by pressure treatment at 100 MPa (Roldán-Marín et al. 2009). Gonzalez and Barrett (2010) suggest that these conditions can also be interesting for fruits and vegetables to improve the bioaccessibility and extraction of vitamins.

24.2.5 High Pressure for Product Modification

Several opportunities for product modification relevant for fruits and vegetables at pressures at 500–700 MPa are:

– Starch gelatinization and denaturation of proteins to prepare gels.
– Preparation of pectin gels (jams, texture modification of vegetables).

High pressure can be used instead of blanching as a pretreatment for other processing. Combination with calcium can be used to improve the texture of vegetables during pasteurization and sterilization (Sila et al. 2008).

As shown here, there is a whole range of applications of high pressure for fruits and vegetables possible, of which high-pressure pasteurization is most commonly applied in industry. The large number of fruit and vegetable products on the market treated with high pressure shows that high pressure in relation to fruits and vegetables is not only interesting from a scientific point of view but also for industrial applications.

24.3 Effects of High Pressure on Characteristics of Fruits and Vegetables

The effects of high pressure on quality aspects such as color, texture, taste, and composition of fruits and vegetables are highly depended on the conditions (pressure, time, temperature) chosen. It is therefore essential to keep the application area in mind when evaluating the effects of high pressure on the quality. Below an overview is given of the main effects of high pressure on fruits and vegetables, with main emphasis on pasteurization and sterilization. The inactivation of microorganisms by high pressure is not discussed here, as this is addressed in other chapters of this book.

24.3.1 High-Pressure Effects on Texture of Fruits and Vegetables

High-pressure treatments often result in good preservation of the texture of fruits and vegetables. When looking in more detail, several processes can be observed that are relevant for the description of the effects of high pressure on the texture of Fruits and vegetables, HP effects:

- *Flexibility of the Product to Withstand Compression.*
  During high-pressure treatment, the air present will be compressed to a very large extent, while water is only compressed for 10–20 % depending on the conditions used. As a result, products with large volumes of air will deform during pressure treatment. This can be observed in strawberries often possessing an internal air hole, resulting in a puree-like product after high-pressure treatment. In contrast, grapes or berries consisting mainly of water remain nearly entirely intact during high-pressure processing at room temperatures. Mushrooms will turn brown during HP pasteurization as a result of the compression or the air holes in the product and the subsequent enzymatic browning (Matser et al. 2000). The amount of air in the product combined with the flexibility of the product (e.g., properties of the skin) is important for the integrity and texture of the product after high-pressure treatment.

- *Permeabilization of Cell Membranes.*
  Due to the increased permeability of cell membranes after treatment, plant cells lose part of their turgor and a softer texture is observed. Crispiness of raw products is often decreased by high-pressure treatment at room temperature. Luscher et al. (2005) showed that for potato tissue, the turgor loss as a result of membrane changes negatively influences the texture. For carrot, potato, and red radish, Park et al. (2013) showed that the electrical conductivity increases after high-pressure treatment due to permeabilization of membranes and that this can be linked to a decrease in crunchiness.

- *Gelatinization of Starch.*
  Starch will gelatinize at pressures above 500 MPa resulting in an obvious change in the texture. For fruits and vegetables containing a large amount of starch, this can influence the texture of the product.

- *Changes in Cell Wall Polymers.*
  Both enzymatic and nonenzymatic reactions are important for the texture in fruit and vegetable products based on cell wall polymers like pectin. Extensive research on this topic has been done by the group of professor Hendrickx of the University in Leuven, showing the effect of pressure-temperature-time combinations on enzymes responsible for pectin degradation and the formation and deformation of pectin networks (Van den Broeck 2000; Sila et al. 2008; Van der Plancken et al. 2012).

  Although the texture of fruits and vegetables is often well preserved during high-pressure treatments at room temperature, especially changes in cell wall

polymers are very important for the exact effects of high pressure on texture of fruits and vegetables. For pressure-assisted thermal sterilization, the additional effect of temperature is important, especially for the effects on cell wall polymers.

24.3.2 High-Pressure Effects on Color of Fruits and Vegetables

Most of the pigments responsible for the color of fruits and vegetables are relatively stable under high-pressure treatment at room temperature. For example, carotenoids responsible for the orange color of carrots remain unaffected at high-pressure pasteurization conditions. Anthocyanins responsible for the red color of strawberries are relatively stable during HP processing at ambient temperatures (Van der Plancken et al. 2012). This is also the case for many other yellow and red colors. This is different when high temperature is combined with high pressure during sterilization treatments, as the high temperature will affect these colors, resulting in a more brownish color.

The green color of vegetables like green beans or spinach is a result of their chlorophyll content, which is highly pressure stable at ambient conditions. Green vegetables that are treated at high-pressure pasteurization conditions often have a more intense green color than the untreated product, possibly caused by leakage of chlorophyll into the intercellular space due to cell disruption during pressure treatment (Krebbers et al. 2002b). During intense heat treatment, the green color of vegetables like green beans is turning into a more olive-green color. This is also the case during pressure-assisted thermal sterilization treatment, due to degradation of chlorophyll (Oey et al. 2008a).

The color of fruits and vegetables can also change due to chemical or enzymatic oxidation processes. The enzyme polyphenoloxidase is responsible for the browning of sliced apples. High-pressure treatment at pasteurization conditions can result in an increase in enzymatic browning due to activation of this enzyme and increased contact between substrate and enzyme due to the increase in cell permeabilization. As polyphenoloxidase is relatively pressure stable, elevated pressures are necessary to inactivate this enzyme completely. Often a residual enzyme activity is present after industrial high-pressure pasteurization. Cold storage retards the activity of this enzyme and therefore decreases the rate of browning. Remaining activities of other enzymes are often influencing the color of fruits and vegetables during storage, for example, glucosidases are influencing anthocyanins (Oey et al. 2008a).

24.3.3 High-Pressure Effects on Flavor and Taste of Fruits and Vegetables

High-pressure treatment effects on flavor and taste are determined by instrumental methods, like GC analysis of flavor components and sometimes also by trained sensory panels that judge the sensorial effects of fruits and vegetables. The number of

publications showing results of a sensory panel is relatively scarce. However, sensorial evaluation of high-pressure-treated products by trained experts is extremely valuable for determining the feasibility of this technology for a specific product. Experts from industrial producers often observe very small changes in the taste balance of a product that are sometimes difficult to determine with analytical methods.

In general, small molecules, responsible for flavor and taste, are not much influenced by high-pressure pasteurization conditions resulting in preservation of the fresh taste of the product. However, enzymatic reactions and texture changes also influence the taste of the product, and therefore changes are observed between treated and untreated products. Laboissièrea et al. (2007) showed that the sensorial quality of passion fruit juices is not influenced by high pressure when compared to untreated products. Pressure-treated products were much better when compared to conventional heat treated product. Vegetable products, for example, have been evaluated by Ximenita et al. (2009). They evaluated the sensorial quality of carrots after different treatments showing that sweetness, flavor, and crunchy texture are well preserved. Furthermore, high-pressure-treated carrots showed a significantly higher intensity perception of the orange color and fibrousnesses compared to carrots subjected to heat treatments, while similar brightness to cooked carrots and odor to raw carrots were observed. This also shows that high-pressure pasteurization can result in some product attributes that are similar to those of raw products, while other attributes resemble more those of cooked products.

24.3.4 High-Pressure Effects on Vitamins and Chemical Composition

High-pressure treatment at room temperature has a limited effect on vitamin content of fruits and vegetables (Oey et al. 2008b). Many researchers investigated the effect of pressure on ascorbic acid, showing that at room temperature, this vitamin is relatively stable in fruit and vegetable products. At high temperature ascorbic acid is degraded resulting in a considerable loss during high-pressure sterilization treatment. Important for the stability is the amount of oxygen present, as oxidation is the major cause of degradation. Eliminating oxygen is therefore a good measure to reduce the degradation of this vitamin. Also other water-soluble vitamins like B1, B2, B6, niacin, and folates (vitamin B9) are relatively stable under high-pressure pasteurization conditions. In general, these vitamins are much better preserved by high-pressure pasteurization than in a conventional thermal pasteurization process. Information on the effect of high-pressure conditions of fat-soluble vitamins (A, D, E, K) is less abundant than for water soluble vitamins. For these vitamins, it is observed that high-pressure pasteurization better preserves these vitamins than conventional processing (Oey et al. 2008b). In some cases an increase in the concentration of these vitamins is observed, probably due to enhanced extraction by high pressure. An example is the increase in lycopene content in tomato puree observed by (Krebbers et al. 2003). In addition to the concentration of the vitamins, the

bioavailability can also be influenced by high-pressure processing resulting in, for example, vegetables with higher folate bioaccessibility (Oey et al. 2008b).

Combination of high pressure with high temperature in high-pressure sterilization treatments, results in a much higher degradation of vitamins than high-pressure pasteurization. Fat-soluble vitamins are less sensitive to combined pressure-heat treatments than water-soluble vitamins (Oey et al. 2008b). However, due to shorter treatment times compared to conventional retort sterilization, high-pressure sterilization often results in a better preservation of these vitamins (Van der Plancken et al. 2012).
In addition to the research on the effect of high pressure on specific components, fingerprinting techniques are used as a non-targeted approach for determining the effect of, e.g., novel processing on the chemical composition of products. Vervoort et al. (2012b, 2013) used headspace GC-MS fingerprinting for the evaluation of high-pressure pasteurized orange juice and high-pressure pasteurized and sterilized carrots. This technology revealed no differences in chemical composition between high-pressure-treated orange juice and untreated juice. Combining high pressure with high temperature resulted for carrots in some changes that can also be observed during heat treatments.

24.4 Conclusion

High pressure is a very attractive technology for preservation of fruit and vegetable products. Especially high-pressure treatment at room temperature is interesting for pasteurization of a range of fruits and vegetables, resulting in products with a shelf life of 4–6 weeks when stored refrigerated. For high-pressure pasteurization, extensive scientific studies are available showing the effect on shelf life, quality, nutrients, and sensorial aspects. In addition to this, high pressure is potentially also interesting under other conditions. The literature on the effects of high-pressure/high-temperature conditions is increasing and shows the potential of these conditions for sterilization of packed food products. For subzero applications of high pressure and applications at relatively low pressure, relatively few publications are available, while this can be also very interesting for fruits and vegetables.

When considering a specific application of high pressure for fruits and vegetables, it is advised to start at the one hand with a feasibility study to determine the effect of the selected conditions on sensorial attributes and at the other hand with a literature search on the specific product. This can be the starting point of more detailed research and industrial application of high-pressure processing.

Feasibility studies will give an indication of the potential of high-pressure products towards shelf life and product quality, as well as estimating process requirements, costs, and gaps in existing knowledge. If these feasibility studies are successful, future studies can be done including specific process conditions for optimizing shelf life and quality of targeted products, shelf life with respect to specific microorganisms and enzymes, pre-processing and packaging requirements, commercial processing and equipment requirements, and a detailed cost-benefit analysis across the supply chain including processing.

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Chapter 25
Processing of Dairy Products Utilizing High Pressure

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Abstract Current knowledge of the main changes induced in milk (including goat, ewe, and buffalo milks) and milk products when treated by high hydrostatic pressure (HHP) is presented. The effects of HHP on casein micelles, whey proteins, lipids, indigenous enzymes, mineral equilibrium, and microorganisms are described. The significance of these effects on the technological properties of milk, particularly in cheese- and yogurt-making applications, and functional properties is also discussed.

Keywords High pressure • Dairy product • Microbial safety • Chemical changes

25.1 Introduction

Milk was one of the first foods to be subjected to high hydrostatic pressure (HHP) in 1897, when Bert H. Hite, a researcher working at the Agricultural Experiment Station, University of West Virginia, began to study the effects of HHP on food preservation. However, at that time, it was technically impossible to work at the industrial level, so there was reduced interest in such treatment. Nowadays, a number of industrially relevant applications exist for fruit juices, meat, and fish (Rastogi et al. 2007), but there are few applications for dairy products, although patents for milk and dairy products have been published, showing the industrial relevance of this technology.

One reason for this lack of application for dairy products might be that high hydrostatic pressure (HHP) treatment affects many constituents of milk, especially protein and mineral equilibrium, and induces changes in the functional properties of such products (Trujillo et al. 1997, 2002; Huppertz et al. 2002, 2006b; Needs 2002; López-Fandiño 2006).

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Current knowledge of the main changes induced in milks (including goat, ewe, and buffalo) and milk products when treated by HHP is presented in this chapter. The effects of HHP on casein micelles, whey proteins, lipids, indigenous enzymes, mineral equilibrium, and microorganisms are described. The significance of these effects on the technological and functional properties of milk, particularly in cheese and yogurt making, is also discussed.

25.2 Effects of HHP on Milk Constituents

25.2.1 Effects of HHP on Casein Micelles

Casein micelles contain four protein species, α_{s1}-, α_{s2}-, β-, and κ-casein (CN), which are bound together by amorphous calcium phosphate and by hydrophobic interactions. These are hydrophobic, phosphorylated proteins, always occurring as large, polydisperse aggregates in aqueous solution at ambient temperature with neutral pH.

Considerable research has been carried out on the effects of HHP on casein micelles using model systems and in milk. HHP treatment induces disruption of casein micelles and dissociation of caseins from the micelle, increasing the level of non-micellar caseins; two main mechanisms are possibly responsible: the solubilization of micellar calcium phosphate and the disruption of intramicellar hydrophobic and electrostatic interactions (Schrader et al. 1997; Needs et al. 2000a; Huppertz et al. 2004a, d, f). Electron microscopy images have indicated that micellar substructures are similar for pressure-modified casein micelles and casein micelles in untreated milk (Knudsen and Skibsted 2010).

Increases in micelle size depend on treatment temperature, time, milk pH, HHP cycles, and the presence of hydrophobic solvents (Gaucheron et al. 1997; Huppertz et al. 2004d, f).

HHP treatment increases the hydration of casein micelles, due to the association of denatured beta-lactoglobulin (β-LG) with the casein micelles, which increases the net negative charge of micelles enhancing micellar solvation, and by the HHP-induced disruption of casein micelles into smaller units, which also increases micellar hydration (Gaucheron et al. 1997; Huppertz et al. 2004a).

HHP-induced increase of non-micellar caseins is affected by pH; HHP treatment of acidified milk ($pH \leq 6$) and at $pH = 7$ increases the level of soluble caseins related to that found at $pH = 6.7$, probably due to destruction of colloidal structure or enhanced electrostatic repulsion, respectively (Arias et al. 2000).

25.2.2 Effects of HHP on Whey Proteins

Unlike caseins, whey proteins are classic globular proteins with a tight tertiary structure, occurring in milk as monomers or oligomers. Two of these proteins are dominant, β-LG and α-lactalbumin (α-LA), although several other proteins are also

present in milk: bovine serum albumin (BSA), immunoglobulins, lactoferrin, and various enzymes.

β-LG is denatured (estimated by the loss of solubility) in milk by pressure over 100 MPa; however, α-LA and BSA appear to be completely resistant to pressures up to 400–500 MPa. Pressurization of milk for 30 min at 200 and 300 MPa brought about a 20 % and 80 % denaturation of β-LG, respectively, but little further denaturation occurs at 400–800 MPa (López-Fandiño et al. 1996; García-Risco et al. 2000; Scollard et al. 2000b). A pressure of 600 MPa for 15–30 min denatures between 15 and 33 % of α-LA (Needs et al. 2000a; Huppertz et al. 2004c).

The higher-pressure resistance of α-LA and BSA than β-LG has been related to the more rigid molecular structure of the former, due to the presence of a higher number of intramolecular disulfide bonds (4 and 17 compared to 2) and to the free sulfhydryl group of β-LG, which can act as an initiator in sulfhydryl-oxidation or sulfhydryl-disulfide interchange reactions (López-Fandiño et al. 1996; Gaucheron et al. 1997).

The majority of denatured β-LG in HP-treated milk is associated with casein micelles, with a small proportion remaining nonsedimentable during ultracentrifugal operation, either in the form of whey protein aggregates or associated with very small casein particles (Felipe et al. 1997; Huppertz et al. 2004d). The free sulfhydryl group of β-LG can interact with other proteins such as κ-casein, α-LA, β-LG, and α_{s2}-CN and also with proteins associated with the milk fat globule membrane through sulfhydryl-disulfide interchange reactions (Huppertz et al. 2004c; Ye et al. 2004; Considine et al. 2007).

The extent of denaturation by HHP of α-LA and β-LG is dependent on different factors; it increases with increasing treatment time (López-Fandiño et al. 1996; Scollard et al. 2000b; Huppertz et al. 2004d), temperature (Gaucheron et al. 1997; García-Risco et al. 2000; Huppertz et al. 2004d), and milk pH (Arias et al. 2000) but is reduced by diminishing the level of micellar calcium phosphate in the milk (Huppertz et al. 2004c). Furthermore, denaturation of these proteins is prevented by adding a sulfhydryl-blocking agent to milk prior to pressurization (Huppertz et al. 2004c), and denaturation of β-LG is enhanced by the addition of a sulfhydryl-oxidizing agent (KIO_3) but is reduced in the case of α-LA (Huppertz et al. 2004c; Zobrist et al. 2005).

HHP-induced denaturation of whey proteins from species other than bovine has received little attention. There are differences in whey protein denaturation by HHP from milk of different species; ewe, goat and buffalo β-Lg denaturation by HHP at 250 MPa occurs at a faster rate than that of cow β-LG in the order buffalo > ovine > caprine > cow (Felipe et al. 1997; López-Fandiño and Olano 1998; Huppertz et al. 2005b, 2006a). In addition, the level of α-La denatured in buffalo milk after treatment of 800 MPa (~90 %) is higher than that in bovine milk treated at the same pressure (~70 %; Huppertz et al. 2005b).

Few data are available on HHP-induced denaturation of other whey proteins. No differences were observed by Felipe et al. (1997) in levels of immunoglobulins (IGs) in goat milk up to 300 MPa, but some aggregation occurred between 400 and 500 MPa (~35 %). Tonello et al. (1992) and Trujillo et al. (2007) determined that

IGs in bovine and caprine colostrums treated up to 200 and 400–500 MPa showed partial damage (~12 % and 19–38 %, respectively). Viazis et al. (2007) and Permanyer et al. (2010) investigated the effects of HHP (400–600 MPa) and pasteurization (62.5 °C, 30 min) on total IG A and lysozyme activities in human milk, showing that HHP-treated human milk retained higher levels of IG A and lysozyme activities compared to heat-treated samples (100–75 % retention versus 51 % and 96–100 % versus 79, respectively). More recently, Mayayo et al. (2014) showed that HP treatment of human milk at pressures of 300–600 MPa for 30 min resulted in retention of immunoreactive lactoferrin concentration of 90–52 %. In contrast, LTLT treatment that is usually applied in human milk banks retained only 20 % of immunoreactive lactoferrin. These data suggest that HHP is a potential alternative to thermal pasteurization of human milk banking that can provide greater retention of some bioactive components.

25.2.3 Effects of HHP on Mineral Equilibria

Milk salts have been recognized as playing a major role in determining the stability of milk to heat treatments. Several authors have investigated the effects of HHP treatment on the distribution of minerals between colloidal and diffusible phases and on the level of mineral ionization. It has been reported that HHP treatment increases (López-Fandiño et al. 1998; Zobrist et al. 2005) or does not increase (Johnston et al. 1992; De la Fuente et al. 1999) the concentration of ionic calcium in milk. According to Zobrist et al. (2005) these differences could be explained by the fact that HHP-induced ionization of calcium is a reversible process on subsequent milk storage.

Desobry-Banon et al. (1994) showed that HHP-induced disintegration of the casein micelles is accompanied by an increase in the levels of diffusible calcium and phosphate from colloidal phase. HHP treatment also increases the levels of diffusible salts in milks from non-bovine species such as ewe, goat, and buffalo (Law et al. 1998; López-Fandiño et al. 1998; Huppertz et al. 2005b).

25.2.4 Effects of HHP on Milk Fat Globules

Milk fat globule size is not affected by HHP treatment (Kanno et al. 1998; Huppertz et al. 2003; Ye et al. 2004), although Kanno et al. (1998) described an increase in the mean diameter and modifications in the size distribution of milk fat globules at pressures between 400 and 800 MPa. Gervilla et al. (2001) showed that the distribution of milk fat globules in ewe milk is modified by HHP up to 500 MPa. Pressure treatment at 25 and 50 °C tended to increase the number of small globules in the range of 1–2 μm, while at 4 °C the tendency was the opposite. However, the milk fat

globule membrane (MFGM) was not damaged, as the lack of lipolysis increased during storage of milk at 4 °C.

During HHP treatment (100–800 MPa), some denatured whey proteins associated with milk MFGM via disulfide bonds could be seen; β-LG was observed in the MFGM material isolated from milk treated at 100–800 MPa for 30 min, and small amounts of α-LA and κ-CN were also observed at pressures ≥700 and 500 MPa, respectively. Of the major original MFGM proteins, xanthine oxidase and butyrophilin are the major proteins involved in HHP-induced interaction with β-LG, but no change in butyrophilin content was observed during HHP treatment of whole milk, whereas xanthine oxidase was reduced to some extent beyond 400 MPa (Ye et al. 2004).

25.2.5 Effects of HHP on Lactose

Lactose in milk and in milk products may isomerize in lactulose by heating and then degrade to form acids and other sugars. No changes in these compounds are observed after pressurization, suggesting that neither Maillard reaction nor lactose isomerization occurs in milk after pressure treatment (López-Fandiño et al. 1996).

25.2.6 Effects of HHP on Milk Enzymes

Significant technological applications of some milk indigenous enzymes include flavor, texture, and stability in milk and dairy products. Because of the relative economic significance of various enzymes in milk, their stability when treated with HHP and their possible use as markers of the severity of treatment have been investigated. Most indigenous milk enzymes are quite resistant to moderate pressures (up to 400 MPa), and the resistance of different milk enzymes is correlated to their structures, as shown by Rademacher et al. (1999), who indicated the following order of enzyme resistance: phosphohexoisomerase < γ-glutamyltransferase < alkaline phosphatase < lactoperoxidase.

Some studies about the effect of HHP treatment on milk indigenous enzymes are given in Table 25.1.

According to Rademacher and Hinrichs (2006), regarding quality control, the kinetics of inactivation of γ-glutamyltransferase at 20 °C and pressures above 500 MPa are sufficiently close to the inactivation of *L. monocytogenes* and *E. coli* and may therefore provide a useful process marker for the destruction of these organisms and could be considered as a process marker in pressurized milk. On the other hand, xanthine oxidase has been also proposed as an indicator of the HHP treatment of milk. This enzyme is resistant at 400 MPa at 25 °C but is inactivated at higher pressures following a first-order kinetics (Olsen et al. 2004).

Table 25.1 Overview of the effects of high hydrostatic pressure on milk enzymes

Enzyme	Medium	HHP conditions	T^a (°C)	Inactivation (%)	Reference
Acid phosphatase	Raw and skim milks, acid and rennet wheys	200–800 MPa, 50 min	25	0–85	Balci et al. (2002)
Alkaline phosphatase	Raw milk	400 MPa, 60 min	20	0	López-Fandiño et al. (1996)
	Tris buffer (pH = 7) and milk	600 MPa, 30 min	55	90 (buffer)	Seyderhelm et al. (1996)
				10 (milk)	
	Goat milk	500 MPa, 10 min	20	0	Felipe et al. (1997)
	Raw milk	500 MPa, 90 min	25 or 50	50	Rademacher et al. (1998)
		600 MPa, 10 min		100	
	Raw milk	0.1–725 MPa	25–63	14–300 min	Ludikhuyze et al. (2000)
Cathepsin D	Ewe milk	200, 450, 650 MPa, 10 min	20, 40, 55	0–58	Moatsou et al. (2008)
γ-Glutamyltransferase	Raw milk	400 MPa	20–25	0	Rademacher et al. (1999)
	Raw milk	300–400 MPa, 0–180 min	3	15	Pandey and Ramaswamy (2004)
				35 activation	
Lactoperoxidase	Raw milk	400 MPa, 60 min	20	0	López-Fandiño et al. (1996)
	Tris buffer (pH = 7) and raw milk	600 MPa, 2 min	25	70	Seyderhelm et al. (1996)
		600 MPa, 5–30 min	25 or 50	30	
	Raw milk and acid whey	150–750 MPa, 0–140 min	15–73	0–25	Ludikhuyze et al. (2001)
				Antagonistic effect of T^a	
Lipase	Raw milk	Up to 600 MPa, 15 min	20	0	Kolakowski et al. (1997a)
		1000 MPa, 15 min	20	6–23	
	Raw milk	300–400 MPa, 0–100 min	3	133 activation	Pandey and Ramaswamy (2004)
Phosphohexose isomerase	Raw milk	400 MPa	20–25	0	Rademacher et al. (1999)

Plasmin	Goat milk	400 MPa, 10 min	2	0	Trujillo et al. (1997)
	Raw milk	400 MPa, 30 min	25	0	García-Risco et al. (1998)
	Raw milk	400 MPa, 15 min	60	86.5	García-Risco et al. (2000)
	Phosphate buffer (pH = 6.7) + sodium caseinate	Up to 600 MPa, 20 min	20	0	Scollard et al. (2000a)
	Phosphate buffer (pH = 6.7) + sodium caseinate + β-lactoglobulin	50–800 MPa, 1–30 min	20	0–85	Scollard et al. (2000b)
	Raw milk	400, 600 MPa, 30 min	20	30, 75	Huppertz et al. (2004f)
	Phosphate buffer (pH = 6.6)	300–800 MPa, 0–60 min	25–65	Stable at room T^a Inactivation 300–600	Borda et al. (2004)
				Antagonistic effect of T^a at >600 MPa	
	Ewe milk	200, 450, 650 MPa, 10 min	20, 40, 55	0–77	Moatsou et al. (2008)
Protease from *Bacillus subtilis*	Raw, pasteurized, and homogenized milk	300–450, 600 MPa, 0–15 min	40, 50, 60	0–35	Bilbao-Sáinz et al. (2009)
Xanthine oxidase	Raw milk	0–600 MPa	20		Olsen et al. (2004)

25.2.7 Effects of HHP on Minor Components of Milk

The effect of HHP on hydrosoluble vitamins (B_1, B_6, and C) in a multivitamin model system and in raw milk has been studied. Treatment of raw milk at 400 MPa for 30 min at 25 °C resulted in no significant losses in vitamins B_1 and B_6 (Sierra et al. 2000). Minor variations have also been found in vitamins after pressurization (200–600 MPa for 30 min at room temperature) of a multivitamin model system. Vitamins B_1 and B_6 undergo no significant losses after treatment, but vitamin C levels, although significant, are not dependent on the intensity of the HHP process (Sancho et al. 1999). More recently, Moltó-Puigmartí et al. (2010) studied the ability of HPP to maintain fatty acid, vitamin C, and vitamin E contents of human milk. Fatty acid proportions in milk, as well as levels of δ-, γ-, and α-tocopherols, did not vary with any of the treatments. Total vitamin C and ascorbic acid levels were maintained after HPP.

The effect of HHP at moderate-temperature processing on the volatile profile of milk, the kinetics of volatile formation in milk subjected to pressure-assisted thermal treatments, and the antioxidant impacts on volatile formation in HHP-treated milk have been investigated under different pressures (482, 586, 620, and 655 MPa), temperatures (25–75 °C), and holding times (1–10 min) and compared to pasteurization treatment (Vazquez-Landaverde et al. 2006, 2007; Vazquez-Landaverde and Qian 2007). These authors showed that heat treatment tends to promote the formation of methanethiol, hydrogen sulfide, methyl ketones, and aldehydes, whereas HHP treatment favors the formation of hydrogen sulfide and aldehydes. BHA and epicatechin, and ascorbic acid and β-carotene, to a lesser extent, are able to effectively inhibit aldehyde formation. On the other hand, pressure-assisted thermal treatments inhibit the formation of volatile sulfur compounds reported to be factors in consumer rejection of cooked milk flavor.

25.2.8 Changes in Characteristics of Milk Due to HHP-Induced Modifications in Milk Constituents

One effect of HHP on milk, caused by the destruction of the colloidal structure into smaller structures, is change in the visual appearance of milk, which becomes translucent and green yellow (Johnston et al. 1992; García-Risco et al. 2000; Needs et al. 2000a). Reduction in micellar size causes milk to lose its ability to scatter light, becoming translucent; this effect is particularly strong in the case of skim milk, since fat globules have light scattering properties themselves, although the effect is dependent on pressure and temperature of the treatment. In this case, skim milk HHP treated at 400 MPa for 15 min at 50 °C is not visually distinguished by sensory analysis compared to untreated milk, and these samples are preferred by taste-test panelists because of their smoother and creamier taste (García-Risco et al. 2000). This is consistent with the increase in micellar size occurring in these conditions, which increases light scattering of milk.

Creaming of raw whole bovine milk at refrigeration temperatures is generally regarded as an undesirable phenomenon; traditionally, creaming is prevented by homogenizing the milk. HHP processing at pressures ≤250 MPa increases the rate and level of creaming, an aspect that could be used to improve cream separation in the production of butter, whereas treatment at >400 MPa reduces both of these parameters, resulting in more stable milks during storage. The amount of milk protein associated with MFGM during HHP treatment and HP-induced aggregation and denaturation of agglutinins and lipoproteins may explain the changes in creaming characteristics of milk reducing rate and level of creaming (Gervilla et al. 2001; Huppertz et al. 2003).

HHP processing increases the viscosity of skim milk, and it could be related to the effect of HHP on casein micelles, e.g., disruption of casein micelles and reduction in particle size, and HP-induced increases in hydration of casein micelles, increasing voluminosity of the micelles, which should also increase the viscosity of milk (Huppertz et al. 2003).

HHP had little overall effect on heat stability of raw, preheated, or serum protein-free skim milk at pH values in the range 6.1–7.0. This result may possibly be related to reversibility of HP-induced changes in milk, either on subsequent storage or on severe heating of milk during determination of heat stability. However, HHP treatment at 600 MPa considerably increases heat stability of concentrated skim milk at pH values >6.7, which may be related to the formation of stabilizing κ-CN/β-LG complexes on the micelle surface (Huppertz et al. 2004c, d).

Ethanol stability of raw skim milk is reduced by HHP treatment, although it is partially reversible on subsequent storage for up to 24 h at 5 °C (Johnston et al. 2002b; Huppertz et al. 2004g). Increases in the level of soluble Ca and the dissociation of κ-CN from the micelle, which reduces steric stability of casein micelles, could facilitate the ethanol-mediated coagulation in HHP-treated milk (Huppertz et al. 2004g).

HHP-treated milk undergoes proteolysis after treatment during storage depending on the pressure and temperature of the treatment applied and the storing temperature. The level of proteolysis in HHP-treated milk at 100–400 MPa at room temperature and in untreated milk was similar during refrigerated storage (García-Risco et al. 2003), although some proteolysis of milk treated at 400 MPa for 30 min and then held at 5 °C was observed by Huppertz et al. (2004e) in comparison to untreated milk. According to García-Risco et al. (2003), although pressure conditions assayed does not lead to great plasmin inactivation (~20 %), it is likely that serum-liberated enzymes become more vulnerable to the action of proteinase inhibitors normally found in the soluble fraction, thus counteracting the enhanced susceptibility of caseins to the enzyme due to micellar disruption and protein solubilization, resulting in proteolysis levels on refrigerated storage similar to those of untreated milk.

However, when HHP-treated milk (300–400 MPa for 30 min) is stored at 37 °C, proteolysis of milk increases as a result of combination of disruption of casein micelles, increased availability of substrate to plasmin, and low inactivation of plasmin (Scollard et al. 2000b; Huppertz et al. 2004e). In contrast, proteolysis is considerably reduced if milk is HHP treated at 600 MPa for 30 min at room temperature, probably due to reduced plasmin activity after treatment (Scollard et al. 2000b).

25.3 Effects of HHP on Milk Microorganisms

25.3.1 Effect of HHP on Milk Shelf Life

Interest in high hydrostatic pressure (HHP) for treatment of milk has increased, mainly due to the possibility of reducing the number of spoiling and pathogenic microorganisms without causing significant effects on flavor and nutritional value. Several studies demonstrated that milk pressurized at 400–600 MPa by 10–60 min presents a microbiological quality comparable to that submitted to standard thermal pasteurization (72 °C during 15 s), but not to sterilized milk (UHT or similar) due to high resistance of bacterial spores to high pressures (Kolakowski et al. 1997b; Mussa and Ramaswamy 1997; Buffa et al. 2001a). Attempts to increase the lethal effect over bacterial spores by combining HHP with mild temperatures were not completely satisfactory on milk (Scurrah et al. 2006).

Molina-Hoppner et al. (2004) reported that HHP treatments at 300 MPa for 5 min on *L. lactis* ssp. *cremoris* MG1363 reduced metabolic activity to 10–12 % with respect to untreated microorganisms; after 12 min of treatment, cells did not show any metabolic activity. Gervilla et al. (1999) reported for *L. helveticus* that pressure treatments were more effective at low (2 and 10 °C) and moderately high (50 °C) temperatures than at room temperature (25 °C).

Bacteria are expected to be injured or inactivated by HHP, depending on the pressure level, species, and strain of the microorganism and subsequent storage conditions. Sublethal injured bacteria may be able to repair in a medium containing necessary nutrients under conditions of optimum pH and temperature, affecting quality and safety, especially in nutritive and low-acid food products such as milk. Two types of injury were described after HHP treatments by Bozoglu et al. (2004): I1 and I2. I2-type injury is a major injury, and after its repair (I2 to I1), cells can form colonies on nonselective but not on selective agar. Therefore, it is imperative that shelf life studies must be conducted over a period of time for potential repair of I2-type injury either to detectable injury (I1) or to active cells (AC) to ascertain the microbiological safety of low-acid food products, such as milk. During storage at low temperatures, milk retains optimum conditions to repair psychrotrophic microorganisms, such as *L. monocytogenes*, *Yersinia enterocolitica*, and *Pseudomonas* spp. (Bozoglu et al. 2004; De Lamo-Castellví et al. 2005).

25.3.2 Effect of HHP on Main Food-Borne Pathogens Present in Milk

25.3.2.1 Bacillus cereus

Bacillus cereus spores most often enter milk from water, soil, feces, bedding, cattle feed, milkstone deposits on farm bulk tanks, pumps, pipelines, gaskets, processing equipment, and packing material of the dairy industry, as well as from udders during

milking or as a result of mastitis (Meer et al. 1991; Andersson et al. 1995), but values of *B. cereus* usually reported in pasteurized milk are less than 3 log cfu/ml (Van Netten et al. 1990; Lin et al. 1998). The amount of viable cells or spores to form enterotoxins seems to vary between about 5 and 7 logs, partly due to large differences in the amount of enterotoxins produced by different strains (Granum 1994; Granum and Lund 1997). López-Pedemonte et al. (2003a) studied the effect of HHP (300, 400, or 500 MPa at 30 °C during 15 min) on inactivation of spores of *B. cereus* ATCC 9139 inoculated into cheese made of raw cow's milk with and without a previous germination cycle of 60 MPa at 30 °C for 210 min, observing that adding the germinative cycle resulted in higher efficiency when applied with a 500 MPa HHP treatment; however, maximum reduction achieved was only about 2.0 log cfu/ml. Van Opstal et al. (2004) identified two possible approaches to inactivate spores of *B. cereus* in milk: a single-step treatment at 500 MPa and 60 °C for 30 min and a two-step treatment consisting of 30 min at 200 MPa and 45 °C to induce spore germination, followed by mild-heat treatment at 60 °C for 10 min to inactivate germinated spores. Both treatments achieved a ≥6 log inactivation, but a small fraction of spores always remained ungerminated. Further, not all germinated spores were inactivated by pressure treatment, even under the most severe conditions, probably due to the existence of a fraction of superdormant spores that resist germination under high pressure.

This combined effect with lysozyme and/or nisin has also been suggested for increasing the lethal effect of HHP. López-Pedemonte et al. (2003b) studied the combined effect of HHP and nisin or lysozyme on the inactivation of spores of *B. cereus* (ATCC 9139) in model cheeses made of raw milk submitted to a germination cycle of 60 MPa at 30 °C for 210 min, to a vegetative cell destruction cycle of 300 or 400 MPa at 30 °C for 15 min, or to both treatments. The combination of both cycles improved the efficiency of the whole treatment, obtaining the highest inactivation (2.4 ± 0.1 log cfu/g) when the second pressure cycle of 400 MPa was applied with the presence of nisin (1.56 mg/l of milk), whereas lysozyme (22.4 mg/l of milk) did not increase sensitivity of the spores to HHP. Black et al. (2008) investigated the germination and inactivation of spores of *B. cereus* suspended in milk. Treating four strains of *B. cereus* at 500 MPa for 5 min twice at 40 °C in the presence of 500 IU/ml nisin proved to be less effective at inactivating the spores compared with *B. subtilis*, where a log reduction of 5.9 was obtained when nisin was added to milk prior to HHP treatment.

25.3.2.2 Escherichia coli

Different types of *E. coli* have been reported as a cause of food-borne diseases. *E. coli* O157:H7 has emerged as a food-borne pathogen of major concern for the food industry due to its ability to cause severe illness, particularly in children. Dairy cattle are considered to be the main reservoir of *E. coli* O157:H7 for human infection (Weeratna and Doyle 1991), with fecal contamination of milk being an important vehicle for its transmission (Borczyk et al. 1987; Gonzalez 2002). Patterson and Kilpatrick (1998) used different combinations of pressures, temperatures, and

times to eliminate the population of *E. coli* O157:H7 inoculated in UHT milk. Population was reduced by 5 log cfu/ml when milk was treated at 400 MPa for 15 min at 50 °C. Linton et al. (2001) reported only a 4 log (cfu/ml) reduction of the two most resistant strains (NCTC 11601 and NCTC 9706) after applying 500 MPa for 40 min. Nevertheless, no survivors of either strain could be detected after an HHP treatment of 600 MPa for 30 min, which would mean a reduction above 7 log cfu/ml.

Dogan and Erkmen (2003) achieved complete inactivation of *E. coli* inoculated in raw milk (ranging from 6.14 to 6.98 log cfu/ml) with HHP treatment of 600 MPa for 30 min. The required time to achieve this goal in milk was significantly higher than in other matrices, like peach and orange juices, where 12 and 10 min were needed, respectively. A protective effect has been described for skim milk on HHP-mediated inactivation and injury of *E. coli.* However, protein fractions derived from skim milk (casein, whey, globulin, and albumin) did not exhibit this protective effect. Microscopy analysis by DAPI/PI staining indicated that some cells were localized in the solid portion of skim milk and that this would protect those cells from the effect of HHP (Narisawa et al. 2008).

25.3.2.3 Listeria monocytogenes

Outbreaks of listeriosis have often been related to the consumption of raw and pasteurized milk, sour milk, chocolate milk, butter, and ice cream. Its presence in milk-based products can be a result of either raw milk direct contamination from dairy cattle, reaching concentrations above 3 log cfu/ml (Sanaa et al. 2004), or post-processing contamination in the case of dairy products like cheese (Borucki et al. 2004; Carminati et al. 2004).

Styles et al. (1991) reported inactivation above 6 log cfu/ml of *L. monocytogenes* Scott A after treating inoculated UHT milk samples at 340 MPa for 80 min. Mussa et al. (1999) observed that *L. monocytogenes* Scott A was more pressure resistant than the natural microbiota of raw milk after applying HHP treatments from 150 to 350 MPa during 0 to 120 min. Erkmen and Dogan (2004) described reductions of about 2.09 and 2.76 log cfu/ml in aerobic bacteria and *L. monocytogenes*, respectively, after 10-min pressure treatment at 400 MPa in raw milk, increasing to 5.09 and 6.47 log cfu/ml, respectively, at 600 MPa.

Chen and Hoover (2003) studied HHP inactivation of *L. monocytogenes* Scott A in whole milk combining higher pressures and temperatures (400 and 500 MPa; at 22, 40, 45, and 50 °C; holding time, 0–120 min) and observed a tailing phenomenon in all survival curves, indicating that linear models were not adequate for describing the effect of HHP. The log-logistic model produced the best fits to all survival curves, but the Weibull model provided good fit at the range 40–50 °C and reasonable predictions of inactivation.

Growth temperatures and growth phases have a significant effect on the inactivation of *Listeria monocytogenes* by HPP into milk. Cell growths at 15 °C were more

sensitive than cell growths at 4, 25 or 35, or 43 °C, which were the most resistant. Inactivation of cell growths at 4, 15, or 25 °C followed first-order kinetics, whereas cells grown at 35 or 43 °C displayed nonlinear inactivation kinetics due to tailing. Growth phase also significantly influenced inactivation of *L. monocytogenes* by HPP. Cells at the mid-stationary phase were significantly more resistant than cells grown at the mid- and late-exponential phase. This was probably due to changes in membrane composition and synthesis of stationary phase proteins and/or stress proteins (Hayman et al. 2007).

Post-processingtemperatures influence in recovering ability of injured *L. monocytogenes*. Koseki et al. (2008) observed that immediately after HPP treatment (550 MPa at 25 °C for 5 min), no *L. monocytogenes* cells were detected in milk regardless of the inoculum level (up to 7 log cfu/ml). However, the number of *L. monocytogenes* cells increased by >8 log cfu/ml after 3–28 days of storage at 4 °C. This recovery was not observed when storage was at 37 °C for 28 days. Mild-heat treatments (37 °C for 240 min or 50 °C for 10 min) following HPP (550 MPa at 25 °C for 5 min) inhibited the recovery of *L. monocytogenes* in milk after HPP during 70-day storage at 25 °C. Low pH also resulted in a noticeable synergistic effect on inactivation of *L. monocytogenes*. Xu et al. (2009) evaluated the effects of pressure come-up and holding times on the inactivation of *Listeria monocytogenes* in milk subjected to high-pressure treatments at 300, 400, and 500 MPa for less than 10 min at 30 °C. Milk showed a considerable baroprotective effect against *L. monocytogenes*. At 300 MPa, the *D* values for *L. monocytogenes* were 9.56, 1.11, and 0.94 min in milk, orange juice, and tomato juice, respectively.

25.3.2.4 Mycobacterium avium ssp. paratuberculosis

Mycobacterium avium ssp. *paratuberculosis* (MAP) causes Johne's disease, a chronic granulomatous enteritis that affects cattle and other ruminants. The isolation of MAP from breast milk, intestinal and lymph nodes, and blood from patients with Crohn's disease suggests a link between MAP and Crohn's disease, although this connection may not definitively be proved to date (Naser et al. 2000, 2004; Schwartz et al. 2000; Greenstein 2003; Selby 2004). MAP has been of concern to the dairy industry due to some reports supporting that it may not be effectively inactivated by conventional HTST pasteurization (72 °C, 15 s) (Lund et al. 2002). Few data have been published concerning the effect of HHP treatments on MAP. López-Pedemonte et al. (2006) inoculated two strains of MAP into sterilized milk to evaluate inactivation by HHP. Significant differences were also found between MAP strains as reported for other microorganisms, obtaining average reductions of 4 log cfu/ml after treatment with 500 MPa, which is comparable to results reported for thermal treatments. Donaghy et al. (2007) determined the effect of HHP alone and in conjunction with pasteurization (72 °C for 15 s) on the viability of two strains of MAP. A significantly greater ($P < 0.001$) reduction in viable numbers (mean log reduction of 6.52 cells/ml) was observed when using 500 MPa compared with 400 MPa (mean

log reduction of 2.56 cells/ml) for 10-min treatments, and the number of survivors was significantly lower ($P<0.001$) after a 10-min treatment with respect to a 5-min treatment. The use of high pressure was even more effective when combined with pasteurization, although there were still survivors when high inoculum levels of MAP were used.

25.3.2.5 Salmonella spp.

Raw and pasteurized milks have been involved in several outbreaks of salmonellosis. *Salmonella enterica* sv. *typhimurium* is most often associated with milk and dairy products; of great concern nowadays is *Salmonella typhimurium* DT 104, which is resistant to multiple antibiotics and has been documented in the UK in several outbreaks, some of which were determined to be caused by unpasteurized milk. In the USA, this serotype of *Salmonella* is the second most commonly reported cause of food-borne salmonellosis (Guan et al. 2005).

Tholozan et al. (2000) obtained complete inactivation of *S. typhimurium* strain Mutton (ATCC 13311) (>8 log reduction) in sodium citrate (pH 5.6) and sodium phosphate (pH 7.0) buffers after applying a 400 MPa HHP treatment for 10 min at 20 °C. However, Guan et al. (2005) reported that 350 MPa applied to UHT whole milk inoculated with *Salmonella typhimurium* DT 104 at ambient temperature (21 °C) during 60 min had little effect on this bacterium and only 3 log reductions were observed after 120 min. Cell count reductions reported at 350, 400, and 450 MPa for 30-min treatment were approximately 0.6, 1.8, and 5.0 log cfu/ml, respectively. Pressures of 500, 550, and 600 MPa reduced counts 4.5–5.1 log cycles within 10 min. A tailing was observed in all survival curves. The log-logistic model produced the best fit to data. Consequently, a 5.0 log reduction of *S. typhimurium* DT 104 in UHT milk required pressurization at 550 MPa for 50 min or 600 MPa for 30 min.

25.3.2.6 Staphylococcus aureus

Staphylococcus aureus are frequent contaminants of raw milk, being widely recognized as a common cause of clinical and subclinical mastitis in dairy cattle, sheep, and goats.

As is the case for most of vegetative bacteria, viability loss of *S. aureus* is enhanced as level of pressure, time, and temperature increases but shows greater resistance to HHP than other non-spore-forming bacteria (Patterson et al. 1995; Alpas et al. 2000; Trujillo et al. 2002). Patterson and Kilpatrick (1998) achieved a maximum reduction of approximately 6 log cfu/ml of *S. aureus* only when milk was treated at 500 MPa for 15 min at 50 °C. Gervilla et al. (1999) also found that in ovine milk *S. aureus* was extremely resistant to pressure and cell reductions above 7 log (cfu/ml) were only achieved after applying treatments of 500 MPa at 50 °C for 15 min.

García-Graells et al. (2003) observed that the lactoperoxidase system increased HHP inactivation of *S. aureus* in skim milk at pressures above 500 MPa at 20 °C

when it was present in loads under 10^6 cfu/ml. Under HHP it is sensitive to lysozyme, although it is not lysed, which suggested the existence of a non-lytic mechanism of bactericidal action of lysozyme against *S. aureus* (Masschalck et al. 2002).

25.3.2.7 Yersinia enterocolitica

Yersinia enterocolitica has been frequently isolated from raw milk and even from pasteurized milk (Larkin et al. 1991), and milk has been implicated in several outbreaks of yersiniosis (Tacket et al. 1984). In pasteurized milk, contamination has been mainly attributed to inadequate pasteurization or post-processing contamination (Klausner and Donnelly 1991; Kushal and Anand 1999). The psychrotrophic nature of this organism is of particular significance in milk and milk products that are normally stored at low temperatures.

Chen and Hoover (2003) studied the survival curves of *Yersinia enterocolitica* ATCC 35669 inactivated by high hydrostatic pressure in UHT whole milk at pressures ranging from 350 to 500 MPa. Tailing was observed in all survival curves and there was strong curvature in the plotted data so that the log-logistic Weibull models showed better R^2 and MSE values than the linear regression model. De Lamo-Castellví et al. (2005) also observed a tailing after 35 min of HHP treatment when the kinetics of population reduction were determined in one of the most baroresistant strains of *Yersinia enterocolitica* (serotype O:8). In that case, quadratic adjustment was the mathematical model that better fitted the results ($R^2 = 0.992$).

25.4 Effects of HHP on Dairy Products

25.4.1 Cheese

HHP treatment is a potential technology in the dairy industry for the manufacture of cheese, due to its effects on rennet coagulation time, cheese yield, ripening characteristics, extent of cheese shelf life, cheese functionality, and development of new textures.

25.4.1.1 Effects of HHP Processing on Cheese-Making Properties of Milk

As reported previously, HHP treatment reduces the number of microorganisms in milk, so it can be used to increase the microbiological safety and quality of milk to produce high-quality cheeses. Drake et al. (1997) reported a comparable microbiological quality of cheeses made with pasteurized milk and HHP-treated milk (3 cycles of 1 min at 586 MPa) with no detrimental effects on cheese flavor. In accordance with this finding, microbiological quality of cheeses made with HHP-treated goat

milk (500 MPa for 15 min at 20 °C) was comparable to pasteurized milk (72 °C for 15 s) cheeses (Trujillo et al. 1999; Buffa et al. 2001b). Cheeses elaborated with HHP-treated milk showed a similar level of lipolysis to cheeses made from raw and pasteurized milk and received the highest scores for overall aroma and taste (Buffa et al. 2001a), suggesting that it is possible to apply HHP processing to milk for making cheese of satisfactory hygienic quality.

In addition to improving the safety of cheeses, the application of HHP treatment of milk causes several protein modifications, such as whey protein denaturation and micelle fragmentation, and alters mineral equilibrium. Consequently, these changes modify the technological capacity of milk for making cheese.

Coagulation Properties

Many authors have found an improvement in the coagulation characteristics of cheese milk by HHP treatments. Desobry-Banon et al. (1994) showed that pressurization from 230 MPa enhanced acid and rennet milk coagulation in reconstituted milk prepared from low-heat skim milk powder. In other skim milk samples, rennet clotting time decreased as a result of pressure treatments up to 200 MPa but increased with higher pressures (Needs et al. 2000b). In agreement, coagulation time in raw bovine whole milk decreased as pressure increased $\leq$200 MPa and then increased again until at 400 MPa, reaching values comparable to that of raw milk (López-Fandiño et al. 1996, 1997). In goat's milk coagulation time did not change significantly with pressures up to 200 MPa, but treatments at 300, 400, and 500 MPa increased the coagulation time of milks (López-Fandiño and Olano 1998; Buffa et al. 2001c). In the case of ewe's milk, coagulation time decreased slightly with pressurization at 100 MPa and then increased significantly at 200 and 300 MPa, decreasing again at 400 MPa, with values similar to untreated milk (López-Fandiño and Olano 1998). Pandey et al. (2003) showed that coagulation rate is a function of pressure and temperature. At lower pressures (200 MPa), a change in treatment temperature from 3 °C to 21 °C increased the coagulation rate; however, at higher-pressure level (400 MPa), the coagulation rate was lower at higher temperatures (21 °C). Coagulation of milk is the result of two processes, i.e., enzymatic hydrolysis of κ-CN, which destabilizes casein micelles, and the aggregation of micelles leading to formation of a gel. These processes are governed by the stability of casein and mineral balances in milk, especially calcium and pH. HHP treatment of milk has been shown to cause a reduction in colloidal calcium phosphate concentration and reduction of enzymatic coagulation time, due to an increase in Ca^{2+} activity (Schrader et al. 1997). Furthermore, pressure treatment of milk affects milk proteins, including reduction in the size of casein micelles and denaturation of β-LG, probably followed by interaction with micellar κ-CN (O'Reilly et al. 2001).

López-Fandiño et al. (1997) found that the initial stage and extent of the enzymatic phase of coagulation were inhibited by pressures over 200 MPa and explained

by the attachment of β-LG to κ-CN under pressure, which restricts the hydrolysis of κ-CN by chymosin. However, Ohmiya et al. (1987) did not observe differences in the primary phase reaction of milk curdling by rennet with pressures up to 130 MPa. Similarly, Needs et al. (2000b) showed that the first stage of coagulation was unaffected by pressure, but HHP processing affected the second phase of rennet coagulation, which was explained with two opposing mechanisms: the effect of pressure on the properties of the micelles resulted in their rapid aggregation, while increasing β-LG denaturation progressively reduced the aggregation rate. In conclusion, it is clear that the effects of HHP on cheese coagulation properties depend on the substrate and the HHP conditions (pressure, time, and temperature).

Cheese Yield

An interesting application of high-pressure treatment on milk in cheese manufacture is the possibility to increase cheese yield.

Cheese yield increases with pressurization at 300 and 400 MPa in raw and heated milks (López-Fandiño et al. 1996; Huppertz et al. 2005a). This increase in cheese yield could be explained by the denaturation and incorporation of additional β-LG into the curd and greater degree of moisture content. Besides the economic interest in increasing cheese yield, gel characteristics of rennet curd is an important parameter in the cheese-making process, because it can affect textural attributes of cheese. A treatment of 300 MPa for 30 min in cow's milk significantly increased the firmness of gels (López-Fandiño et al. 1996). Pandey et al. (2000) studied the effect of HHP treatment and temperature on the water holding capacity and gel strength of rennet curds and found that lower pressures (200 MPa) and lower temperatures (3 °C) decreased water holding capacity but increased gel strength. Queso fresco made from pressure-treated milk (400 MPa, 20 min, 20 °C) contained more moisture and was less firm, less crumbly, and more sticky than cheeses made from raw milk and decreased in firmness during storage (Sandra et al. 2004). Nevertheless, pressurization (400 MPa, 22 °C, 15 min) of reduced-fat cow's milk prior to cheese making increased yield and improved cheese texture and accounted for higher overall acceptability (Molina et al. 2000). In a study of cheddar cheese made with high-pressure-treated milk, HHP treatment of raw milk (483 and 676 MPa) augmented cheddar cheese yield with better curd formation properties. HHP-treated cheeses at 10 °C showed higher cheese yield and protein retention compared to cheeses made from raw or pasteurized milk, which can be attributed to a combined action of protein and moisture retention (San Martín-González et al. 2007). Similarly, the yield of cheddar cheese made from HHP-treated milk (three cycles of 1 min at 586 MPa) was 7 % higher than that from raw or pasteurized milk (Drake et al. 1997); however, texture defects were present in pressurized milk cheeses, which were attributed to an excess of moisture. Arias et al. (2000) reported that curd yield and moisture retention in the curd increased with an increase in pH of milk, in the range 5.5–7.0, for milk treated at 400 MPa. In agreement, yield of curd from cow's milk was

slightly influenced by treatment at pressures ≤250 MPa, but treatment at 400, 600, or 800 MPa for 30 min significantly increased curd yield by 10 %, 24 %, or 25 %, respectively (Huppertz et al. 2004b). Needs et al. (2000b) did not observe significant differences in syneresis from curds prepared from untreated milk and milks pressure treated at 200 or 400 MPa; however, a treatment of 600 MPa significantly decreased the syneresis of the curd, possibly due to the effects of a finer gel network and increased inclusion of whey protein. The microstructure of curds formed from pressure-treated (600 MPa) milks showed a gel formed with a dense network of fine strands, which appeared to be continuous over long distances, as a result of an increase in the number of protein particles and modification of the properties of these particles that occurred during pressure treatment, for example, through disruption of casein micelles and denaturation of β-LG (Needs et al. 2000b).

Goat's milk behaves very similarly to cow's milk, and treatments at 300 and 400 MPa applied for 30 min in milk significantly improve cheese yield (López-Fandiño and Olano 1998). However, in goat's milk, firmness of curd is also improved with pressurization. Trujillo et al. (1999) found that the yield of cheese made from goat's milk treated at 500 MPa for 15 min was 5 % higher than that from pasteurized milk (Trujillo et al. 1999). Texture characteristics of cheeses made with HHP-treated milk were firmer and less fracturable than pasteurized milk cheeses but less cohesive than raw milk cheeses. HHP-treated milk cheeses have the most regular and close protein matrix, with small and uniform fat globules, resembling the structure of raw milk cheeses (Buffa et al. 2001d).

In the case of ewe milk, cheese yield also increases with pressurization from 200 to 400 MPa (López-Fandiño and Olano 1998). Higher increase in cheese yield of ewe milk at lower pressures is in accordance with the higher level and higher denaturation of β-LG in ewe milks (López-Fandiño and Olano 1998). However, firmness of curds was not affected by pressure treatment.

Rennet coagulation time of buffalo milk increases with increasing pressure, whereas the strength of the coagulum formed decreases after treatment at 250–800 MPa (Huppertz et al. 2005b).

25.4.1.2 Use of HHP Processing to Reduce or Inactivate Microorganisms in Cheese

HHP application in cheese can reduce total numbers of microorganisms or inactivate pathogenic microorganisms, thus increasing the shelf life and safety of the product. However, the application of pressure treatment could affect the sensorial characteristics of cheeses, which will be dependent on the type of cheese, HHP conditions, and cheese age at the time of treatment.

Ewe's milk cheeses pressurized at 15 days of ripening exhibit lower counts than those treated at day 1, demonstrating that the degree of maturity of cheeses affects the degree of microbial inactivation. However, pressurization at day 1 significantly changed the sensorial characteristics of cheeses, with a more elastic, softer, and less

crumbly texture (Juan et al. 2007d, 2008). In agreement, HHP treatments at 300 or 400 MPa on 2 or 50 days of ripening significantly reduced counts of undesirable microorganisms, improving the microbiological quality and safety of La Serena cheese immediately after treatment and at the end of the ripening period (Arqués et al. 2006). Cheeses treated by HHP on day 2 presented higher fracturability, hardness, and elasticity values than untreated cheeses or cheeses treated on day 50 (Garde et al. 2007). The aroma also was affected by HHP treatments on day 2, showing lower quality and intensity scores in comparison with untreated cheeses. On the other hand, HHP treatment on day 50 did not influence the sensory characteristics of 60-day-old cheeses (Arqués et al. 2007).

Gallot-Lavallée (1998) studied the efficiency of HHP treatment for destruction of *L. monocytogenes* in goat cheese from raw milk, finding that 450 MPa for 10-min or 500 MPa for 5-min treatments achieve more than 5.6 log units of reduction of this microorganism without significantly affecting sensory characteristics of cheeses.

Calzada et al. (2013) pressurized blue-veined cheeses made from pasteurized ovine milk at 400 or 600 MPa after 3, 6, or 9 weeks of ripening. On day 90, treatments at 400 MPa had lowered counts of lactic acid bacteria and *P. roqueforti* by less than 2 log units, whereas treatments at 600 MPa had reduced lactic acid bacteria counts by more than 4 log units and *P. roqueforti* counts by more than 6 log units. Differences in sensory characteristics between pressurized and untreated cheeses were generally negligible, with the only exception being treatment at 600 MPa at 3 weeks, which affected sensory characteristics of cheese.

HHP processing can be applied to increase fresh cheese shelf life to maintain its organoleptic acceptance. Capellas et al. (1996) showed that treatment at 500 MPa for 5 min at 25 °C delayed cheese spoilage during 2 and 3 months when applied during 5 or 30 min, respectively. When HHP treatment was combined with nisin, the extended cheese shelf life was more effective. In agreement, HHP treatment of fresh lactic curd cheese between 300 and 600 MPa for 5 min at ambient temperature effectively controlled the occurrence of spoilage yeasts and extended product shelf life for up to 8 weeks (Daryaei et al. 2008) without adverse effects on sensory and textural attributes of the product (Daryaei et al. 2006). In contrast, a significant effect of HHP on textural properties of rennet-coagulated fresh Scottish cheese was observed by Okpala et al. (2010). Fresh cheeses treated at 300 and 400 MPa for 5 min, stored at 4 °C, presented a shelf life of 14 and 21 days, respectively, compared to untreated control cheese, which presented a shelf life of 7 days. On the other hand, HHP treatments produced firmer texture and more yellow color (Evert-Arriagada et al. 2012). On the other hand, the application of 500 MPa (5 min, 16 °C) on fresh cheeses produced a considerable increase in shelf life, achieving 19–21 days when stored at 4 °C, whereas untreated cheese became unsuitable for consumption on days 7–8. Cheeses that were HHP treated at 500 MPa were firmer and more yellow than untreated ones. However, these changes, which were detected by instrumental and sensory analysis, did not affect the preference for pressurized cheese (Evert-Arriagada et al. 2014).

25.4.1.3 Use of HHP Processing for Cheese Ripening Acceleration

Ripening of cheese is a long and costly process; consequently, many methods to shorten ripening have been studied, including the use of elevated ripening temperatures, addition of enzymes, use of cheese slurries and selection, and attenuation or genetic modification of the starter. Ripening of cheeses is determined mainly by proteolysis; however, glycolysis and lipolysis are also necessary for the development of flavor and texture characteristics. Many authors have reported the possibility to ripening acceleration by HHP processing. Yokohama et al. (1992) described in a patent proposal the potential use of HHP technology for accelerating the ripening of cheddar cheese. Cheese samples were exposed to pressure from 0.1 to 300 MPa at 25 °C for 3 days. O'Reilly et al. (2000) tested the same conditions and showed an increase in proteolysis rates. Furthermore, studying different ranges of pressures and times to determine optimal conditions for acceleration the ripening of cheddar cheeses, O'Reilly et al. (2003) found that use of pressures below 150 MPa gave the largest increases in proteolysis parameters, in terms of %pH 4.6 SN/TN. However, levels of free amino acids (FAA) progressively decreased as the pressure was increased above 50 MPa. The increase in primary proteolysis in cheese by HHP may be explained by conformational changes in casein structure post-pressurization, making the protein more susceptible to the action of proteases. In Camembert cheese, the highest degree of proteolysis was observed with 50 MPa for 4 h and was dependent on the maturity of the cheese. Application of 50 MPa when treatment was applied on 4-day-old cheeses enhanced the primary proteolysis of cheeses, while the subsequent peptidolysis remained unaffected (Saldo et al. 2001). Increased levels of proteolysis have been found for surface-mold ripened cheese (Paillardin cheese, containing a secondary inoculum *Penicillium camemberti*) which was HHP treated at 50 MPa for 8 h at 20 ° C (Messens et al. 2001).

Other HHP conditions have been tested for accelerating cheese ripening that involves high HHP treatments (400–600 MPa) at short times (5–15 min) or an initial high HHP treatment at short times followed by low HHP treatment (50 MPa) for long times (72 h) in different cheese varieties. Saldo et al. (2002) subjected goat milk cheeses to pressures of 400 MPa for 5 min and lowered the breakdown of α_s-CNs, explained by the reduction of residual rennet and activity of some proteases. However, the HHP treatment increased FAA release, reaching twice the value found in untreated cheeses after 28 days, possibly as a result of higher peptidase activity. These authors observed that pressurization of cheese increased pH levels, which could enhance enzymatic activity. Levels of proteolysis of goat milk cheeses increased when the previous treatment (400 MPa for 5 min) was followed by 50 MPa for 72 h (Saldo et al. 2000). These results suggest that a combination of shock high-pressure treatment, which causes enzyme release, followed by a long and moderate-pressure treatment, which enhances enzymatic activity, would be best for accelerating the ripening of goat milk cheeses (Saldo et al. 2000). In Spanish cheese manufactured with a mixture of cow and ewe milks, HHP treatment of 400 MPa for 5 min accelerated casein degradation and increased FAA; however, this treatment did not influence the taste quality of cheeses (Ávila et al. 2006).

On the other hand, treatment of 400 MPa for 10 min at room temperature applied on 1-day post-manufacture on full-fat cheddar cheese did not influence the primary or secondary proteolysis of cheeses but could be discriminated from untreated cheeses by higher cooked animal flavor and butter odor at 90 days of ripening. When cheeses were ripened until 180 days, HHP-treated cheeses presented lower intensity of flavor attributes (Rynne et al. 2008).

Juan et al. (2004, 2007a) studied the possibility of accelerating the ripening of ewe milk cheeses by HHP treatments from 200 to 500 MPa for 10 min applied on 1- and 15-day-old cheeses. They found that primary proteolysis was enhanced by pressures of 300 and 400 MPa applied on the first day of ripening with 200–500 MPa applied at 15 days of manufacturing and attributed this to conformational changes in casein structure produced by pressure, which makes protein more susceptible to the action of proteases. Secondary proteolysis was enhanced by treatment at 300 MPa. Cheeses HHP treated on 1 day of ripening presented the highest FAA levels, whereas those treated at the same pressure on day 15 of manufacturing had the highest levels of water-soluble nitrogen (Juan et al. 2004). The lipolytic process of ewe milk cheeses was also improved by pressurization at 300 MPa on 1 day of ripening, showing twice the level of FFA after pressurization, which could be caused by the early lysis of cells and better interaction of microbial lipase with fat (Juan et al. 2007b).

On the other hand, pressurization at 500 MPa drastically reduced proteolysis and lipolysis of ewe milk cheeses, probably due to the reduction of starter bacteria and enzyme inactivation by pressure, suggesting that it may be useful for slowing or arresting cheese ripening at the optimum stage of ripening (Juan et al. 2004, 2007c).

The potential application of HHP processing for cheese ripening is evident from the described results; however, it depends on the stage of ripening and the variety of cheese. HHP treatment increases cell membrane permeability (Cheftel 1992), which favors the release of intracellular material to the medium, and consequently improves the access of enzymes to their substrates. Furthermore, HHP causes destabilization of micelles, which might render caseins more susceptible to the action of proteolytic enzymes. It seems that the application of HHP during the early stages of ripening tends to have a greater effect the on ripening process than when HHP is applied at a later stage of ripening. Furthermore, the application of higher pressures ($\geq$500 MPa) drastically reduces microbial counts and inactivates enzymes, so it may be useful to arrest the ripening of cheeses and maintain the optimum characteristics at a given time (Juan et al. 2004, 2007c; Calzada et al. 2014a, b).

25.4.1.4 Effects of HHP Processing on Sensorial Characteristics of Cheese

Despite the acceleration of the ripening of cheeses, HHP treatment can alter their rheological characteristics. Torres-Mora et al. (1996) suggested the possibility of the generation of desirable new cheese textures and reduced variability of moisture contents within blocks of reduced-fat cheddar cheese by the use of HHP, which produced a more continuous microstructure in cheddar cheese curd post-pressurization.

HHP treatment of 50 MPa for 72 h resulted in a softer texture of goat milk cheeses, with a more uniform structure (Saldo et al. 2001). When these cheeses were pressurized at 400 MPa or 400 MPa followed by 50 MPa, their elasticity and mouthfeel increased, and crumbliness decreased, but did not affect the acceptability of cheeses. Textural changes seem to be related to changes in calcium equilibrium, which is reestablished after HHP treatment, but with different associations between caseins (Saldo et al. 2000). Texture of cheddar cheese HHP treated at 50 MPa for 72 h became softer (Saldo et al. 2001) but did not show gross structural changes in the cheese matrix (O'Reilly et al. 2000). However, cheddar cheeses pressurized at 350 MPa for 70 h at 25 °C showed more fat emulsification, and the fat appeared to be encapsulated by protein (O'Reilly et al. 2003). Serrano et al. (2004, 2005) described that the application of moderate (345 MPa) and higher (483 MPa) pressures for 3 and 7 min accelerated the shredability of cheddar cheeses, showing similar attributes to those produced after 27 days of ripening. Pressure treatments reduced the presence of crumbles, increased mean shred particle length, improved length uniformity, and enhanced surface smoothness, so this treatment can be used to shred cheddar cheese immediately after block cooling, thus reducing refrigerated storage costs and simplifying the handling of cheese for shredding. In a half-fat cheddar cheese, pressurization from 100 to 800 MPa for 2 h induced softening of cheeses and increased meltability, cohesiveness, and chewiness (Johnston et al. 2002a). Increase in cohesiveness with pressure also was observed by Nienaber et al. (2000) in cheddar cheese with the same pressure treatments, explained by the physical effect of HHP that fuses particles together and strengthens internal binding forces. Full-fat cheddar cheese HHP treated at 400 MPa for 10 min at room temperature presented lower fracturability and flowability and higher deformability, explained by increases in cheese pH due to HHP that would be conductive to decreases in the ratio of soluble-to-colloidal calcium and degree of paracasein hydration and an increase of paracasein aggregation (Rynne et al. 2008).

O'Reilly et al. (2002) described that it is possible to accelerate the ripening process of low-moisture mozzarella cheese by pressurization of 400 MPa for 20 min at 25 °C. HHP treatment enhanced the development of cooking-related functional characteristics, which would normally happen gradually during storage time, resulting in an increase in water holding capacity of the matrix and an increase in the flowability and fluidity of heated cheeses. Hence, HHP treatment reduced the time required to attain satisfactory cooking performance (O'Reilly et al. 2002). Furthermore, Johnston and Darcy (2000) found that ripening acceleration of mozzarella cheese was possible with 200 MPa during 60 min, resulting in a decrease in hardness and an increase in flow of the heated cheese. On the other hand, pressurization (400 MPa for 5 min) of reduced-fat mozzarella cheese did not affect its rheological properties (Sheehan et al. 2005).

For ewe milk cheeses, Juan et al. (2007d) observed that pressurization of cheeses (from 200 to 500 MPa) on the first day of ripening decreased fracturability and increased deformability of cheeses. This phenomenon could be explained by the increase in water retention capacity and the more homogeneous microstructure produced by HHP, thus reducing possible areas of fracture. The highest treatment (500 MPa) significantly changed the texture of cheese, producing the least crumbly,

most elastic, and softest cheese. When ewe raw milk cheeses were HHP treated at 300 or 400 MPa for 10 min on day 2 of ripening, fracturability, hardness, and elasticity values increased (Garde et al. 2007). In this case, pressurized cheeses presented lower breakdown of α_{s1}-CN, which could produce firmer cheeses. According to Juan et al. (2007d), when pressure treatment was applied at 50 days of ripening, cheese texture was not affected.

25.4.1.5 Other HHP Processing Applications in Cheese

Besides the reduction of microbial counts, increase in cheese yield, modification of cheese ripening, and development of products with new sensory characteristics, other applications of high pressure in cheese have been proposed.

The possibility of accelerating cheese brining by HHP treatment has been suggested in Gouda (Messens et al. 1998, 1999) and Manchego (Pavia et al. 2000) cheeses, but salt uptake and salt diffusion were not accelerated by the pressure conditions tested (100–500 MPa, 15–130 min in Gouda and 50–200 MPa in Manchego, respectively).

Another proposed application was use of HHP processing to attenuate starter bacteria to be used as adjuncts in cheese manufacture. Attenuated starter bacteria cannot produce acid during cheese manufacture, but they do contain enzymes that contribute to cheese ripening. Casal and Gómez (1999) suggested that *Lactococcus lactis* ssp. *lactis* treated at 300 MPa and *Lactobacillus casei* ssp. *casei* treated at 350 MPa may be added during cheese making to provide an extra supply of enzymes with potential debittering properties, which may be used to accelerate cheese ripening. Upadhyay et al. (2007) observed that pressurization at 200 MPa for 20 min at 20 °C was successfully used to attenuate *L. lactis* ssp. *cremoris*, which may be used in combination with primary strains in cheddar cheese making, producing higher levels of FAA and acceleration of secondary proteolysis in cheese.

25.4.2 Yogurt and Acid Gels

Commercial yogurt is available in different presentations such as set gel, stirred gel, or liquid. Varieties of presentations are also related to milk composition, especially solids and fat content, which depend on milk origin and the preliminary step of skimming. The composition of yogurt cultures is typically a mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, but increasingly, a probiotic strain is being added for health purposes. The effect of HHP on yogurt characteristics has been studied by several authors, and some of the abovementioned types of raw material commercial presentation and approaches have been raised (Table 25.2).

Yogurt quality must meet two relevant aspects. One is that the milk must be of very good hygienic quality. Also, from a physical point of view, yogurt should be stable during its commercial life (approximately 1 month), which in most cases

Table 25.2 Overview of the effects of high hydrostatic pressure on yogurt and acid gels

Type of yogurt	HHP (MPa) conditions Time (min)	*T* (°C)	Parameter evaluation	Authors
Stirred skim milk acid gels (GDL)	200, 400, 600	NS	Viscosity	Johnston et al. (1994)
Set yogurts treated	200–1000 (15)	Ambient	Activity of starters	Reps et al. (1998a)
Set yogurts from ewe's milk	200, 350, 500 (15)	10, 25, 55	Firmness Coagulation properties Syneresis	Ferragut et al. (2000)
Stirred low-fat yogurts	100–400 (15)	20	Culture counts Organic acids Free amino acids Viscosity Sensory evaluation	de Ancos et al. (2000)
Set skim fortified milk yogurts	600 (15)	Ambient	Color Texture Viscoelasticity Whey protein denaturation Microstructure	Needs et al. (2000a)
Acid-set gels (GDL): WP treated (H, HHP, native) added to simulated milk treated (H, HHP, native)	250, 400, 600, 700 (20)	25	Gel formation Whey protein denaturation Acidification curves	Walsh-O'Grady et al. (2001)
Set skim milk with WPC	600 (15)	Ambient	Consumer test Texture	Capellas et al. (2002)
Set whole milk fortified with WPC	676, (5, 30) 193, (5, 30)	Ambient	Yield stress Water holding capacity Microstructure	Harte et al. (2002)
Low-fat set yogurt	300, 400, 500, 676 (5) and combined with HT(HHP + H and H + HHP)	Ambient	Luminosity Yield stress, G' Penetration Water holding capacity Microstructure (TEM)	Harte et al. (2003)
Set yogurt of fortified (WPC) or concentrated skim milk	600, 15 min	Ambient	Syneresis Texture	Capellas and Needs (2003)
Stirred low-fat fortified with different cultures including *Bifidobacterium longum*	676, 5 min HT HHP HHP + H	Ambient	Yield stress, K, n TPA	Penna et al. (2006)

(continued)

Table 25.2 (continued)

Type of yogurt	HHP (MPa) conditions Time (min)	*T* (°C)	Parameter evaluation	Authors
Stirred low-fat fortified with different cultures including *Bifidobacterium longum*	676 (5) H HHP HHP + H	Ambient	Yield stress, K, n Water holding capacity Microstructure	Penna et al. (2007)
Low-fat stirred yogurt	100, 250, 400 (10)	25, 70, 90	Viscosity Whey protein denaturation Water holding capacity Particle size Microstructure	Udabage et al. (2010)
Acid gels (GDL) of pressurized skim milk between pH 6.4 and 7.3	200–600 (30)	Ambient	Particle size Whey protein denaturation Gel formation	Anema (2010)

means accomplishing good water retention capacity of the gel, minimizing the expulsion of whey (minimum syneresis) and good texture characteristics (firmness and creamy mouthfeel of gels).

To discover the best conditions of HHP treatment for yogurt making and mechanisms involved in acid gel formation, studies performed in this field have used different degrees of raw material complexity and treatment combinations and sequences (heat and HHP treatments).

Needs et al. (2000a) compared set yogurts made from skim fortified milk HHP treated (600 MPa, 15 min, ambient temperature) and heat treated (85 °C, 20 min). Viscoelastic characteristics of yogurts when strain sweeps were performed showed lower resistance to strain (loss of linear behavior) of HHP than those produced from heated milk. However, G' (elastic modules related to the interacting forces in the solid structure) values in the linear region were higher in HHP than in heat-treated yogurts. This behavior could be interpreted as HHP yogurt having more interacting micelles, probably with less intense forces, than yogurts produced from heat-treated milk, in which interactions were produced through more resistant strands, giving a deformable and resistant character to breakdown of the latter. In a study performed by Harte et al. (2002), the application of 676 MPa and long holding times (30 min) to milk (full fat) resulted in yogurts with equivalent rheological properties and WHC yogurts made from heat-treated (85 °C, 30 min) milk. In a further work, Harte et al. (2003) studied the effect of combining HHP (300, 400, 500, and 676 MPa for 5 min at ambient temperature) and the same heat treatment in low-fat fortified milk. Results showed improvement in the most important quality characteristics of yogurts when HHP and heat treatment were combined; in most conditions the combination was better than when each treatment was applied alone, although the order of treatment application gave different results. Generally, better WHC and mechanical

properties were observed in milk heat treated and then HHP treated, particularly in the intermediate range of 400–500 MPa. However, microstructure of yogurts was similar independently of whether thermal treatment was applied after or before HHP. Penna et al. (2006, 2007) studied mechanical properties, microstructure, and water retention capacity of stirred low-fat fortified yogurts made with two different probiotic starter cultures. HHP conditions applied were 676 MPa, 5 min at ambient temperature. The effect of HHP was compared to heat treatment (85 °C, 30 min) alone and with yogurts obtained from milk processed by combining HHP and heat treatment. Milk treatment was applied before yogurt fermentation, type of starter culture, and inoculation rate modified the gel properties. Combined HHP and heat treatment of milk with 0.1 % inoculation rate (for both cultures) led to attractive rheological and textural properties, i.e., creamy and thick consistency with good water retention properties, although WHC was not always the best compared to heat and HHP treatments applied alone. The authors explained these results by focusing mainly on yogurt microstructure characteristics, and they proposed a scheme of microstructure gel formation process as pH dropped during fermentation (Penna et al. 2007), in which the microstructure of heat-treated milk yogurt was composed of fewer interconnected chains of irregularly shaped casein micelle structures, forming a network that enclosed the void spaces, while the microstructure of HHP yogurt presented more interconnected clusters of densely aggregated protein with reduced and uniform particle size. The combined HHP and heat milk treatments led to compact yogurt gels with larger casein micelle clusters interspaced by void spaces and exhibited a higher degree of cross-linking. These aggregates in association with clumps of dense amorphous material resulted in improved gel texture and viscosity.

More recently, Udabage et al. (2010) also studied physical properties of low-fat stirred yogurts prepared from reconstituted skim milk (14 % solids) treated in some combinations of HHP (100, 250, or 400 MPa at 25, 70, or 90 °C for 10 min) before (HHP + H) and after (H + HHP) heat treatment (90 °C, 10 min). HHP treatment of skim milk at 25 °C with prior or later heat treatment resulted in stirred yogurts of similar consistency to those produced by heat treatment alone. The application of only HHP at 70 and 90 °C did not produce any improvement in yogurt characteristics. However, as HHP promotes whey protein denaturation, there is a possibility to reduce the severity of the standard heat treatment traditionally applied to yogurt milk by incorporating an HHP processing step in yogurt production. This treatment combination may be a strategy to incorporate heat-sensitive ingredients in yogurt formulation.

The observation that heating was more efficient in producing casein/whey protein interaction products as acid gels was also observed by Walsh-O'Grady et al. (2001) in a study of acid-set simulated yogurt milk gels in which HHP was applied and compared to heat treatment. In this study it was confirmed that to obtain acid gel of certain consistency ($G' > 500$ Pa), denaturation of whey protein is a prerequisite.

A more recent contribution to understanding the HHP acid gel formation by GLD in skim milk has been made by Anema (2010), who studied the effect of initial pH of milk (from 6.4 to 7.3). Anema begins with the idea that denaturation of whey

proteins and the disruption of casein micelles could not entirely account for changes in the rheological properties of acid gels, as denaturation of up 50 % of the whey proteins produced acid gels with very low G' and yield stresses. It is proposed that the pH and magnitude of pressure treatment affect the interactions of denaturated β-LG with casein in the acid gel structures. At low pressures and/or initial pHs, denatured β-LG acts predominantly as an inert filler in the acid gel structures, whereas, at higher pressures and/or initial pHs, denatured β-LG actively participates in the formation of the gel network during acidification. However, to confirm this hypothesis, further studies about the kinetics of β-LG denaturation and localization of the denatured whey protein are needed.

Apart from yogurt characteristics produced from HHP-treated milk, few studies have been focused on the application of HHP to the gelled final product. Reps et al. (1998b) applied pressures in the range of 200–1000 MPa in 200 MPa intervals for 15 min to yogurts to study the effect on microflora. It was found that pressure of 400 MPa was the minimum pressure necessary to inactivate *Lactobacillus delbrueckii* sp. *bulgaricus*. However, *Streptococcus thermophilus* was resistant to pressurization. During storage of yogurt after pressurization, a decrease in *Streptococcus thermophilus*counts was observed. During cold storage of yogurt pressurized under 400 MPa and higher, no further acidification was observed.

De Ancos et al. (2000) studied the effects of HHP (100–400 MPa for 15 min) on physicochemical, microbiological, and sensory characteristics of packed stirred low-fat yogurt. Pressures over 200 MPa prevented post-acidification of yogurt during cold storage. HHP-treated yogurts presented higher viscosity than did untreated controls, and these differences were maintained during storage. HHP treatments of 300 and 400 MPa reduced the number of viable cells of lactobacilli to below the legal minimum permitted in many countries.

25.4.3 Other Dairy Products

The effect of HHP on other dairy products such as cream, ice cream, and whey protein concentrate used as an ingredient have been conducted in different studies.

HHP modification of whey protein concentrates (WPC) added in low-fat whipping cream has been studied by Padiernos et al. (2009). Whipping cream containing HHP-treated WPC (300 MPa at 25 °C for 15 min) was compared with samples not containing WPC or with untreated WPC. Overall, the most relevant quality characteristics of whipping cream, i.e., viscosity, water retention capacity and overrun, and formulations containing HHP-treated WPC, were the best, indicating improvement of foaming properties by using this technology in WPC as an ingredient. A similar study was performed by the same research group with the same HHP conditions, using HHP-treated WPC added to low-fat ice cream mixes (Lim et al. 2008). Ice cream containing HHP-treated WPC exhibited the greatest overrun and foam stability, confirming the effect of HHP on foaming properties of whey proteins in a complex system. Improvements of overrun and foam stability were

observed when HHP-treated whey protein was used at a concentration as low as 10 % (wt/wt) in ice cream mix.

Other studies in dairy products have been related to physical changes in milk fat. HHP-induced crystallization of milk fat is one of the main effects observed in cream and butter. Buchheim and Abou El Nour (1992) applied HHP (100–500 MPa at 23 °C for 1–15 min) to dairy cream. Crystallization was observed in fat droplets, mainly in the globule periphery, and increased with duration and magnitude of pressure application (maximum at 300–500 MPa). It was also observed that after HHP treatment at 23 °C and subsequent storage at the same temperature, crystallization was maintained. The authors mentioned two potential applications of this observed phenomenon: fast aging of ice cream mix and physical ripening of dairy cream for butter making. Another possible consequence of HHP-induced crystallization of milk fat was the improvement in whipping properties observed by Eberhard et al. (1999). The best conditions were between 400 and 600 MPa for up to 2 min. However, at higher pressures, denaturation of whey protein occurs, causing destabilization of whipping cream.

For water and nonfatty products, adiabatic heat is approximately 3 °C per 100 MPa. Fats have larger adiabatic heat (up to 10 °C per 100 MPa) due to higher compressibility of fat compared to water (Ting et al. 2002). Depending on the fat studied and HHP conditions applied, adiabatic temperature is variable and also different for compression and decompression processes (Buchheim et al. 1999). These observed differences are mainly due to transitions between polymorphic states of the fats. In the case of milk fat, the increase of pressure magnitude leads to an increase of crystallization and melting temperatures, approximately 16 °C per 100 MPa (Frede and Buchheim 2000).

Another aspect studied on cream is the physical stability of emulsion when submitted to HHP. Dumay et al. (1996) compared the effect of HHP (450 MPa at 25 °C for 15 or 30 min or at 10 or 40 °C for 30 min) on pasteurized and UHT dairy creams (35 % fat). Pressure applied at 10 or 25 °C did not affect fat globule size distribution or rheological behavior, and samples were stable during cold storage for 8 days. However, the application of 450 MPa at 40 °C induced surface changes in fat globules, which were reversible in part during cold storage. UHT-treated creams were more sensitive to globule aggregation than pasteurized creams. This observation suggests a possible application to cream churning or whipping.

HHP may have interesting applications related to processes in which phase transitions are implied. HHP reduces the freezing and melting points of water to a minimum of −22 °C at 201.5 MPa. The primary applications of pressure in relation to water phase diagram are increased freezing rates obtained using pressure-assisted freezing (resulting in rapid and uniform nucleation and growth of ice crystals on releasing the pressure), increased thawing rates, and also the possibility of nonfrozen storage at subzero temperatures (Kalichevsky et al. 1995). Pressure-assisted freezing may be of special interest to avoid coarse ice crystallization and obtaining a smooth texture in various types of ice creams (including low fat) or sherbets. The Unilever company has patented combinations of HHP processing and freezing for improving consistency and smoothness and slower melting of ice creams (Keenan et al. 1998).

25.5 Conclusions

High hydrostatic pressure induces inhibition and destruction of microorganisms, influences the physicochemical and technological properties of milk, and is able to produce high-quality dairy products with improved characteristics. However, and despite these possibilities, the application of this technology on milk and dairy products at industrial-level HHP is scarce, given the limited number of dairy companies applying this technology. However, a series of dairy products are already being treated using this technology, such as: (1) cold pasteurized milk (Villa de Patos, Mexico); (2) free-starter fresh cheese with a shelf life of 45 days in cold storage (Pastoret La Segarra, Spain); (3) cheese-based sandwich fillings (Rodilla, Spain); (4) different yogurt-based products (drinkable yogurt, yogurt dressing, "clouds of yogurts") by Pulmuone (Korea), Bolthouse (USA), and Romantics (Spain), respectively; (5) colostrum-based beverages that preserve the functionality of heat-sensitive bioactive components present in colostrum such as immunoglobulins, lactoferrin, and growth factors (New Image Group, New Zealand); and (6) different snacks made from mozzarella and beef jerky (Snack Patrol, USA) or ham pork and cheddar cheese (Deli24, UK). In addition, there are other very interesting research dairy applications, including cheese ripening acceleration, the arresting of cheese ripening at the optimal point, and the improvement of set yogurt characteristics (water retention and texture) which have not been transferred to the market, although they possess numerous potential advantages. Although the industrial HHP equipment used at present is discontinuous, from 200 to 600 L of capacity, current HHP machines are able to process from 1000 kg to 2.2 tn/h (www.hyperbaric.com), so the implementation of this technology in the dairy industry today is possible, with a reasonable price.

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Chapter 26
Processing of Meat Products Utilizing High Pressure

Anna Jofré and Xavier Serra

Abstract High pressure processing is an alternative to thermal treatment for the production of safe meat and meat products which retains quality and freshness. Due to its effectiveness and acceptability by consumers, a considerable number of meat companies worldwide apply this technology to extend the shelf life of a wide variety of meat products without using chemical additives. Research studies have shown that the inhibitory effect of HPP on microorganisms depends on both the cycle parameters (especially pressure intensity and holding time) and the physicochemical characteristics of the meat product. While low water activity decreases the inhibitory effect of HPP, acidity and the combination of HPP with other factors such as natural preservatives enhance it. In addition to the inactivation of microorganisms, HPP can also affect the appearance, flavor, and texture of meat products. The extent and importance of these changes ultimately determine the commercial suitability of HPP for use on food products.

Keywords High pressure processing • Meat • Quality • Food safety

26.1 Introduction

High pressure processing (HPP) facilitates the improvement of the microbial safety of meat and meat products (Cheftel 1995; Cheftel and Culioli 1997; Hugas et al. 2002). Also known as cold pasteurization, HPP is often presented as the main alternative, with good consumer acceptability (Baron et al. 1999; Nielsen et al. 2009), to thermal pasteurization of meat products. The main objectives in industrial HPP are to destroy the pathogenic and spoilage microorganisms and to extend shelf life, while maintaining the characteristics and the quality of meat and meat products almost intact (Balasubramaniam et al. 2008). In some cases, as in dry-cured meat products, HPP is the only feasible pasteurization process which has minimal effects on appearance,

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flavor, texture, and nutritional value (Hugas et al. 2002). Nonetheless, different effects of HPP on meat and meat products, other than microbial inactivation, have been studied and described by many authors, as reported in numerous reviews (Cheftel and Culioli 1997; Dumoulin and Hayashi 1998; San Martín et al. 2002; Suzuki et al. 2006; Rastogi et al. 2007; Norton and Sun 2008; Sun and Holley 2010; Zhou et al. 2010).

26.2 Microbiological Aspects

26.2.1 High Pressure and Hurdle Technology

Hurdle effect refers to the concept of controlling the growth of spoilage or pathogenic microorganisms by combining in series, or parallel, a number of intrinsic and/or extrinsic factors which individually would not be adequate. In the food industry, this has led to the application of hurdle technology, where the survival of microorganisms is greatly decreased when they are confronted with multiple antimicrobial factors such as temperature, water activity (a_w), pH, and redox potential (Leistner and Gorris 1995). With the aim of reducing the extent of processing and due to demand for lightly processed or fresh-like products, new hurdles such as high pressure processing and combinations of hurdles are applied to obtain safe value-added food products.

A great number of studies have shown that HPP improves the safety of raw meat and various other kinds of meat products (Table 26.1); the success of this technology is confirmed by the fact that pressurized meat products are commercially available

Table 26.1 Collection of fresh, cooked, and dry-cured meat products assayed by HPP

Meat products	References
Fresh Pork slurries, minced beef, raw ground chicken, mechanically recovered poultry meat, raw smoked pork loin, bovine muscle, raw marinated beef loin, ground pork, ground beef, duck liver, chicken mince, poultry sausages	Shigehisa et al. (1991), Carlez et al. (1993, 1994), O'Brien and Marshall (1996), Gola et al. (2000), Yuste et al. (2000a, 2001), Karlowski et al. (2002), Cruz et al. (2003), Jung et al. (2003), Garriga et al. (2004), Hayman et al. (2004), Linton et al. (2004), Lindsay et al. (2006), Morales et al. (2008), Jofré et al. (2009b), Black et al. (2010)
Cooked Cooked ham, poultry cooked sausages, canned Vienna sausages, blood sausage (*morcilla de Burgos*), cooked poultry meat, Bologna-type sausage	Yuste et al. (2000b), Karlowski et al. (2002), Garriga et al. (2004), Aymerich et al. (2005), Chung et al. (2005), Jofré et al. (2007), Pietrzak et al. (2007), Diez et al. (2008a, b, 2009a, b), Jofré et al. (2008b, c, 2009b), Marcos et al. (2008a, b), Morales et al. (2009), Slongo et al. (2009), Patterson et al. (2010), Han et al. (2010)
Dry-cured Dry-cured ham, dry-cured beef *Cecina de León*, fermented sausages (*chorizo*, *salchichón*, Hungarian beef salami, Genoa salami), and low-acid fermented sausages (*fuet* and *chorizo*)	Garriga et al. (2004, 2005), Marcos et al. (2005, 2007), Morales et al. (2006), Latorre-Moratalla et al. (2007), Rubio et al. (2007a, b), Ruiz-Capillas et al. (2007), Campus et al. (2008), Gill and Ramaswamy (2008), Jofré et al. (2008a, c, 2009a, b), Pal et al. (2008), Ananou et al. (2010), Porto-Fett et al. (2010), Bover-Cid et al. (2011)

Table 26.2 Commercially available meat products treated by HPP

Company	Country	Products
Maple leaf	Canada	RTE meat-based meals with rice, pasta, or vegetables
Abraham	Germany	Dry-cured ham
Vismara	Italy	Dry-cured ham, salami, etc.
ITOHAM	Japan	Nitrite-free bacon, sausages, and sliced meat
Espuña	Spain	Cooked ham and *Tapas al minuto* (mini *chorizos*, fermented sausages, etc.)
Campofrío	Spain	Cooked ham, dry-cured ham, fermented sausages, *Vuelta y vuelta* (gross slices of ham, turkey, and chicken)
Hormel Foods	USA	Deli meats (sliced ham, turkey, and beef)
Tyson (Ameriqual)	USA	Oven roasted chicken (whole bird, breasts, drumsticks, etc.)
Oscar Mayer	USA	Deli meats (sliced ham turkey and chicken)
Perdue Farms	USA	Poultry strips
Kayem Foods	USA	Chicken sausages
Foster Farms	USA	Chicken breast slices and strips
Fresherized foods (AvoMex)	USA	RTE meat meals

Table 26.3 Inactivation levels of *L.monocytogenes* in different meat products submitted to HPP

Product	HP treatment (MPa, min, °C)	Inactivation (log units)	References
Cooked ham model	300, 10, 16	2.63	Jofré et al. (2008a)
Dry-cured ham model	300, 10, 16	0.57	Jofré et al. (2008a)
Fermented sausage (*fuet*)	300, 10, 17 before ripening	1.05	Marcos et al. (2005)
Fermented sausage (*chorizo*)	300, 10, 17 before ripening	0.99	Marcos et al. (2005)
Cooked ham	400, 10, 17	2.01	Aymerich et al. (2005)
Fermented sausage (*fuet*)	400, 10, 17 after ripening	0.58	Jofré et al. (2009a)
Fermented sausage (*fuet*)	400, 10, 17 after ripening	0.47	Garriga et al. (2005)
Fermented sausage (*chorizo*)	400, 10, 17 after ripening	0	Garriga et al. (2005)
Dry-cured ham	347, 9, 16	0.05	Bover-Cid et al. (2011)
Dry-cured ham	450, 5, 11	0.32	Bover-Cid et al. (2011)
Cooked ham	600, 5, 15	≥ 3.79	Jofré et al. (2008d)
Fermented sausages	600, 5, 15	0.96	Aymerich et al. (2009)

in the USA, Europe, and Japan (Table 26.2). However, it has also been shown that the intrinsic properties of the meat products used highly influence the effectiveness of this technology. While high inactivation rates have been demonstrated in raw and cooked meat products, a baroprotective effect has been observed in dry-cured meats. As shown in Table 26.3, inactivation of *L. monocytogenes* after high-pressure (HP) treatments at a similar level is higher in cooked ham than in some other

dry-cured meat products (dry-cured ham and fermented sausages). In contrast, during refrigerated storage, the recovery and growth of survivors is prevented or delayed by the presence of hurdles such as low a_w, acidity, or bacteriocins (Leistner and Gorris 1995; Aymerich et al. 2005; Jofré et al. 2010).

Currently, HP treatments of up to 600 MPa are being successfully applied in the food industry. The availability of equipment able to reach pressures higher than 600 MPa is limited; therefore, research performed at those ultra-high-pressure levels is scarce. The application of 200–900 MPa treatments to *L. monocytogenes*, *Salmonella*, and *S. aureus* grown overnight in models of cooked and dry-cured ham showed that treatments ≥600 MPa decreased *L. monocytogenes* and *Salmonella* to levels below the detection limit (10 CFU/g), while *S. aureus* could be detected even after a treatment of 5 min at 900 MPa (16–18 °C) in some of the replicates (Jofré et al. 2008a). Again, dry-cured ham exerted a baroprotective effect, which could be related with stress cross-protection and interaction among hurdles (Scheyhing et al. 2004; Sagarzazu et al. 2010), and control (absence of hurdles) showed the highest recovery rates. Accordingly, even after treatments at 900 MPa, composition of the food product determines both the immediate effect of pressurization and the ability of the resulting sublethally injured cells to recover. Apart from increasing pressure intensity, inactivation can be enhanced by combination with other factors such as antimicrobials, temperature, etc.

26.2.2 Fresh Meat Products

Muscle tissue of healthy animals is considered to be essentially sterile. However, animals harbor large numbers of various microorganisms on body surfaces that are exposed to the environment, e.g., skin, hooves, and mucosal membranes of digestive and respiratory tracts (Paulsen and Smulders 2003) which can contaminate muscle tissues during slaughtering. Thus, hygiene is the primary factor for controlling initial microbial contamination of fresh meat surfaces, meat being a rich medium, and therefore encouraging the growth of mesophile and psychrophile microorganisms during storage and distribution.

High pressure is a decontamination method that has been described as increasing the quality and safety of fresh meat by inactivating both spoilage and pathogenic microorganisms. Since the 1990s, several studies have been performed on different types of raw meat products, and a wide variety of microorganisms, especially bacteria (pathogens and spoilers), have been shown to be significantly inactivated. In ground beef, a highly perishable derivate of fresh meat, Carlez et al. (1993) observed that pressurization above 200, 280, and 400 MPa for 20 min completely eliminated *Pseudomonas fluorescens*, *Citrobacter freundii*, and *L. innocua*, respectively. The highest inactivation was observed at low (4 °C) and markedly at high (50 °C) holding time temperatures. When studying the evolution of the bacteria during storage, the same authors found that microbial growth was delayed from 2 to 6 days after 200–300 MPa at 20 °C. After treatment at 400–450 MPa *Pseudomonas* sp.,

Lactobacillus sp., and coliforms were completely inactivated after HPP, but recovered after storage at 3 °C in air. When storage was performed under vacuum, only *Pseudomonas* recovered (after 9 days) (Carlez et al. 1994). O'Brien and Marshall (1996) also demonstrated an extension of the shelf life of raw ground chicken. Treatments at 408, 616, and 888 MPa (maximal temperatures during holding time ranged from 14.4 to 28.4 °C) showed estimated microbial spoilage times of 27 days, 70 days, and >98 days, respectively.

E. coli O157:H7 is of major concern to the meat industry. Despite improvements in farming and slaughterhouses, beef products remain a common source of outbreaks of *E. coli* O157:H7 in ground meat, mainly beef (Black et al. 2010). Microbiological studies performed to date have shown that HPP has potential as an additional hurdle for ensuring the safety of ground beef. Gola et al. (2000) showed immediate reductions up to 5 log units by pressure treatments up to 700 MPa at 15 °C in raw minced meat. In ground beef, Morales et al. (2008) compared inactivation by single and multiple-cycle treatments of 400 MPa at 12 °C and demonstrated higher lethality for the latter for the same total length of treatment. For example, to achieve a reduction of 4.4 log CFU/g, a single cycle of 20 min or four 1 min cycles was necessary. More recently, Black et al. (2010) compared the effect of pressurization at 400 MPa for 10 min at −5 or 20 °C and storage at the same temperatures in ground beef. They concluded that the highest reductions in the levels of *E. coli* O157:H7 (3 log CFU/g) were achieved after pressurization at 20 °C followed by storage at −20 °C (an additional 1.5 log reduction). The authors also observed sublethal injury of the surviving cells, which were inhibited by low pH, bile salts, and mild cooking, plus an impairment of toxin production.

The HPP approach has proved to be successful in ensuring the safety of other fresh products apart from raw meat such as pork slurries (Shigehisa et al. 1991), raw smoked pork loin (Karlowski et al. 2002), and duck liver (Cruz et al. 2003). In pork slurries, Shigehisa et al. (1991) showed that Gram-positive bacteria were more HPP resistant (treatments of 10 min at 25 °C) than Gram-negative, concretely, *Campylobacter jejuni*, *P. aeruginosa*, *Salmonella typhimurium*, and *Yersinia enterocolitica* which were inactivated at pressures higher than 300 MPa; *Escherichia coli*, *Saccharomyces cerevisiae*, and *Candida utilis* at pressures higher than 400 MPa; and *Micrococcus luteus*, *Staphylococcus aureus*, and *Streptococcus faecalis* at 600 MPa. *B. cereus* spores were less than 1 log CFU/g inactivated at 600 MPa. In marinated beef loin, treatment at 600 MPa for 6 min at 16 °C (experimental process parameters estimated to be the maximum industrially acceptable according to cost and the available industrial equipment) decreased levels of total aerobics, psychrotrophs, LAB, enterobacteria, yeasts, and *E. coli* to levels below detection limit (1 or 2 log CFU/g). These low levels lasted for 120 days of storage at 4 °C in vacuum packaging. Pressurization also eliminated the presence of *Salmonella* and *Listeria monocytogenes* which were found in several of the beef loin samples (Garriga et al. 2004). In a subsequent study, the same authors artificially contaminated marinated beef loin with ca. 3.5 log CFU/g of food-borne pathogens *L. monocytogenes*, *Salmonella enterica*, *S. aureus*, *Y. enterocolitica*, and *C. jejuni*, spoilage lactic acid bacteria (LAB), *Escherichia coli,* and yeast *Debaryomyces hansenii*. Pressurization

at 600 MPa kept the levels of all studied microorganisms, except for LAB, below detection limit for 120 days of storage at 4 °C under vacuum (Jofré et al. 2009b). An increase in shelf life after HPP was also found in raw smoked pork loin treated at 600 MPa for 10 min (Karlowski et al. 2002) and duck liver treated at 550 MPa for 20 min at 55 °C (Cruz et al. 2003).

The combination of HPP with other hurdles for microbial growth such as natural antimicrobials has not been widely studied in raw meat. Yuste et al. (2000a) evaluated the combined effect of HPP with nisin or lysozyme on aerobic mesophile and psychrotroph populations of mechanically recovered poultry meat and observed enhanced inactivation when nisin and HPP were combined. In contrast, no synergism was observed between HPP and lysozyme.

26.2.3 Cooked Products

Pasteurization destroys vegetative spoilage organisms and food-borne pathogens. Therefore, microbiota of cooked products is mainly represented by microorganisms resulting from recontamination (Thippareddi et al. 2009). According to hurdle technology, perishability of cooked meat products will depend on their physicochemical characteristics, i.e., the number of obstacles to microbial growth they contain.

Cooked ham is a highly perishable product due to its high a_w, pH close to neutrality, and the absence of competing microbiota. Once sold, sliced post-processing contamination is the greatest hazard. However, research has shown that HPP is a useful technology for inactivating food-borne pathogens and extending the shelf life of cooked ham and cooked meat products in general. Indeed, the first pressurized meat product commercialized in the world was sliced cooked ham, which was launched by the Spanish enterprise Espuña in 1998.

The effectiveness of HPP has been widely studied in sliced cooked ham. Karlowski et al. (2002) showed that application of treatments of 300–400 MPa for 10 min did not extend the shelf life of the product; 500 MPa produced a decrease in levels of total bacterial count, psychrophilic bacteria, acidophilic bacteria and *Enterococci*; but application of 600 MPa for 10 min was necessary to reduce the number of all investigated microorganisms by 5–6 log units and extend shelf life to 6–8 weeks. Treatment at 600 MPa for 6 min at 16 °C also demonstrated the ability to avoid growth of yeasts and enterobacteria which can potentially produce off-flavors and delay the growth of LAB as spoilage microorganisms at 4 °C (Garriga et al. 2004). Another study showed that pressurization also reduces safety risks associated with *Salmonella* spp. and *L. monocytogenes*. Treatment of sliced ham at 400 MPa for 10 min at 17 °C did not completely eliminate *L. monocytogenes* nor *Salmonella* spiked at 2.5 log CFU/g, but decreased their levels to <1 log CFU/g. During subsequent storage at 1 °C, both pathogens were inhibited. In contrast, at 6 °C, *L. monocytogenes* increased to 6 log CFU/g after 84 days (Aymerich et al. 2005). In a similar experiment performed at a higher pressure level (600 MPa for 5 min at 10 °C), both refrigeration temperatures inhibited the growth of pathogens, which remained absent in 25 g of product for 90 days of storage. Conversely, baroresistant

S. aureus only diminished 0.9 log units after 5 min at 600 MPa, although the growth of the pathogen was prevented by refrigerated storage (Jofré et al 2008a). In another study at 600 MPa (for 6 min at 31 °C during holding time), a poor decrease of *S. aureus* but complete inactivation of *L. monocytogenes*, *Salmonella*, *C. jejuni*, and *Y. enterocolitica* was reported in cooked ham (Jofré et al. 2009b).

Combined application of HPP and natural antimicrobials such as bacteriocins from LAB and lactate salts has been shown to be very useful for increasing the safe shelf life of sliced cooked ham. Aymerich et al. (2005) showed that treatment of 10 min at 400 MPa (17 °C) reduced counts of *L. monocytogenes* from 2.7 log CFU/g to 0.7, 0.3, and 1.3 log CFU/g in the control, nisin (800 AU/g), and 1.8 % potassium lactate (3 % Purasal® P/Hi Pure 60, Purac Biochem) batches, respectively. During subsequent storage, the presence of nisin inhibited recovery of *L. monocytogenes* for 42 days, while lactate inhibited the pathogen during the entire storage time (84 days at 6 °C). When bacteriocins and lactate were applied through active packaging (interleavers) in slices of cooked ham inoculated with 4.5 log CFU/g of *L. monocytogenes* and submitted to the same HP treatment, the highest reductions (4 log units) and the lowest counts (<1.51 log CFU/g) after 3 months of storage at 6 °C were found in batches containing bacteriocins (i.e., 200 AU/cm^2 of nisin, 200 and 2000 AU/cm^2 of sakacin, and 200 and 2000 AU/cm^2 of enterocins A and B). In contrast, application of lactate through active packaging was not effective and only moderately reduced *L. monocytogenes* counts, which reached levels of 6.5 log CFU/g at the end of storage (Jofré et al. 2007). In contrast to *L. monocytogenes*, *Salmonella* was completely eliminated (from 25 g of ham) by pressurization with nisin-containing interleavers (Jofré et al. 2008b). In another kind of active packaging, alginate films containing enterocins A and B, the efficiency of combining both technologies was also demonstrated using different storage temperatures. While at 1 °C the application of a HP treatment of 10 min at 400 MPa and 17 °C was enough to maintain levels of *L. monocytogenes* < 1.5 log CFU/g, the application of active alginate films was necessary to maintain those levels when storage was performed at 6 °C and after a cold chain break of 24 h at 20 °C (Marcos et al. 2008a). At 600 MPa, the combination of HPP with 800 AU/g of nisin, 1.8 % lactate, and nisin + lactate was more effective in the control of *Salmonella* and *L. monocytogenes* inoculated at 4 log CFU/g than at 400 MPa. After pressurization (600 MPa, 5 min, 10 °C), levels of both pathogens decreased to <1 log CFU/g and were kept at this level for 3 months at both 1 and 6 °C. However, the proportion of samples with the absence of the pathogen in 25 g of product was higher for *L. monocytogenes* (97.6 % of the samples) than for *Salmonella* (88.7 %) and even higher for control and nisin-containing ham batches than lactate. Both HPP and combined treatments were much less effective against *S. aureus*, although additional reduction was observed in the presence of nisin (Jofré et al 2008d). Due to the inability of *S. aureus* to grow at refrigeration temperatures together with the fact that at least 10^5 CFU/g is required to produce enough toxin to elicit symptoms (Food and Drug Administration 2003), *S. aureus* would not be a major concern if food products were properly refrigerated. In another cooked product, Vienna sausages, the application of HPP treatment at 600 MPa for 5 min at 28 °C applied alone resulted in only a modest decrease in the number of positive samples for *L. monocytogenes* 48 h after treatment. Enhanced inactivation

was observed by the combination of HPP with 300 ppm of tert-butylhydroquinone (TBHQ) or 100 ppm of TBHQ plus 100 IU/g of nisin, which eliminated the pathogen from all samples (Chung et al. 2005). Thus, the combination of HPP at 600 MPa combined with antimicrobials appears to be an effective way of obtaining value-added cooked meat products with safe long-term storage life.

Pressurization has also been shown to improve the shelf life and/or safety of other meat products. Blood sausages are traditional cooked meat products popular in many parts of the world. High-pressure treatment of *morcilla de Burgos*, a popular Spanish blood sausage made with onion, rice, animal fat, blood, and different spices, was studied with the aim of extending shelf life, which under refrigeration and vacuum packaging is around 14–21 days. Diez et al. (2008a) showed that the application of HPP treatments of 300, 500, and 600 MPa for 10 min at 15 °C reduced enterobacteria and pseudomonads to levels below their LOD. In contrast, LAB, typical spoilage microbiota under vacuum, were only slightly reduced. After treatment at 600 MPa for 10 min at 15 °C, counts of LAB decreased 1.3 log CFU/g and shelf life was extended by 15 days (Diez et al. 2009a). This shelf-life extension was related to a reduction of bacterial populations and changes in the prevalence of LAB during storage, mainly *W. viridescens* and *L. mesenteroides*, the most intensive spoilage microorganisms in *morcilla* (Diez et al. 2008b, 2009a). In contrast to what occurs with *L. monocytogenes* in cooked ham, the combination of pressurization at 600 MPa with potassium and sodium lactate (3 % Purasal™ Lite S/6) in *morcilla de Burgos* did not enhance the quality of the product (spoilage reduction) when compared with HPP treatment applied alone. In vacuum-packaged cooked poultry meat, *W. viridescens* was found to be the dominant microorganism and no obvious signs of spoilage were observed even when counts were >7 log CFU/g. Due to its high-pressure resistance (<1 log reduction after 2 min at 600 MPa and 18 °C) and ability to inhibit Gram-positive and Gram-negative pathogens, *W. viridescens* could be used to extend the shelf life and microbiological safety of the product (Patterson et al. 2010).

Predictive microbiology offers various tools in the form of mathematical models that can be useful to determine inactivation or growth of microorganisms in foods. Slongo et al. (2009) developed a model of the growth of LAB during storage of vacuum-packaged sliced cooked ham processed by HPP applying Gompertz and logistic models. The main factor influencing the growth of LAB and, consequently, shelf life of the product was pressure intensity. The highest inhibition was found after application of the most severe treatment (400 MPa, 15 min, and 27 °C), which extended product shelf life (LAB levels at 10^7 CFU/g) from 19 days in non-pressurized slices to 85 days.

26.2.4 Dry-Cured Meat Products

Traditionally ripened meat products can be made from chopped meat (fermented sausages) or from whole pieces of meat (dry-cured ham) and have been produced since ancient times to extend the longevity of meat. In fermented sausages, stability is due to acidulation caused by the production of lactic acid and a_w reduction caused by the addition of salts (curing by NaCl, nitrite, and/or nitrate) and drying. In dry-cured ham,

the amount of NaCl is higher than in sausages and the main hurdle to microbial growth is low a_w. However, traditional manufacturing practices cannot assure the elimination of meat-borne pathogens; the application of high pressure processing appears to be a nonthermal processing technology that improves the safety of such products without affecting sensory quality.

26.2.4.1 Fermented Sausages

Fermentation is a widely used preservation method in which microorganisms change the sensory and functional properties of meat products. Fermented sausages often have a long shelf life, but traditional manufacturing practices cannot assure the elimination of meat-borne pathogens such as *S. aureus*, *L. monocytogenes*, *Salmonella*, and verotoxigenic *E. coli* from the final product, especially in low-acid and semidry sausages.

Porto-Fett et al. (2010) demonstrated the effectiveness of HPP applied after fermentation and drying to inactivate *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. (inoculated at 7 log CFU/g of batter) in four types of *Genoa* salami differing in casing diameter (65 and 105 mm) and a_w produced with trichinae-infected pork. After fermentation the pH of the salamis decreased to 4.6–4.8 and pathogen levels decreased, but were not eliminated from any of the salamis. The lowest levels were for *Salmonella* (around 2.2 log CFU/g) and the highest for *L. monocytogenes* (6 log CFU/g in 105 mm diameter casings). Pressurization at 483 MPa for 12 min and 600 MPa for 5 min at 20 °C completely eliminated *Salmonella* and *E. coli* O157:H7, while *L. monocytogenes* reductions ranged from 2 to 6 log units depending on the type of salami. During subsequent storage of 28 days at 4 °C, *Salmonella* remained absent in all replicates and *E. coli* O157:H7 in the majority of the replicates. *L. monocytogenes* only was completely eliminated when treated for 5 min at 600 MPa from the salami with the lowest pH (4.7). However, HPP proved to be useful as a post-process intervention to meet performance standards and/or compliance guidelines for the three pathogens and appeared as an alternative method to heating, curing, or freezing to eliminate *Trichinella spiralis*. The effect of HPP at 600 MPa for 3, 6, and 9 min (at ambient temperature) on the inactivation of *E. coli* O157:H7 was also tested in two types of salami: *Hungarian* (pork meat, pH 4.8, and a_w 0.927) and *All Beef*, less acid (pH 6.3) and with higher a_w (0.968). HPP reduced *E. coli* levels on both salamis by more than 4 log CFU/g. During storage at 15 °C, the numbers of *E. coli* O157 increased on *All Beef* salami, but remained static on the *Hungarian* salami, which had a restrictive pH and a_w (Gill and Ramaswamy 2008).

In slightly fermented sausages, typically produced in Mediterranean countries and characterized by a relatively high pH, microorganisms contaminating raw materials (pathogens and aminogenic bacteria) might not be totally inhibited during the manufacturing process and can compromise the safety and stability of the final product. The application of HPP before fermentation was an additional hurdle to control *Salmonella* but had a negative effect on *L. monocytogenes* due to inactivation of endogenous LAB and consequent delay in pH drop (Marcos et al. 2005). In contrast, pressurization after ripening produced better results at both sensory and microbiological levels. However, treatments of 400 MPa (for 10 min at 17 °C)

applied alone were not enough to completely eliminate *L. monocytogenes*, and the combination of HPP with the use of starter cultures was necessary to eliminate *L. monocytogenes* and *Salmonella*, to control enterobacteria and *Enterococci*, and to reduce the content of biogenic amines in the final product (Garriga et al. 2005; Marcos et al. 2007). The combination of HPP with bacteriocins (enterocins A and B and enterocin AS48) also proved to be useful in reducing *L. monocytogenes* and *Salmonella* spiked at 3 log CFU/g to levels ≤ 1 log CFU/g during storage under refrigeration but especially during storage at room temperature where decrease of a_w was higher. These hurdles could not decrease the levels of *S. aureus*, a baroresistant and low a_w-tolerant bacterium (Jofré et al. 2009a; Ananou et al. 2010). Given the capacity of certain pathogenic bacteria to survive in low-acid fermented sausages, selection of high-quality raw materials and good manufacturing practices are crucial.

26.2.4.2 Dry-Cured Ham

Whole-muscle dry-cured meat products, such as dry-cured ham, have pH values close to neutrality, and low a_w is the main hurdle for the growth of spoilage and pathogenic microorganisms. In dry-cured ham, the inhibitory effect of HPP has been shown to vary depending on a_w of the product. Morales et al. (2006) compared inactivation of *L. monocytogenes* in Iberian and Serrano ham by treatment at 450 MPa for 10 min at 12 °C and observed higher inactivation in Iberian ham ($a_w = 0.904$ and 1.5 log unit decrease) than in Serrano ham ($a_w = 0.880$ and 1.16 log decrease). The authors attributed these differences to the higher concentration of NaCl and lower fat content of Serrano ham. Another study demonstrated that after treatment at 600 MPa for 6 min at 16 °C (31 °C during holding time), sensory freshness of sliced dry-cured ham was maintained due to reduction of aerobic bacteria from 4.8 to 2.1 log CFU/g and diminution of psychrotrophs, LAB, and yeasts to levels below plate detection limit. During 120 days of storage at 4 °C, populations maintained similar levels (Garriga et al. 2004). Furthermore, when dry-cured ham was spiked with a wide range of pathogenic and spoilage microorganisms (i.e., *L. monocytogenes*, *Salmonella*, *S. aureus*, *Y. enterocolitica*, *C. jejuni*, LAB, *E. coli*, and *D. hansenii*) and treated at 600 MPa for 6 min at 31 °C during holding time, all microorganisms decreased to below the limit of detection (3–4 log unit reduction) with the exception of *S. aureus* and LAB, which decreased only 0.5 and 1.6 log CFU/g, respectively. During 120 days of storage at 4 °C, both pressurized and non-pressurized microorganisms remained at the same level (Jofré et al. 2009b). Recently, modeling of HPP inactivation of *L. monocytogenes* in dry-cured ham has shown that pressure time and intensity, but not temperature (in the range of 2.3–24.4 °C), influence inactivation of the pathogen. According to the validated model, treatment of 807 MPa for 5 min would be necessary to attain the 6D reduction recommended by the US Government for RTE products. However, given the inability of *L. monocytogenes* to grow in dry-cured ham, in addition to the assumed levels of post-processing contamination of 10 CFU/g, Hoz et al. (2008) proposed a 2.39D process to meet the US *zero tolerance* policy, which requires, according to the model proposed, treatment of 613 MPa for 5 min (Bover-Cid et al. 2011).

As shown for other meat products, combination of HPP with bacteriocins increases the inhibitory effect of HPP in dry-cured ham. Application of sakacin K, enterocins A and B, and especially nisin together with HPP treatment of 600 MPa for 5 min at 15 °C eliminated *Salmonella* and *L. monocytogenes* spiked at 3.5 log CFU/g and decreased *S. aureus* to <1 log CFU/g in the batch containing nisin. During storage at temperature of abuse (15 °C), none of the pathogens recovered (Jofré et al. 2008c).

In conclusion, from a microbiology point of view, the main goal of HPP in the food industry is to ensure absence or low levels of pathogens and spoilage microorganisms in foods at the time of consumption. In meat products, HPP appears to be a promising technology which is already used by companies and accepted by consumers. Intrinsic and extrinsic factors associated with each meat product result in high- and low-risk products and determine levels of microbial inactivation by HPP and growth during storage. Thus, research efforts are needed to generate realistic data and to determine which combinations would be more effective to ensure destruction of food-borne pathogens in a wide variety of optimal quality meat products. At the same time, development of cost competitive industrial equipment able to reach pressure intensities higher than 600 MPa is necessary.

26.3 HPP Effects on Color, Texture, and Other Quality Characteristics

26.3.1 Raw Meat

In the last 15 years, the HPP effect on the color of raw minced beef, pork, and poultry meat and whole muscles has been studied by many authors. The main reason for this interest is because the most frequent visible effect of HPP on raw meat is a dramatic color change, i.e., meat discoloration, due to protein denaturation caused by the pressure increase necessary to reach microbial inactivation (i.e., >400 MPa). Many studies have focused on the HPP effect on meat color as the main aim of their investigation (Carlez et al. 1995; Goutefongea et al. 1995; Jung et al. 2003), but also in many other studies the effect on color was complementary information to their main objective, i.e., effect on pathogenic and/or spoilage microorganisms and shelf-life extension, among others. In any case, most studies agree on reporting lightness (L^*) increase with increasing pressure, whereas yellowness (b^*) either increases or is not affected, and redness (a^*) is the more variable and dependent on experimental design (i.e., meat species, minced or whole muscle, and HPP conditions).

26.3.1.1 Minced Meat Color

Minced beef from *semimembranosus* muscle subjected to HPP (200–500 MPa, 10 min, 10 °C) showed paler color at pressures above 200 MPa. Lightness (L^*) values increased from 200 to 350 MPa, whereas redness (a^*) values decreased with increasing pressure, and yellowness (b^*) did not change significantly. The pH of minced

meat increased with HPP from 5.55 to 5.78 (Carlez et al. 1995). Discoloration of HPP red meat is caused both by a whitening effect, probably related to globin denaturation, that would occur between 200 and 350 MPa (causing an increase in L^* values and turning the red color of beef into a paler pink), and by a loss of red color between 400 and 500 MPa (causing a decrease in a^* values and turning the pale pink color into a pale gray-brown) which could be attributed to the oxidation of ferrous myoglobin into ferric metmyoglobin (Carlez et al. 1995). Frozen ground beef (−15 °C) submitted to high-pressure thawing (210 MPa, 30 min, and 280 MPa, 25 min, room temperature) showed higher lightness (L^*) and lower redness (a^*) at 280 MPa than at 210 MPa or samples thawed in a cooler at 3 °C (Zhao et al. 1998). Fresh minced pork (*m. gluteus superficialis*) and beef (*m. semimembranosus*) meat showed brownish-grayish color after HPP (600 MPa, 30 min, 20 °C), which was consistent with significantly higher L^*, a^*, and b^* values and oxymyoglobin oxidation to metmyoglobin (Goutefongea et al. 1995). Ground beef lean meat subjected to HPP (400 MPa, 1–20 min single cycles and multiple cycles of up to 3 × 5 min, 12 °C) showed higher lightness (L^*) and yellowness (b^*) values, in the exterior of beef patties, with increasing treatment length and number of cycles. Similarly, color measurements of the inner part of beef patties showed higher lightness but lower redness (a^*) (Morales et al. 2008). Pressurization above 300 MPa (HPP: 200–800 MPa, 20 min, 20 °C) caused oxymyoglobin denaturation (ferric form) in post-rigor minced pork and pH increase from 5.90 to 6.06 (measured in muscle suspension) as a result of protein denaturation (Cheah and Ledward 1996). Pork loin slurries subjected to HPP (100–600 MPa, 10 min, 25 °C) showed discoloration with increasing pressure. Higher lightness (L^*) and lower redness (a^*) were observed above 300 MPa, whereas yellowness (b^*) increased above 400 MPa (Shigehisa et al. 1991). Minced chicken thighs (HPP:500 MPa, 60 min, −10, +5, 20, and 50 °C) showed a cooked appearance (lightness increase and redness decrease) at all temperatures tested (Beltran et al. 2004). Thawed minced chicken breast mixed with spices (HPP:300, 600, and 800 MPa, 10 min, initial $T = 5$ °C and reaching max. $T = 22.6$, 40.2, and 49.7 °C, respectively) showed increased lightness and reduced redness with increasing pressure, irrespective of use of spices (Mariutti et al. 2008).

26.3.1.2 Whole Muscle Color

In beef *biceps femoris* muscle (2.5 × 2.5 × 3.0 cm samples), instrumental color parameters (L^*, a^*, and b^*) increased significantly after HPP (520 MPa, 260 s, 10 °C) at 2 days post-mortem. However, color differences after cooking between HPP and unprocessed samples were reduced to less than one third (Jung et al. 2000). Redness (a^*) values of beef *biceps femoris* muscle (2.5 × 2.5 × 2.5 cm samples) (HPP: 50–600 MPa, 20–300 s, 10 ± 3 °C) increased up to 350 MPa, but above this pressure started to decrease. Metmyoglobin content followed redness evolution with increasing pressure, but conversely, i.e., metmyoglobin content decreased with increasing pressure up to 300 MPa approximately and then increased up to 600 MPa. Total color difference between HPP and unprocessed meat increased significantly with

increasing pressure. In concordance with these results, reflectance spectra showed that HPP meat had higher lightness than the control. However, reflectance spectra of raw HPP samples were very similar to those of the cooked control, suggesting that HPP could induce myoglobin modifications similar to cooking. Furthermore, no color differences between cooked control and raw pressurized samples after cooking were observed. Color evolution up to 4 days of storage showed higher redness in HPP meat at 130 MPa (10 °C; 260 s) than in control samples, whereas meat at 520 MPa showed lower values. In contrast, after 7 days of storage, meat at both 130 and 520 MPa showed lower redness than the control. Evolution of redness (a^*) during storage was well correlated with metmyoglobin content (Jung et al. 2003). Beef *semitendinosus* muscle steaks subjected to HPP (100–500 MPa, 5 min, 15 ± 3 °C) showed significant lightness (L^*) increase with increasing pressure above 200 MPa and up to 500 MPa. Redness (a^*) decreased at 500 MPa, whereas yellowness (b^*) increased above 300 MPa (Kim et al. 2007). Raw beef *longissimus dorsi* samples (cylinders: 2.5 cm Ø × 6.0 cm length) showed increased lightness (L^*) and yellowness (b^*) and reduced redness (a^*) values after HPP (650 MPa, 10 min, 20 °C) with respect to the control. In contrast, HPP (650 MPa, 10 min, −35 °C) of raw beef frozen at −30 °C did not affect L^* and b^* parameters measured after thawing, but a^* values were lower than the control. Despite the redness reduction observed, freezing would apparently protect beef meat against negative color changes, i.e., lightness increase, caused by HPP (Fernández et al. 2007). Beef *longissimus dorsi* muscle (2.5 × 2.5 × 3 cm samples) subjected to HPP (200–600 MPa, 20 min, 10–30 °C) showed lightness (L^*) increase in all pressure × temperature combinations tested. Yellowness (b^*) increased only at 400 and 600 MPa, whereas redness (a^*) was not affected. However, when comparing HPP, independently from process temperature, redness reduction was observed at 600 MPa, which could be related to ferric metmyoglobin formation. The highest lightness values were observed at 400 MPa, followed by 600 MPa. Meat at 400 and 600 MPa showed the highest yellowness and total color increase values. The temperature effect, independently of pressure level, lightness, and total color increase, showed the highest values at 30 °C (Marcos et al. 2010). Beef *pectoralis profundus* steaks showed a higher lightness (HPP:200–400 MPa, 20 min, 20 and 40 °C) in all processes except for the mildest one at 200 MPa and 20 °C. Temperature effect, independently of pressure level, showed the highest lightness and the lowest yellowness values at 40 °C (McArdle et al. 2010). Fresh beef (*m. semitendinosus*) and pork (*m. longissimus dorsi*) submitted to HPP (200 MPa, 2.5 h, −20 °C, plus 0.5 h at 30 °C before depressurization) and pressure shift freezing (200 MPa, 2.5 h, −20 °C) showed higher lightness, redness (only in beef), and yellowness than raw samples, after overnight thawing at 20 °C, in both treatments (Fernández-Martín et al. 2000). Fresh pork loin (post-rigor) submitted to HPP (414 MPa, 9 min at 2 °C and 13 min at 25 °C) showed higher lightness (L^*) and yellowness (b^*) than control samples, but no redness (a^*) differences. These color differences continued during storage at 4 °C up to 33 days. However, these color differences disappeared after cooking (Ananth et al. 1998). Fresh pork rib portions (cylinders: 5 cm Ø × 16 cm length) submitted to pressure shift freezing (100, 150 and 200 MPa, 3 MPa s^{-1}, −11, −16.5, and −21.5 °C, respectively) showed increase

in lightness, yellowness, and total color difference with increasing pressure, after thawing at room temperature (20 °C) (Zhu et al. 2004). Pork meat (cylinders, 3.2 cm Ø × 2.8 cm length; HPP, 600 MPa, 10 min, ambient *T*) showed higher lightness (L^*) and yellowness (b^*) and lower redness (a^*) than control samples (Wackerbarth et al. 2009). Different meat species (pork, *m. longissimus dorsi*, turkey and chicken, *m. pectoralis superficialis*) showed clear color change after HPP (100–600 MPa, 1 min, 10 °C) with increasing lightness (L^*) above 300 MPa (above 200 MPa in chicken) (Tintchev et al. 2010). Chicken breast fillets subjected to HPP (300–600 MPa, 5 min, 15 ± 3 °C) showed higher L^*, a^*, and b^* values with increasing pressure. However, redness reduction was observed at 600 MPa, though it was still higher than the control (Kruk et al. 2011). In another study, chicken breast fillets submitted to HPP (400 MPa, 1–20 min single cycles and multiple cycles of up to 10 min overall, 5 °C) showed higher L^*, a^*, and b^* values in all treatments (Del Olmo et al. 2010).

26.3.1.3 Texture and Other Quality Characteristics

During HPP (isostatic compression), although no shear forces are produced, some texture changes occur as a result of the increasing pressure effect on the structure and functionality of proteins (Cheftel and Culioli 1997). Quality changes in post-rigor meat and muscle protein gelation, i.e., texture changes induced by pressurization, have been studied by different authors at both nonthermal denaturing temperatures (before reaching 30–35 °C) and in heating under pressure (thermal denaturing conditions >40 °C) (Jiménez-Colmenero 2002). Generally, HPP at non-denaturing temperatures has been reported to increase cooking loss and texture parameters such as TPA hardness and Warner-Bratzler shear force. In contrast, heating under pressure (HPP) seems to decrease texture characteristics like shear force and hardness. In addition, some studies with seemingly contradictory results could probably be explained by different effects, often opposite, resulting from different combinations of pressure, temperature, and other HPP parameters.

In a nonthermal denaturing study, frozen ground beef (−15 °C) submitted to high-pressure thawing (280 MPa, 25 min, room temperature) showed higher cooking loss than conventional thawing, although no differences in penetration force were observed (Zhao et al. 1998). Ground beef lean meat subjected to HPP (400 MPa, 1–20 min single cycles and multiple cycles of up to 3 × 5 min, 12 °C) showed higher Kramer shear force and energy values than the control (Morales et al. 2008). Fresh pork loin showed no differences in moisture content, water-holding capacity, or instrumental texture (peak load) between HPP (414 MPa, 9 min at 2 °C and 13 min at 25 °C) and control samples (Ananth et al. 1998). Fresh pork (*m. longissimus dorsi*) and beef (*m. semitendinosus*) submitted to pressure shift freezing (200 MPa, 2.5 h, −20 °C) showed higher drip loss (centrifugal method) than HPP meat (200 MPa, 2.5 h, −20 °C plus 0.5 h at 30 °C before depressurization) and raw meat (Fernández-Martín et al. 2000). Beef *biceps femoris* (BF) and *longissimus dorsi* (LD) muscles (HPP:520 MPa, 260 s, 10 °C) showed higher Warner-Bratzler shear force (toughness increase) and cooking loss values than the control meat during

aging (2–17 days post-mortem). HPP appeared to delay natural tenderization (i.e., shear force decrease) in both BF and LD muscles (Jung et al. 2000). Fresh pork rib portions submitted to pressure shift freezing (100–200 MPa, 3 MPa s^{-1}, −11 to −21.5 °C) were tougher, i.e., showing higher Warner-Bratzler shear force values, than unprocessed meat (Zhu et al. 2004). Similarly, beef *semitendinosus* muscle steaks (HPP:100–500 MPa, 5 min, 15 ± 3 °C) showed an increase in Warner-Bratzler shear force and TPA (texture profile analysis) hardness at 500 MPa. However, a decrease in these two parameters was observed at 300 MPa. Cooking loss values increased with increasing pressure and stabilized at 300 MPa, whereas water-holding capacity decreased at 200 MPa and remained stable. The pH values (measured in muscle suspension) increased with increasing pressure above 200 MPa (Kim et al. 2007). Raw HPP beef *longissimus dorsi* (650 MPa, 10 min, 20 °C) showed higher expressible moisture (centrifugal method) than unprocessed meat. In contrast, air-blast frozen-HPP raw beef (650 MPa, 10 min, –35 °C) showed lower expressible moisture than raw HPP beef, indicating a freezing protective effect against HPP protein denaturation (Fernández et al. 2007). Water-holding capacity (WHC) of beef *longissimus dorsi* muscle (measured as expressible moisture with a centrifugal method) decreased with increasing pressure at 400 and 600 MPa, irrespective of the process temperature (HPP:200–600 MPa, 20 min, 10–30 °C) (Marcos et al. 2010). Beef *pectoralis profundus* HPP steaks (200–400 MPa, 20 min, 20 and 40 °C) showed the highest cooking loss at 400 MPa and 20 °C, whereas no differences were observed at 40 °C. HPP at 300 and 400 MPa increased cooking loss and pH values irrespective of process temperature (McArdle et al. 2010). Chicken breast fillets subjected to HPP (400 MPa, 1–20 min single cycles and multiple cycles of up to 10 min overall, 5 °C) showed higher shear force values in single-cycle HPP for 10 min with increasing number of 1 min cycles (Kramer cell) and in single-cycle HPP for 15 min and in 2× 1 min (Warner-Bratzler cell). However, instrumental texture parameters in the most severe multiple-cycle HPP were similar to controls (Del Olmo et al. 2010). HPP chicken breast fillets (300–600 MPa, 5 min, 15 ± 3 °C) showed higher cooking loss and TPA hardness and chewiness at 450 and 600 MPa than unprocessed meat or at 300 MPa (Kruk et al. 2011).

In a study combining nonthermal denaturing temperatures and heating under pressure, beef *longissimus dorsi* muscle (HPP:200–800 MPa, 20 min, 20–70 °C) showed pH increase (between 0.06 and 0.19 units) with increasing pressure, irrespective of the temperature applied. Concerning texture, instrumental hardness (TPA) increased with increasing pressure (HPP at 20 °C) up to 400 MPa and then decreased slightly up to 800 MPa, but was still above the control values. Similar behavior was observed for HPP at 40 °C, although hardness continued to increase slightly up to 800 MPa. In contrast, HPP at both 60 and 70 °C led to hardness decrease at 200 MPa, but above this pressure hardness increased again. In general, all other TPA parameters (chewiness, cohesiveness, springiness, etc.) increased with increasing pressure at both 20 and 40 °C (Ma and Ledward 2004). Beef *longissimus* muscle at different aging times (1–16 days) cooked after HPP (samples preheated at 45 °C for 45 min before HPP: 150 MPa, 30 min, 60 °C) showed lower shear force (Warner-Bratzler) than cooked control samples. In HPP samples, shear

force was very low and did not vary much throughout the aging period. In contrast, control samples showed higher values, the highest being 1 and 8 days of aging. HPP cooking loss was quite similar throughout the aging period and higher than that of the control at aging days 1 and 3 (Bertram et al. 2004). Post-rigor chicken (*pectoralis fundus* muscle, samples: 3×2.5×6 cm) submitted to HPP (200–800 MPa, 30 min, 20–70 °C) showed increase in TPA hardness with increasing pressure from 20 to 50 °C. The highest increase was observed between 200 and 400 MPa from 20 to 50 °C. In contrast, from 60 to 70 °C hardness increased at 200 MPa and subsequently decreased between 200 and 400 MPa, where it stabilized (Zamri et al. 2006). Post-rigor beef neck muscle (*sternomandibularis*; samples: 15×3.5×3.5) submitted to HPP (200 MPa, 20 min, 60 °C) showed higher pH values and lower Warner-Bratzler peak force than the raw control (Sikes et al. 2010).

With regard to sensory characteristics of meat, only a few studies have been focused on the HPP effect on flavor. Suzuki et al. (1994) studied the effects of HPP (100–400 MPa, 5 min, 2 °C) on flavor-related components in beef shoulder lean meat, concluding that HPP caused similar flavor changes to those caused during meat conditioning. Similarly, Schindler et al. (2010) studied aroma development in HPP beef sirloin and chicken breast meat (400 and 600 MPa, 15 min, 5 °C) and reported no significant changes when compared with raw and cooked meat.

26.3.2 Cooked and Raw Salted Meat Products

In general, HPP effect on muscle proteins and minced meat products depends on the combination of pressure, temperature, time, and product type (Jiménez-Colmenero 2002). Many studies of minced meat products have reported HPP effects on color: lightness (L^*) increase with increasing pressure and, to a lesser extent, a decrease in redness (a^*) and affecting yellowness (Jiménez-Colmenero et al. 1997; Carballo et al. 2000; Sikes et al. 2009). Furthermore, HPP at non-denaturing temperatures has been reported to improve texture in low-salt minced meat products by improving protein gelation and thus increasing the water-holding capacity (Iwasaki et al. 2006; Sikes et al. 2009). In contrast, heating under pressure (HPP with thermal denaturation) produces less protein denaturation than thermal treatment alone, and the resulting gel structures have improved water-holding capacity, although texture characteristics like hardness, cohesiveness, and chewiness are decreased, i.e., producing weaker gels (Jiménez-Colmenero et al. 1998b; Carballo et al. 2000; Fernández-Martín et al. 2002; Jiménez-Colmenero 2002). A compilation of studies carried out over the last 15 years on the HPP effects on the quality characteristics of processed minced meat products, i.e., sausage batters, patties, gels, and emulsions from different meat species in combination with other effects, is presented in Table 26.4.

Cured minced beef meat (100–200 mg/kg sodium nitrite, 10 g/kg NaCl, vacuum-packaged and stored overnight at 5 °C to allow nitrosomyoglobin formation) showed higher lightness (L^*) values with increasing pressure (HPP:350–500 MPa; 10 min; 10 °C) but no differences in redness (a^*) and yellowness (b^*). In cured meat products,

Table 26.4 Compilation of studies carried out over the last decade regarding HPP effects on the quality characteristics of processed minced meat products (i.e., sausage batters, patties, gels, and emulsions) from different meat species in combination with other effects. Studies are listed according to HPP increasing temperature (i.e., from non-denaturing temperatures to heating under pressure)

Meat species and product	HPP conditions	Other effects	Quality characteristics evaluated	References
Beef patties	300 MPa, 5 min, 4 °C	HPP vs. ionizing irradiation	Cooking loss and consumer acceptability	Schilling et al. (2009)
Beef patties with pork backfat	100 and 300 MPa, 5 and 20 min, 5 °C	Different fat levels	Water and fat binding properties, instrumental texture (Kramer) and color	Carballo et al. (1997)
Pork batters (emulsion)	100 and 300 MPa, 5 and 20 min, 6–8 °C	Different fat levels	Emulsion stability, instrumental texture (Kramer) and color	Jiménez-Colmenero et al. (1997)
Beef batters	100–400 MPa, 2 min, 10 °C	Different salt levels	Cooking loss, instrumental texture (TPA) and color	Sikes et al. (2009)
Pork batters	400 MPa, 10 min, 10 °C	Addition of different amounts of walnut and heating at 70° for 30 min after HPP	Weight loss, instrumental texture (penetration test and TPA) and color	Ayo et al. (2005)
Pork patty	100–400 MPa, 10–20 min, room temperature	Different salt levels and heating at 70° for 20 min after HPP	Cooking loss and apparent elasticity	Iwasaki et al. (2006)
Duck muscle gels with curdlan	100–500 MPa, 10–40 min, 20–22 °C	Heating at 80° for 30 min after HPP	Cooking loss, water-holding capacity, instrumental texture (TPA) and color	Chen et al. (2010)
Pork batter	600 MPa, 20 min, 10–40 °C	Different salt and nitrite levels	Instrumental texture (compression) and color	Hajós et al. (2004)
Chicken meat and egg gels without phosphates	500–900 MPa, 30 min, 40 °C	Transglutaminase and final heating at 75 °C for 5 min	Yield, expressible moisture, instrumental texture (TPA) and color	Trespalacios and Pla (2007a)
Chicken meat gels	500 MPa, 30 min, 40 °C	Transglutaminase and final heating at 75 °C for 5 min	Expressible moisture, instrumental texture (TPA) and color	Trespalacios and Pla (2007b)
Pork patties	300 MPa, 15 min, non-denaturing and denaturing temperatures: 5–50 °C	–	Instrumental color	López-Caballero et al. (2002)

(continued)

Table 26.4 (continued)

Meat species and product	HPP conditions	Other effects	Quality characteristics evaluated	References
Turkey gels	250–300 MPa, 15 min, non-denaturing and denaturing temperatures: 4–50 °C	Transglutaminase and prior to HPP: cooking at 90 °C for 20 min or set at 25 °C/40 °C for 2 h or cooked after setting for 2 h	Torsion test	Ashie and Lanier (1999)
Ostrich batter (*yor*, Thai sausage)	600 MPa, 40 min, heating under pressure: 50 °C	Hydrocolloid effect: carboxymethylcellulose, locust bean gum, and xanthan gum	Rheological measurements	Chattong et al. (2007)
Pork batters	300 MPa, 30 min, 10 °C and 300 MPa, 30 min, heating under pressure: 70 °C	Raw meat subjected to different freeze-thaw cycles prior to batter elaboration. Samples from HPP at 10 °C were subsequently heated at 70 °C for 30 min	Weight loss and instrumental texture (TPA) and color	Carballo et al. (2000)
Chicken batters	400 MPa, 30 min, 10 °C, and 400 MPa, 30 min, heating under pressure: 70 °C	Cathepsin D inhibitor	Weight loss and instrumental texture (dynamic rheological assessment, penetration test, and TPA)	Cofrades et al. (2003)
Pork batters	200 and 400 MPa, 30 min, heating under pressure: 10–70 °C	–	Cooking loss and rheological assessment (penetration force, apparent elasticity and gel strength)	Fernández-Martín et al. (1997)
Pork and chicken batters	200 and 400 MPa, 30 min, heating under pressure: 60–80 °C	Different salt levels	Weight loss, instrumental texture (TPA) and color	Jiménez-Colmenero et al. (1998b)
Pork and chicken batters	200 and 400 MPa, 30 min, heating under pressure: 70 °C	–	Weight loss and instrumental texture (TPA)	Jiménez-Colmenero et al. (1998a)
Chicken batters	200 and 400 MPa, heating under pressure: 70 °C	Addition of starch, egg white, and Iota-carrageenan	Weight loss, instrumental texture (TPA) and color	Fernández et al. (1998)
Pork batters	400 MPa, 30 min, heating under pressure: 70 °C	Salt and phosphate and heating at 70 °C for 30 min without previous HPP	Weight loss and instrumental texture (TPA)	Fernández-Martín et al. (2002)

nitrosomyoglobin resists the HPP-induced oxidation into ferric form. However, the HPP-induced "whitening" effect (protein denaturation) is not prevented (Carlez et al. 1995). In another study, raw sausage batter with and without NaCl and nitrites subjected to HPP (600 MPa, 20 min, room temperature) showed visual discoloration, higher lightness (L^*), and lower redness (a^*) than the control samples. Furthermore, discoloration was higher in HPP batters without nitrites than in HPP batters with nitrites. HPP reduced batter softness, especially in batters with salt (Farkas et al. 2002).

26.3.2.1 Ready-to-Eat Meat Products

Frankfurter-type sausages, elaborated with different percentages of mechanically recovered poultry meat and minced pork meat and submitted to HPP (500 MPa, 30 min, 50–75 °C), showed higher lightness (L^*), yellowness (b^*), and cohesiveness (TPA) and lower hardness and springiness than cooked sausages (30 min, 75 °C) (Yuste et al. 1999). Raw minced beef (HPP:150 and 300 MPa; 5 min; 20 °C) was used for the elaboration of frankfurters with reduced salt content (1.5 % and 2.5 % NaCl). Frankfurters with HPP beef at 150 MPa showed lower cooking loss values than controls, whereas emulsion stability (% expressible fluid) was similar to controls. In contrast, frankfurters with HPP beef at 300 MPa showed the lowest emulsion stability, i.e., higher expressible fluid. No HPP effect on color was observed. In general, HPP frankfurters were more juicy but with lower overall texture and acceptability scores than the controls. Salt-reduced frankfurters (1.5 %) with 300 MPa treated beef had less smoky and spicy flavor values. Instrumental texture parameters (TPA) decreased at 300 MPa in comparison with controls and 150 MPa, irrespective of salt level. Furthermore, HPP (300 MPa) salt-reduced frankfurters had lower TPA hardness and chewiness, i.e., better texture attributes than the controls (Crehan et al. 2000). In another study, frankfurter-type cooked sausages (made with poultry and pork meat) submitted to HPP (500 MPa, 5 and 15 min, 65 °C) showed higher total color difference values than cooked sausages with conventional heat pasteurization or non-pasteurized cooked sausages (although no differences in $L^*a^*b^*$ values were observed). HPP sausages showed higher TPA cohesiveness and lower hardness (less firmness) and weight loss than cooked sausages. According to sensory analysis results, HPP sausages were preferred because of their better appearance (with less gelatin on the surface due to lower formation of exudates), a stronger and more pleasant taste, and better texture, i.e., more juicy, less grainy, and more uniformly consistent (Mor-Mur and Yuste 2003). Raw sausages formulated to obtain low-acid fermented sausages (*fuet* and *chorizo*) were subjected to HPP (300 MPa, 10 min, 17 °C) 1 day after stuffing (i.e., prior to pH decrease and dehydration typical of the ripening stage). Visual discoloration was observed after HPP in both types of sausages. The instrumental color measurements showed a lightness (L^*) increase, but no changes in redness (a^*) were observed due to the protective effect of nitrification against HPP, i.e., nitrosomyoglobin formation. Yellowness (b^*) decreased in HPP *fuet* but not in *chorizo*, probably due to the *chorizo* formulation which includes paprika and cayenne pepper (Marcos et al. 2005). Ostrich-meat *yor* (Thai sausage) heated under pressure (HPP:300–700 MPa, 40 and 60 min, 40 and 60 °C) showed higher

lightness (L^*) and yellowness (b^*) and lower redness (a^*) with increasing pressure and temperature. The amount of released plus expressible water of HPP sausages decreased with increasing pressure and temperature, indicating an increase in cooking yield. Furthermore, gel strength and elasticity of HPP sausages increased gradually with increasing pressure and temperature (Supavititpatana and Apichartsrangkoon 2007). In another study, ostrich-meat *yor* submitted to HPP (200–600 MPa; 40 and 60 min, 40 and 50 °C) showed that increasing pressure, temperature, and holding time increased water-holding capacity and solid-like behavior, indicating a stronger gel structure. Sensory analysis (hedonic) comparing *yors* processed at 600 MPa at 50 °C for 60 min (completely denatured protein) with *yors* elaborated using a conventional steaming process showed that texture, color, juiciness, and acceptability of HPP *yors* were higher than results for the steamed sausages. However, no flavor differences were observed (Chattong and Apichartsrangkoon 2009). Prepacked, sliced, refrigerated, ready-to-eat (RTE) commercial meat products like low-fat pastrami (a cooked, cured, whole beef muscle product), Strasburg beef (a cooked, cured, comminuted beef product), export sausage (a cooked, cured, comminuted beef product), and Cajun beef (a cooked, uncured, whole beef muscle encrusted with spices) were evaluated during shelf life after HPP (600 MPa, 3 min; 20 °C). HPP did not affect pH or water activity (a_w) of RTE products. Consumer acceptability evaluation during shelf life showed no differences between HPP and unprocessed RTE meats (Hayman et al. 2004). Cooked ham (cured and cooked at 68 °C) showed no color changes after HPP (600 MPa; 30 min; 20 °C). In contrast, HPP raw-cured (salted) minced pork and beef meats showed higher lightness (L^*) than unprocessed meat. Furthermore, HPP raw-cured beef also showed higher redness (a^*) and yellowness (b^*) values than the control, whereas raw-cured pork was not affected (Goutefongea et al. 1995). Vacuum-packaged, sliced cooked ham submitted to HPP (200 and 400 MPa; 5 and 20 min; 7 °C) showed no pH or purge loss (liquid loss) differences when compared with controls during chilled storage at 2 °C (López-Caballero et al. 1999). In another study with sliced cooked ham (HPP:300 MPa, 15 min, 5–50 °C), no color changes were observed between HPP ham and control (López-Caballero et al. (2002)). HPP raw smoked pork loin (500 MPa, 10 and 30 min) showed higher lightness values than controls, whereas in HPP cooked pork ham (300–500 MPa, 10 and 30 min) lightness decreased slightly, but visual color and sensory characteristics were not affected (Karlowski et al. 2002). Cooked pork ham (with low or high salt and sodium nitrite contents) submitted to HPP (600 MPa, 10 min, 20 °C) showed higher weight loss during storage than the controls. HPP did not affect color, instrumental texture, (penetration force) or sensory characteristics of ham (Pietrzak et al. 2007).

26.3.2.2 Raw Salted Meat Products

HPP has also been used in marinated or salted raw meat studies. Marinated sliced beef loin (1 % NaCl and without nitrites) subjected to HPP (600 MPa, 6 min, 16 °C) showed visual color modifications, i.e., grayish color, whereas HPP in sliced cooked ham helped to prevent off-odors, ropiness, and color changes, thereby contributing

to the maintenance of sensory freshness for at least 60 days (Garriga et al. 2004). Salted raw beef (*m. longissimus dorsi*; 1 % NaCl) air-blast frozen and with subsequent HPP (650 MPa, 10 min, −35 °C) showed lower expressible moisture than HPP salted raw beef (650 MPa; 10 min; 20 °C) and unprocessed salted raw beef. With regard to color, HPP salted raw beef showed higher lightness (L^*) and yellowness (b^*) than air-blast-frozen-HPP and unprocessed salted raw beef. These results indicate a protective effect of freezing against HPP protein denaturation. Concerning redness, both HPP and air-blast HPP samples showed lower values than the control (Fernández et al. 2007). Turkey breast cubes (2 × 2 × 2 cm cut from thawed muscle) vacuum-packaged, immersed in a NaCl solution (50 g/l), and submitted to HPP (50–300 MPa, pressure holding time = 0.1 s, 25 ± 1 °C) showed higher moisture and NaCl contents with increasing pressure up to 150 MPa. In contrast, moisture and NaCl contents decreased between 200 and 300 MPa, probably due to a decrease in protein solubility. HPP reduced salting time and enhanced moisture content in comparison with salting at atmospheric pressure. HPP with increasing holding time (50–300 MPa, 1–15 min, 25 ± 1 °C) increased NaCl content and decreased moisture content, although these were higher than those in unprocessed samples. TPA hardness and chewiness were lowest at 150 MPa (pressure holding time = 0.1 s), but their values increased at higher pressures. Furthermore, HPP at 100–150 MPa and 15 min holding time also showed the lowest hardness and chewiness values. In general, HPP at 15 min holding time showed higher hardness, cohesiveness, and chewiness than at 0.1 s, at all the pressures tested. The authors concluded that pressure treatment resulted in a tenfold increase in NaCl diffusion coefficient in turkey meat in comparison to salting at ambient pressures (Villacís et al. 2008).

26.3.2.3 Pressure-Assisted Thermal Sterilization

HPP can also be used for food sterilization, although its industrial application is not yet available. Lau and Turek (2007) studied the quality differences between low-acid foods sterilized by HPP and retorting. Fresh chicken breasts sprinkled with barbecue spice rub, grilled for 30 s, and vacuum-packaged were equilibrated, first in a 40 °C water bath for 15 min and subsequently pre-heated in a 90 °C hot water bath until reaching an internal temperature of 80 °C. After heating, the chicken breasts were subjected to HPP by means of a two-pulse process (690 MPa, 90s pressure come-up time and 1 min holding time; 106 ºC maximum temperature attained during pressurization) with pressure release between the two consecutive pulses. The sensory evaluation results showed that HPP chicken "retained the flavor of a fresh roast chicken" and was moist and tender in contrast with the retorted chicken that had a "flavor similar to stewed or canned chicken" and was fibrous, tender, and soft. Furthermore, HPP chicken showed a muscle structure that was more intact than retorted chicken, which had lost muscle integrity and "tended to 'fall apart' parallel to the grain." However, the instrumental texture test (single blade shear) showed no differences between HPP and retorted chicken. With regard to color evaluation, HPP chicken was lighter (higher L^*) and yellower (higher b^*) than retorted chicken.

26.3.3 Dry-Cured Meat Products

Conventional thermal pasteurization is not a good option for application to dry-cured meat products due to the dramatic color and texture changes these products undergo when heated. Alternatively, HPP allows the submission of dry-cured meat products to cold pasteurization with minimal changes in product quality (Hugas et al. 2002). Therefore, HPP meets the needs of industry for greater microbiological safety and longer shelf life of sliced meat products. As evidence of its effectiveness and good consumer acceptability (Baron et al. 1999; Nielsen et al. 2009), several food companies throughout the world are currently using HPP on an industrial scale to cold pasteurize meat products (see Table 26.2) (Balasubramaniam et al. 2008) and, particularly, sliced ready-to-eat meat products, including a wide range of dry-cured meat products such as low-acid and acid fermented sausages and dry-cured ham and loin (Hyperbaric 2006; Avure 2009).

26.3.3.1 Fermented Sausages

Low-acid cured fermented sausages (Catalan *fuet* and Spanish *chorizo*, pH > 5.3) after 28 days of ripening (water activity, 0.854–0.878) were subjected to HPP (400 MPa, 10 min, 17 °C). HPP low-acid fermented sausages showed no instrumental color changes, although a slight decrease in color intensity was observed by the trained sensory panel in HPP *chorizo*. The texture profile analysis (TPA) showed higher cohesiveness, chewiness, and springiness in both HPP *fuet* and *chorizo*. However, no sensory texture differences were observed in the sensory analysis by trained panelists (Marcos et al. 2007). Sliced cured fermented sausages (Spanish *salchichón*, pH = 5.1–5.2 and $a_w = 0.827–0.853$) at the end of the ripening process were subjected to HPP (500 MPa; pressure come-up time, approx 4 min; pressure holding time, 5 min; decompression time, instantaneous, 18 °C). HPP fermented sausages showed no instrumental color differences and no changes in sensory attributes in comparison with the controls throughout the storage period (from 1 day to 210 days). However, by the end of the storage period, both HPP and control samples showed lower scores in sensory analysis (Rubio et al. 2007a). In the aforementioned studies, the authors suggest that the ripening process leads to color stabilization due to transformation of myoglobin into nitrosylmyoglobin and nitrosyl-haemochrome, as described previously (Carlez et al. 1995; Cheftel and Culioli 1997).

26.3.3.2 Dry-Cured Ham and Other Dry-Cured Muscles

Many recent studies have dealt with HPP effects, not only on dry-cured whole-muscle products such as pork dry-cured ham and loin but also dry-cured beef muscles (*cecina*). In a study with vacuum-packaged pieces of Parma ham (elaborated without nitrites and ripened for 14 and 18 months) subjected to HPP (600 MPa, 9 min), pressurized hams showed a lightness (L^*) and yellowness (b^*) increase and a

redness (a^*) decrease in both HPP 14– and 18–month ripened hams, although redness decrease was lower in HPP 18-month hams. Instrumental color measurements were in concordance with the visual color evaluation, since lower visual color intensity was observed in HPP hams, with differences being more marked in 14–month hams. With regard to sensory evaluation, HPP hams showed higher salty taste, fibrousness on chewing, and consistency (described as resistance to compression) than unprocessed hams. However, HPP differences were lower in hams aged for 18 months in comparison with those aged for 14 months. Longer-ripened hams (18 months) undergo higher dehydration and, presumably, have higher pigment stability and are therefore less exposed to HPP changes because of the protective effect of the lower moisture content on color and sensory characteristics (Tanzi et al. 2004). Parma ham elaboration does not include the use of nitrites, which increase myoglobin stabilization by means of nitrosomyoglobin formation, as reported by Carlez et al. (1995), and this could explain the redness decrease induced by HPP. In another study, sliced dry-cured ham (elaborated with nitrites and with an average moisture content of 50.2 % in the final product) subjected to HPP (600 MPa, 6 min, 16 °C) showed no visual color differences and maintained sensory freshness for up to 120 days (Garriga et al. 2004). Iberian dry-cured ham (samples $4 \times 3 \times 0.3$ cm) submitted to HPP (200–800 MPa, 15 min, 20 °C) showed a lightness (L^*) decrease with HPP, although at 600–800 MPa L^* values increased back to unprocessed samples. Redness (a^*) decreased with pressure increase up to 600 MPa, whereas at 800 MPa a^* values increased again above 400 MPa values (Andrés et al. 2004). In a similar study, slices of Iberian dry-cured ham submitted to HPP (200 and 400 MPa, 15 min, 20 °C) showed a lightness (L^*) increase, especially at 400 MPa and a redness (a^*) decrease (Andrés et al. 2006). Sliced Iberian and Serrano dry-cured hams subjected to HPP (450 MPa, 10 min, 12 °C) did not show color differences except for yellowness (b^*) in HPP Iberian ham, which was higher than before HPP. No detrimental effect of HPP was observed as to the sensory characteristics (score preference of visual appearance, flavor, and texture) of either Iberian or Serrano ham (Morales et al. 2006). The effect of HPP (400 MPa and 600 MPa, 10 min, 10 °C) on the sensory properties of commercial dry-cured hams with texture problems, i.e., defective texture such as pastiness and excessive softness, was evaluated by Serra et al. (2006) in vacuum-packaged slices (1.5 mm thick) alternately assigned to either control or HPP. The flavor attributes of HPP dry-cured hams with defective texture were not affected, except for the BF saltiness, which increased at 600 MPa (scores control vs HPP, 2.9 vs 4.0). However, HPP reduced adhesiveness and pastiness and increased hardness and fibrousness in both *biceps femoris* (Fig. 26.1a) and *semimembranosus* (Fig. 26.1b) muscles. These results show that HPP can be used to improve the sensory texture of pasty and excessively soft dry-cured hams by reducing sensory pastiness and increasing hardness. Furthermore, HPP increase in fibrousness does not affect dry-cured ham texture negatively (Serra et al. 2006).

The effect of HPP (400 MPa, 6 min, 11 °C and 600 MPa, 12–35 min, 20 °C) on whole frozen hams at two early stages of the dry-cured ham process (green hams and at the end of the resting stage) was studied by Serra et al. (2007b). HPP at the green ham stage increased lightness (L^*) of the *biceps femoris* muscle (BF) in the final product (i.e., dry-cured ham) both at 400 MPa and 600 MPa. Similarly, visual

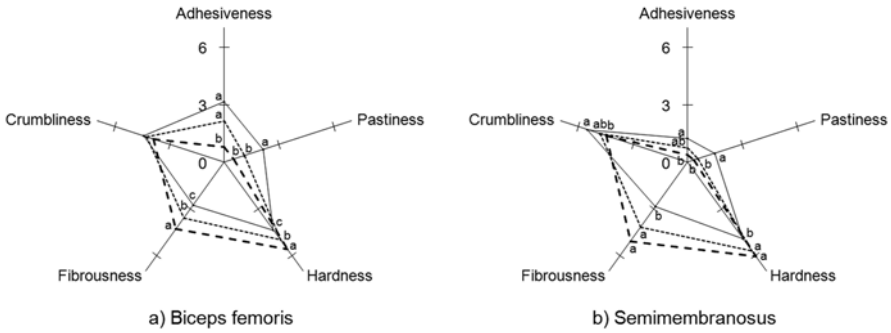

Fig. 26.1 Results of sensory analysis of (**a**) *biceps femoris* and (**b**) *semimembranosus* muscles from commercial dry-cured hams with defective texture, i.e., pastiness and excessive softness (– Control; ⋯ 400 MPa; - - - 600 MPa). Least squares means with a common letter are not significantly different ($P>0.05$)

color intensity was lower (higher discoloration) with increasing pressure. At the end of the resting stage, HPP effect on the color of dry-cured ham was limited to 600 MPa. In general, HPP did not much affect the sensory attributes of dry-cured ham. Nonetheless, at the green ham stage HPP at 600 MPa decreased sensory crumbliness of BF muscle, whereas at the end of the resting stage, HPP at 600 MPa decreased crumbliness and increased fibrousness in both BF and *semimembranosus* muscles of the final dry-cured ham (Serra et al. 2007a). In a study with a slightly different dry-cured ham type, transglutaminase restructured dry-cured hams (boned hams with reduced salt content and potassium lactate; final product, 60–65 % moisture content) subjected to HPP (600 MPa, 6 min, 10 °C) showed a pH increase (0.2–0.3 units) and a water-holding capacity decrease (centrifugal method). The instrumental color of the *biceps femoris* (BF) muscle was affected by HPP, showing an increase in lightness (L^*), redness (a^*), and yellowness (b^*) in comparison with unprocessed samples. Similarly, HPP also affected the slice appearance by increasing brightness and iridescence and decreasing color homogeneity. Pressurization affected both sensory and instrumental (tensile test) texture attributes. HPP increased sensory hardness, gumminess, and fibrousness, whereas adhesiveness and pastiness incidence and its intensity decreased. The tensile test results showed that the apparent Young's modulus (high values indicate lower elasticity) and the breaking stress increased with HPP. Regarding sensory flavor, HPP increased saltiness perception, sweetness, and umami flavors (Fulladosa et al. 2009). Sliced *biceps femoris* muscle from dry-cured Iberian ham and dry-cured Iberian loin submitted to HPP (200 and 300 MPa, 15 and 30 min, <14 °C) showed no effect on color lightness (L^*) and yellowness (b^*). In contrast, redness (a^*) decreased in dry-cured ham with increasing pressure, as it did in HPP dry-cured loin with respect to unprocessed loin, although no a^* differences were observed between the different pressure levels (Cava et al. 2009). Iberian dry-cured ham *biceps femoris* muscle (vacuum-packaged slices and pieces) subjected to HPP (600 MPa, 6 min, 12 °C) showed no color changes in lightness (L^*) or yellowness (b^*), although redness (a^*) decreased slightly and the lean

appearance was less bright. Concerning sensory attributes, HPP samples were harder, chewier, and less juicy and showed higher saltiness, bitterness, cured, and overall flavor scores than unprocessed samples (Fuentes et al. 2010). Commercial dry-cured hams (5 cm thick slices) of two different salt levels submitted to HPP (500 MPa, 7 min, 7 °C) showed higher pink color appearance (i.e., visual lightness), sweetness, saltiness, hardness, and stringiness (fibrousness) and lower crumbliness than unprocessed samples, irrespective of the salt content (Guerrero et al. 2010). Commercial vacuum-packaged sliced dry-cured pork loin subjected to HPP (300–400 MPa, 10 min, 20 °C) showed a lightness (L^*) increase and a redness (a^*) decrease above 300 MPa, which were observed throughout the storage time (Campus et al. 2008). Vacuum-packaged pieces of pork *longissimus* muscle, salted either with NaCl or KCl, without nitrites and dried at different moisture contents, were submitted to HPP (600 MPa, 6 min, 12 °C). Irrespective of the salting treatment, HPP increased lightness (L^*) and sensory fibrousness at moisture contents above 50 %, as shown in Figs. 26.2 and 26.3, respectively. However, in the driest samples (below 50 % moisture content), no differences were observed between HPP and unprocessed loins. The visual color appearance was lighter in HPP samples in agreement with the L^* increase. Furthermore, visual redness was lower in HPP, although no differences were observed for either a^* or b^* (Serra et al. 2009). Dry-cured beef *Cecina de León* (a salted, smoked, and dried traditional product from northwestern Spain) was subjected to HPP (500 MPa, 5 min, 18 °C) in vacuum-packaged cuts (4–5 cm) and slices (1.5 mm). HPP did not affect *Cecina de León* instrumental color (L^*, a^*, and b^*), texture (TPA), or sensory attributes (Rubio et al. 2007b).

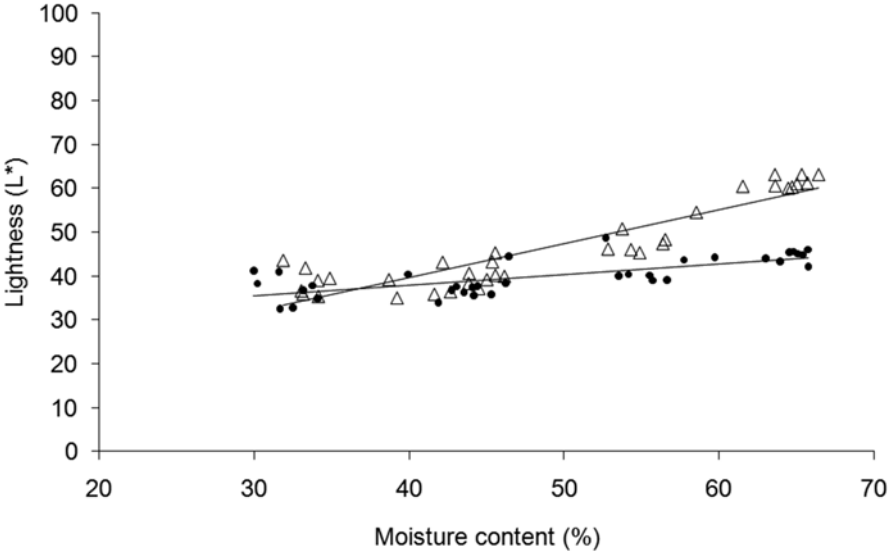

Fig. 26.2 Effect of HPP on the relationship between instrumental lightness (L^*) and moisture content (%) in pork *longissimus* muscle salted and dried at different levels (*filled circle* Control; *open triangle* 600 MPa)

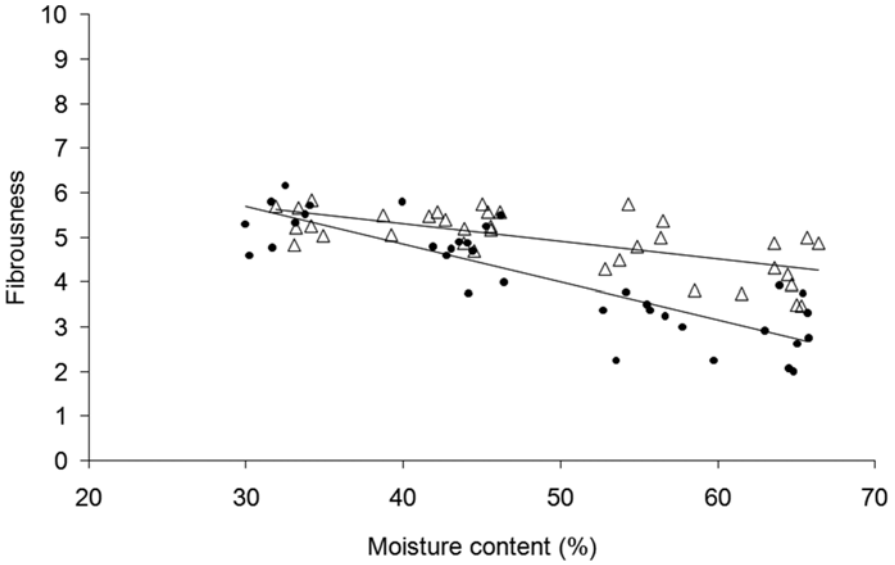

Fig. 26.3 Effect of HPP on the relationship between sensory fibrousness and moisture content (%) in pork *longissimus* muscle salted and dried at different levels (*filled circle* Control; *open triangle* 600 MPa)

As a general conclusion, HPP of dry-cured meat products increases lightness (L^*), decreases redness (a^*), and affects both sensory texture and flavor by increasing fibrousness, hardness, and saltiness. However, it is worth mentioning that in most cases these changes fall within the intrinsic product variability range and, therefore, changes are not to be seen as negative, but as slight product modifications. Furthermore, the extent of most HPP changes in dry-cured meat products is determined by the moisture content of the product, i.e., the moister the product, the bigger the HPP changes expected.

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Chapter 27
Pressure Effects on Seafoods

Gipsy Tabilo-Munizaga, Santiago Aubourg, and Mario Pérez-Won

Abstract Pressure treatment, in combination with refrigeration storage and handling practices, can extend seafood product shelf life and can be effective in reducing microorganisms. This chapter will discuss the effects of high-pressure processing (HP) on seafood products which focused on the sensory and physical properties, microbiological activities, chemical constituents and nutritional values, and existing development patents based on these aspects.

Keywords High pressure • Seafood • Shelf life • Microbial behavior • Patents

27.1 Introduction

27.1.1 Microbiological, Chemical, and Technological Characteristics of Marine Species

Marine foods are known to include high proportions of important constituents for the human diet such as (1) nutritional and digestive proteins including high levels of essential amino acids (lysine, methionine, and others), (2) lipid-soluble vitamins (namely, A and D), (3) microelements (I, F, Ca, Cu, Zn, Fe, and others), and (4) highly unsaturated fatty acids (Simopoulos 1997). Among such components, the lipid fraction is now the subject of a great deal of attention due to its high content of ω-3 polyunsaturated fatty acids (PUFA), which have shown a positive role in preventing certain human diseases.

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In spite of this profitable and valuable composition, marine products are known to be highly perishable. It should be stressed that such food products arise from poikilothermic organisms with both high-water and nonprotein-nitrogen content, a soft muscular and skin structure, and low-collagen content. In addition, the mentioned highly unsaturated lipid composition has shown to be specially prone to lipid oxidation development. Such features mean that these products can be considered to be among the most perishable foods, this demanding a rapid and efficient processing and storage after capture or harvest. Different damage pathways have been identified as responsible for this quality loss, being summarized as microbiological development, endogenous enzyme activity, nonenzymatic lipid oxidation, and browning and enzymatic browning. The relative incidence of each damage mechanism will depend on the kind of technological process applied and on the concrete composition of the marine species involved (Pigott and Tucker 1990; Aubourg 2008).

Most fish and invertebrate marine species give rise to products of great economic importance in many countries. The demand for such products has been increasing steadily during the last century and shows no signs of decreasing. Thus, fishing captures have shown a regular production (FAO 2007a), while a slight but constant increase is being observed for aquaculture production (FAO 2007b). Marine products can be obtained from fresh species through a wide range of technological processes. For years, different traditional strategies have been applied such as cooling (chilling, freezing, and frozen storage), heating (cooking, canning, smoking), salting, and others.

In connection with this trend of seafood consumption, public health concerns have become an issue requiring careful attention, not only to ensure quality and nutrition, but also safety, as the major challenges faced by marine food trade and technologists. The potential health risks associated with seafood, together with the high demand far from local fishing ports, make the need for advanced preservation techniques to be addressed continually. According to the actual need for high-quality fresh products, different recent and advanced technologies have been tested in order to retard marine product damage and accordingly extend the shelf life. One such technology is high-pressure (HP) processing.

27.1.2 General Aspects of HP and Presentation of the Chapter

The modern consumer requires foods that are safe and nutritious, free from additives, taste good, and, for certain products, have a longer shelf life. High-pressure processing (HP), also referred to as high hydrostatic pressure or ultrahigh-pressure processing, is one technology that has the potential to fulfill both consumer and scientific requirements (Patterson et al. 2007). Pressure treatment, in combination with good refrigeration and handling practices, provides a means to increase seafood product shelf life, being effective in reducing microorganisms, and is known as a good method for inactivating pathogens in food materials (Linton et al., 2003; Gómez-Estaca et al. 2007; Cruz-Romero et al., 2008; Erkan and Uretener 2010; Montiel et al. 2012). This technology has demonstrated potential application in the

seafood industry for surimi and kamaboko production as assisting in thawing and thermal processing and for cold-smoked fish preparation.

This chapter will discuss the effects of high-pressure processing (HP) on seafood products which focused on the sensory and physical properties, microbiological activities, chemical constituents and nutritional values, and existing development patents based on these aspects. Following the death of fish, many chemical, biochemical, and physical changes occur leading to the development of postmortem tenderness. A greater understanding of these changes should make an important contribution to the production of high-quality seafood products. While the degradation of myofibrillar and cytoskeleton proteins is desirable for postmortem tenderization of mammalian and avian muscles, such changes can lead to unfavorable changes in fish muscle. Texture remains one of the most important quality attributes affecting consumer acceptance of meat and fish products. In meat products, however, it is tenderness that is important to the consumer, while firmness is key to fish quality (Chéret et al. 2007). Considerable advances have been made over the past two decades with a better understanding of the molecular mechanisms involved in tenderization and textural changes in seafoods' muscle (Eskin et al. 2013).

27.1.2.1 Effect of Pressure Treatment on Sensory and Physical Properties

As a result of an increasing consumer demand for high-quality fresh products, fish technologists and the fish trade have developed different advanced processing systems. Among them, high-pressure technology has shown to maintain sensory and nutritional properties while inactivating microbial development and leading to shelf-life extension and safety enhancement. Nowadays, there is a growing interest in consuming raw or minimally processed foods, and carpaccio is among the most popular when considering fishery products. High-pressure technology can offer an alternative way of treating this ready-to-eat and highly perishable commodity, because pressurization at low or moderate temperatures does not alter the sensorial quality (taste, flavor, and often color) of foods. The appearance of pressurized fish muscle is affected by the treatment used and mainly depends on the pressurization level, i.e., the more the pressure is increased, the more opaque and whiter the muscle becomes, especially at ambient temperature or higher, rather than at low temperatures. Nevertheless, the high sensibility to denaturation of fish myofibrillar proteins, even when subjected to low or moderate pressure, can lead to changes in color and texture, making the treatment unfeasible. This fact, however, may vary from one fish species to another (Gómez-Estaca et al. 2009). Mootian et al. (2013) showed that the volume of clam meat (processed in shell) increased with negligible change in mass after exposure to pressure at 552 MPa for 3 min, while the drip loss was reduced. Clams processed at 552 MPa were softer compared to those processed at 276 MPa. However, all high-pressure-processed clams were found to be harder compared to the unprocessed ones. Pressure-treated clam meat had a glossy appearance compared to the control (Fig. 27.1), and lightness ($L*$) of the meat increased although the redness ($a*$) decreased with increasing pressure.

On the other hand, several studies have reported the effects on physicochemical properties in seafood products, and the results are summarized in Table 27.1.

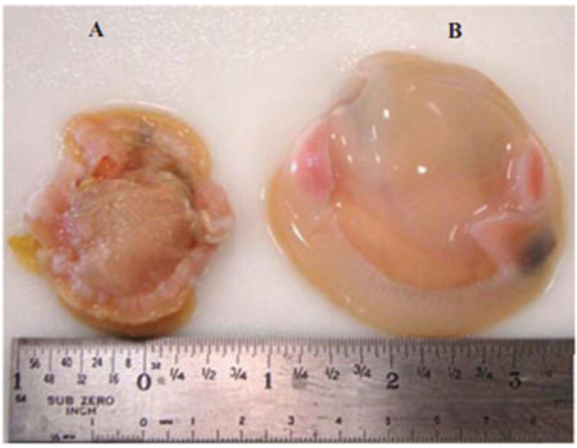


Fig. 27.1 Effect of pressure on the appearance and volume of clam meat. Clams were processed at 552 MPa for 3 min. HP clam (**b**) is larger in volume and lighter in color compared to unprocessed clam (**a**) (from Mootian et al. 2013)

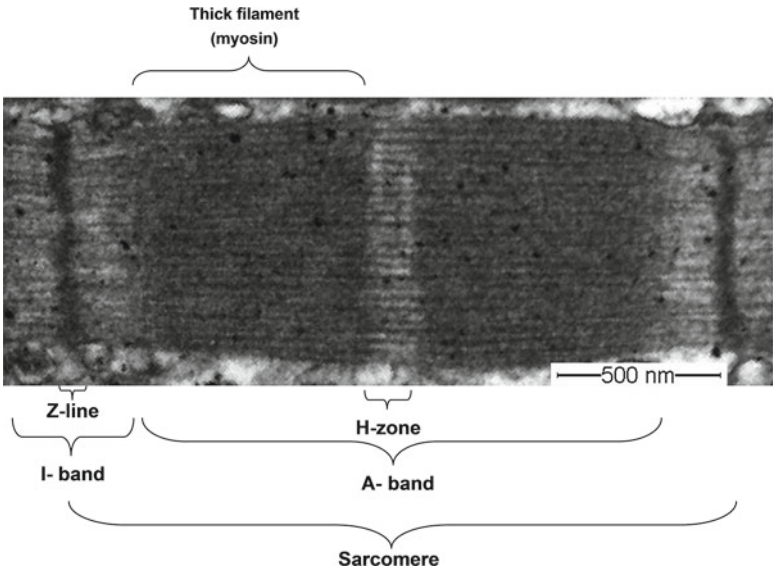


Fig. 27.2 Microphotograph of myofibrillar tissue of jack mackerel. The morphological observations of the myofibrils were done by transmission electron microscopy (26,500×) (from Briones-Labarca et al. unpublished data)

27.1.2.2 Effect of HP on Microbiological Activity

Microbial inactivation is one of the main tasks for the application of HP, and many reports have demonstrated the inactivation effect of HP on microorganisms, extending in this way the microbial shelf life and improving the microbial safety of food products. It has been demonstrated that bacterial spores require more extreme

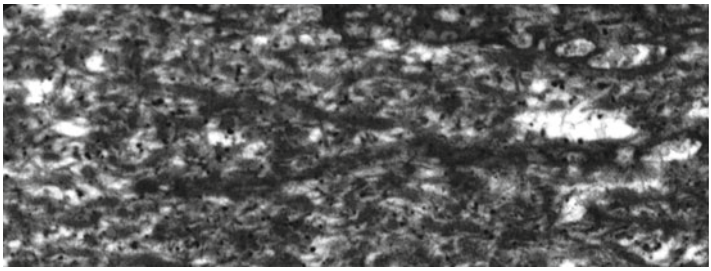


Fig. 27.3 Microphotograph of intramuscular tissue of jack mackerel pressure treated at 550 MPa/4 min (from Briones-Labarca et al. unpublished data)

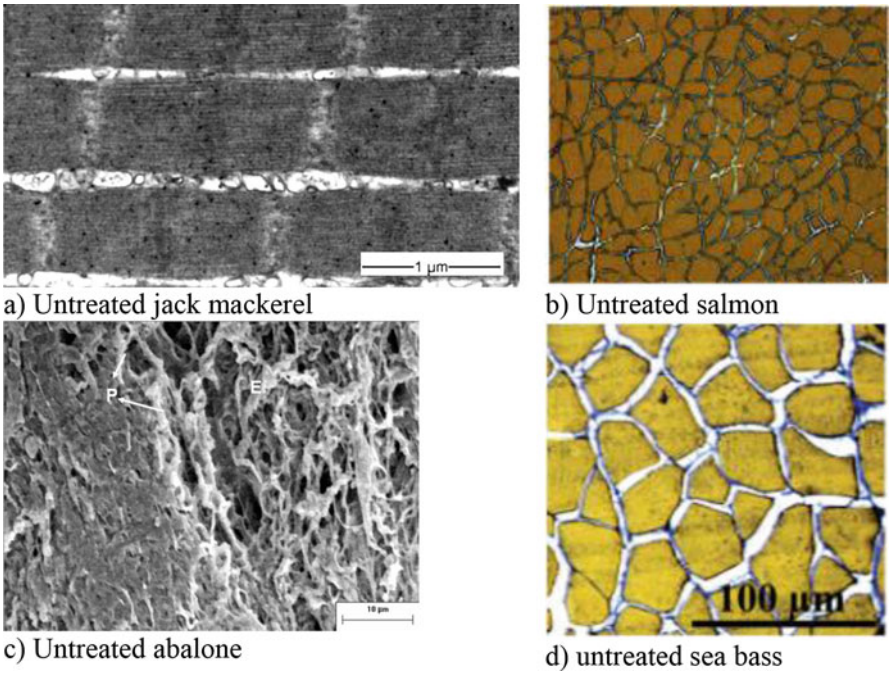


Fig. 27.4 Microphotograph of structure of myofibrillar tissue, post-rigor mortis, of: (**a**) jack mackerel, transmission electron microscopy (16,500×); (**b**) salmon (350×); (**c**) abalone, scanning electron microscopy (350×); and (**d**) sea bass (400×) (from Briones-Labarca et al. 2012, unpublished data; Gudbjornsdottir et al. 2010; Chéret et al. 2005)

conditions of pressure and temperature and longer treatment times to be inactivated. In general, vegetative cells are inactivated at low-pressure levels, around 400–600 MPa, while more resistant bacterial spores can survive pressures higher than 1000 MPa (Yaldagard et al. 2008).

Fish and shellfish are highly perishable food products, due to their high a_w, neutral pH, and presence of autolytic enzymes. During handling and storage, quality

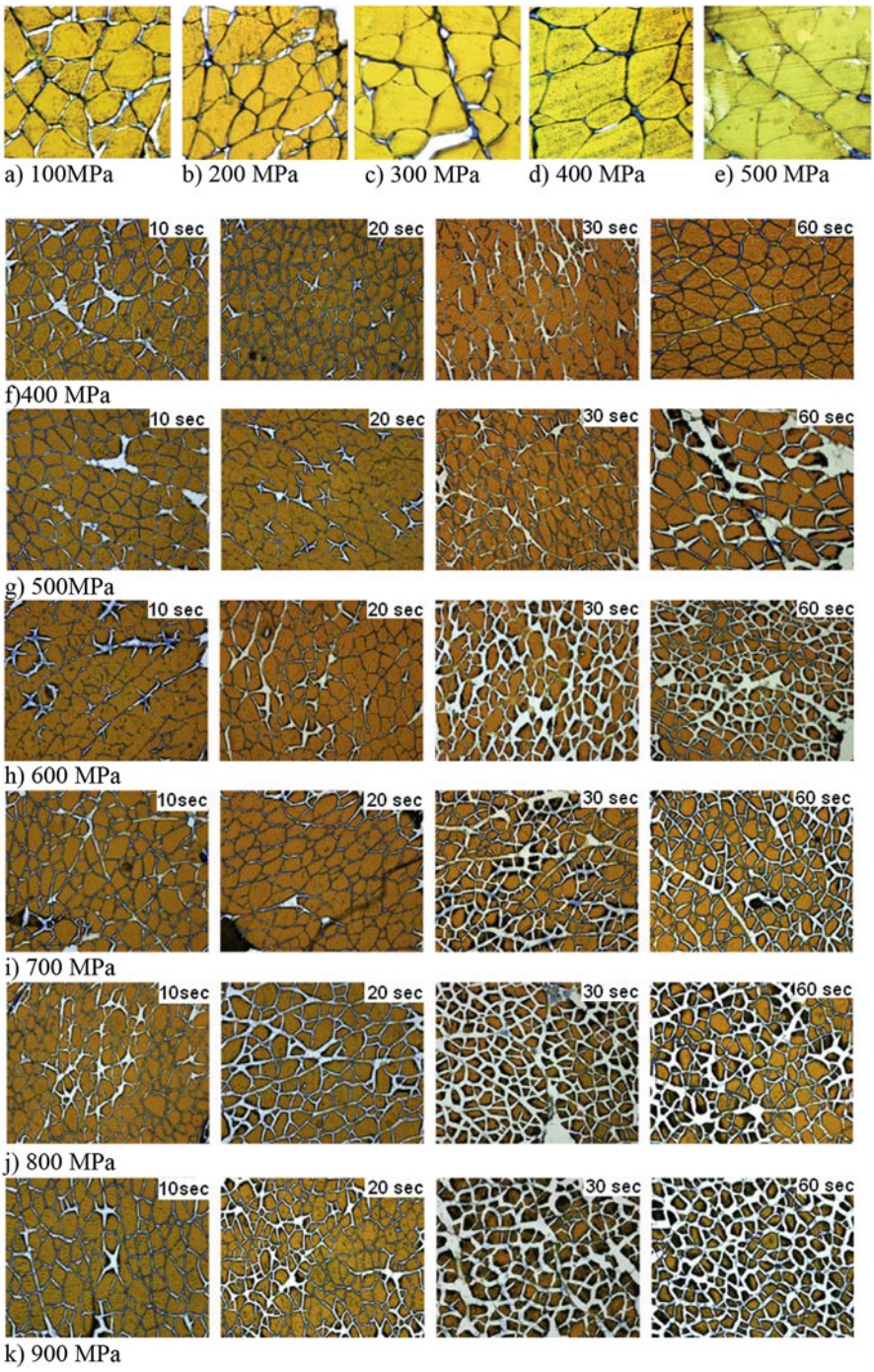


Fig. 27.5 Effect of high-pressure processing on the microstructure of seafoods: (**a**–**e**) optical microphotography (400×) of the sea bass, (**f**–**k**) optical microphotography (350×) of intramuscular tissue of salmon, (**l**–**n**) microphotography of intramuscular tissue of abalone adductor muscle by

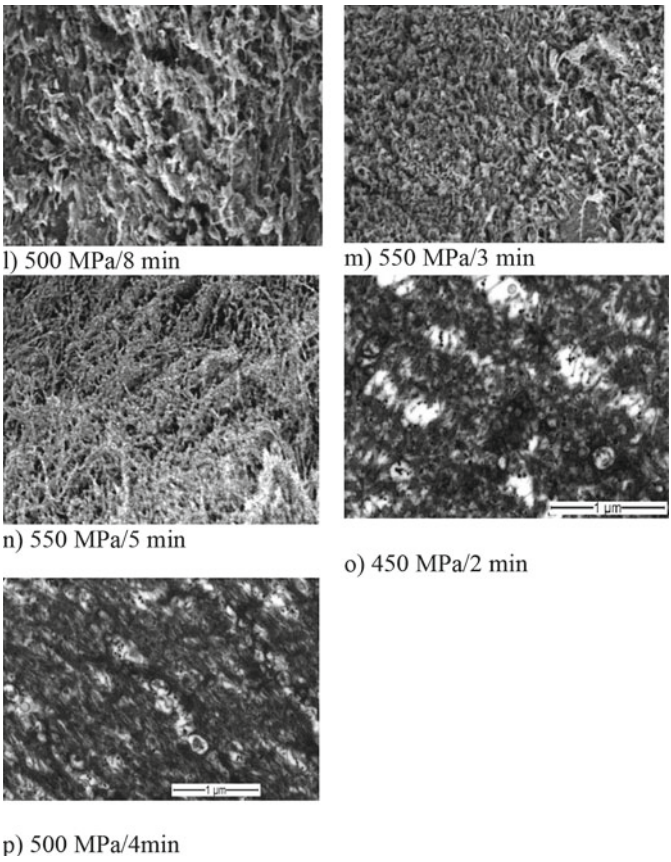
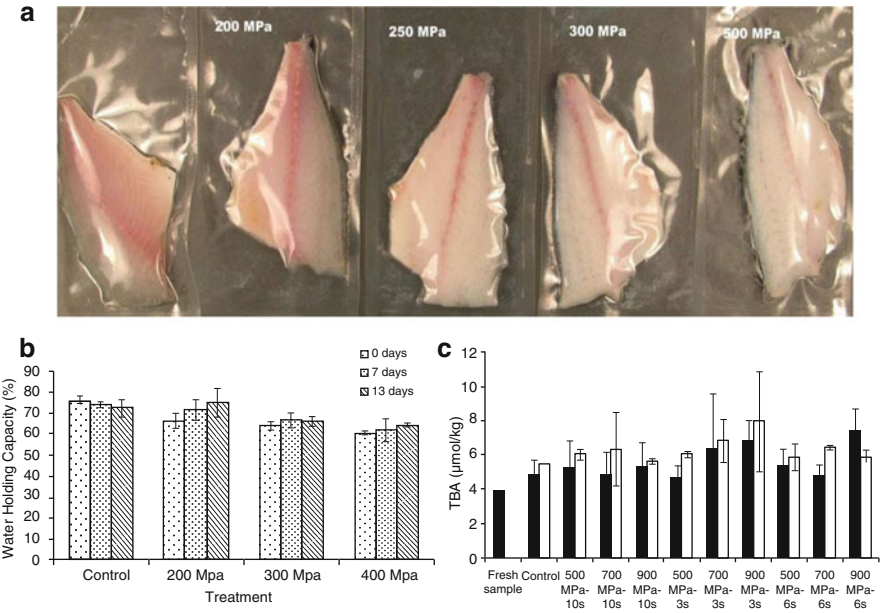


Fig. 27.5 (continued) scanning electron microscopy (350×), (**o**–**p**) microphotography of intramuscular tissue of jack mackerel muscle by transmission electron microscopy (16,500×) (from Chéret et al. 2005; Gudbjornsdottir et al. 2010; Briones-Labarca et al. 2012, unpublished data)

deterioration of fresh fish rapidly occurs and limits the shelf life of the product (Erkan et al. 2010). It is known that loss of freshness and spoilage pattern in fish markedly varies from species to species, physiological condition, environmental influences, and post-harvest handling and storage conditions. Once the fish dies, several postmortem changes take place, which are due to the breakdown of the cellular structure and biochemistry as well as to the growth of microorganisms that are either naturally associated with the fish or associated to contamination during handling (Ocaño-Higuera et al. 2011). Deterioration of fish occurs mainly as a result of bacteriological activity. Although the flesh of freshly caught fish is normally sterile, microorganisms will be found on the skin, on the gills, and in the gastrointestinal tract, and it is these bacteria which promote subsequent spoilage (Karim et al. 2011).



d

TPA parameter	Storage time (day)	Control	Pressure processing during 5 min 100 MPa	200 MPa	300 MPa	400 MPa	500 MPa
Hardness	0	29.82±2.13 $^{a\alpha}$	24.17±3.99 $^{bc\alpha}$	22.78±2.86 $^{b\alpha}$	26.04±3.89 $^{c\alpha}$	31.24±2.24 $^{a\alpha}$	32.70±4.70 $^{a\alpha\beta}$
	7	25.53±4.32 $^{a\beta}$	27.50±3.49 $^{a\alpha}$	20.44±2.72 $^{b\alpha}$	26.39±2.22 $^{a\alpha}$	38.04±2.76 $^{c\beta}$	33.14±4.00 $^{d\beta}$
	14	25.25±3.50 $^{a\beta}$	24.55±4.49 $^{a\alpha}$	23.26±3.85 $^{a\beta\alpha}$	21.09±1.51 $^{b\beta}$	35.01±2.94 $^{c\gamma}$	28.93±3.38 $^{c\alpha}$
Cohesiveness	0	0.52±0.04 $^{a\alpha}$	0.53±0.06 $^{a\alpha}$	0.55±0.03 $^{a\beta\alpha}$	0.56±0.03 $^{a\beta\alpha}$	0.55±0.08 $^{a\beta\alpha}$	0.58±0.04 $^{b\alpha}$
	7	0.25±0.04 $^{a\beta}$	0.42±0.05 $^{b\alpha\beta}$	0.41±0.04 $^{b\alpha\beta}$	0.40±0.05 $^{b\beta}$	0.45±0.07 $^{c\alpha\beta}$	0.46±0.05 $^{d\beta}$
	14	0.30±0.04 $^{a\gamma}$	0.36±0.04 $^{b\gamma}$	0.40±0.04 $^{b\alpha\beta}$	0.43±0.06 $^{c\beta}$	0.50±0.06 $^{d\alpha\beta}$	0.56±0.03 $^{e\alpha}$
Springiness	0	0.69±0.04 $^{a\alpha}$	0.63±0.05 $^{b\alpha}$	0.61±0.04 $^{b\alpha}$	0.65±0.04 $^{a\beta\alpha}$	0.69±0.04 $^{a\beta\alpha}$	0.74±0.06 $^{a\alpha}$
	7	0.51±0.03 $^{a\beta}$	0.55±0.06 $^{ab\beta}$	0.59±0.05 $^{b\alpha\alpha}$	0.54±0.05 $^{ab\beta}$	0.57±0.06 $^{b\beta}$	0.62±0.03 $^{c\beta}$
	14	0.47±0.04 $^{a\gamma}$	0.51±0.05 $^{b\beta}$	0.57±0.05 $^{c\beta\alpha}$	0.54±0.05 $^{bc\beta}$	0.60±0.03 $^{d\beta}$	0.66±0.04 $^{e\beta}$
Gumminess	0	15.32±1.22 $^{a\beta\alpha}$	12.67±1.99 $^{c\beta\alpha}$	12.60±1.52 $^{c\alpha}$	14.49±1.92 $^{a\beta\alpha}$	17.19±3.05 $^{b\alpha\alpha}$	18.97±2.33 $^{a\alpha}$
	7	6.35±1.47 $^{a\beta}$	11.39±1.23 $^{b\alpha}$	8.40±1.25 $^{c\beta}$	10.46±1.34 $^{b\beta}$	17.07±2.31 $^{d\alpha}$	15.21±2.35 $^{e\beta}$
	14	7.51±1.53 $^{a\beta}$	8.91±2.10 $^{ab\beta}$	9.36±2.00 $^{b\beta}$	9.13±1.42 $^{ab\beta}$	17.44±2.06 $^{c\alpha}$	16.24±1.16 $^{c\beta}$
Resilience	0	0.34±0.04 $^{a\beta\alpha}$	0.35±0.04 $^{b\alpha}$	0.32±0.04 $^{a\beta\alpha}$	0.31±0.02 $^{b\alpha}$	0.30±0.06 $^{c\alpha}$	0.32±0.03 $^{a\beta\alpha}$
	7	0.13±0.02 $^{a\beta}$	0.23±0.04 $^{b\beta}$	0.18±0.02 $^{c\beta}$	0.18±0.03 $^{c\beta}$	0.22±0.04 $^{b\alpha\beta}$	0.20±0.03 $^{c\alpha\beta}$
	14	0.14±0.02 $^{a\beta}$	0.19±0.03 $^{b\gamma}$	0.17±0.03 $^{b\beta}$	0.18±0.03 $^{b\beta}$	0.23±0.03 $^{c\beta}$	0.25±0.02 $^{c\gamma}$
Chewiness	0	10.62±1.12 $^{a\beta\alpha}$	8.04±1.58 $^{c\beta\alpha}$	7.68±1.15 $^{c\alpha}$	9.45±1.31 $^{b\beta\alpha}$	11.92±2.46 $^{b\alpha}$	14.11±2.12 $^{a\alpha}$
	7	3.27±0.92 $^{a\beta}$	6.24±1.06 $^{b\beta}$	4.97±1.00 $^{b\beta}$	5.70±0.91 $^{b\beta}$	9.84±1.93 $^{c\beta}$	9.50±1.53 $^{c\beta}$
	14	3.49±0.69 $^{a\beta}$	4.57±1.24 $^{b\gamma}$	5.23±0.94 $^{b\beta}$	4.92±1.02 $^{b\beta}$	10.52±1.50 $^{c\alpha\beta}$	10.63±0.31 $^{c\beta}$

For each criterion, all values were means ± standard deviation of ten values
Same letter (a, b, c, d, e) in the same row indicate no significant differences between means ($p \leq 0.05$)
Same greek letter (α, β, γ) in the same column indicate no significant differences between means ($p \leq 0.05$)

Fig. 27.6 (**a**) Color changes in sea bream, (**b**) water-holding capacity in gilthead sea bream, (**c**) TBARS in cold-smoked salmon, (**d**) texture profile analysis in sea bass (from Campus et al. 2010; Gudbjornsdottir et al. 2010; Chéret et al. 2005)

However, the conditions during storage determine which bacteria are responsible for spoilage (Briones et al. 2010). Bacterial spores are generally more resistant than cells; moreover, Gram-positive bacteria are inactivated at higher pressure than Gram-negative ones, due to the rigidity of the teichoic acids in the peptidoglycan layer of the cell wall (Corbo et al. 2009). Regarding to the mode of action, pressure

Table 27.1 Physicochemical changes in seafoods' muscle: fresh and HP treated

Changes in seafoods' muscle: fresh and HP treated	Sample/treatment	Achievements	References
Pre-rigor mortis	Seafoods' muscle	Relaxed extensible sarcomeres, actin and myosin microfibrils uncoupled, pH about 7, high protein extractability and hydration, firm, cohesive	Connell (1980) Leten et al. (2006)
Rigor mortis	Seafoods' muscle	Partly contracted sarcomeres, actomyosin, partly ruptured myocommata, pH about 6, low-protein extractability and hydration, rigid, cohesive	
Post-rigor mortis	Seafoods' muscle	Partly hydrolyzed sarcoplasmic proteins, slightly disintegrated sarcomeres due to hydrolytic changes in troponin Z-line and M-line disrupted collagen structure, pH about 7, high protein extractability and hydration, plasto-elastic (see Fig. 27.4)	
Reported changes in fresh muscle seafood	Mediterranean horse mackerel (*Trachurus mediterraneus*) and blue jack mackerel (*Trachurus picturatus*)	pH initial for Mediterranean horse mackerel was 6.2 and blue jack mackerel was 6.4. The pH values of both fish species increased significantly ($p<0.05$) during ice storage	Tzikas et al. (2007)
	Horse mackerel (*Trachurus mediterraneus*)	The moisture was between 74 % and 78 % and lipid was between 0.8 % and 2 %. The pH was 6.28.WHC was 98.9 %, TVB-N was 18.7 mg TVB-N/100 g muscle, FFA was 0.83, and TBA was 163.2 μg MDA/kg	Tzikas et al. (2009) Song et al. 2011
	Bream (*Megalobrama amblycephala*)	pH, TVB-N, and TBA values of bream were 7.05, 12.62 mg/100 g, and 0.25 mg MDA/kg, respectively	Thorarinsdottirs et al. (2001)
	Salted cod (*Gadus morhua*)	pH, protein content, and WHC values of bream were 6.6 %, 17.5 %, and 86 %, respectively	Goulas and Kontominas (2005)
	Chub mackerel (*Scomber japonicus*)	The moisture, salt content, pH, TVB-N, TMA-N, and TBA were 75.30 %, 0.1 %, 6.12, 10.93 mg N/100 g, 1.22 mg N/100 g, and 0.23 mf of malondialdehyde/kg, respectively	Goulas and Kontominas (2007)

(continued)

Table 27.1 (continued)

Changes in seafoods' muscle: fresh and HP treated	Sample/treatment	Achievements	References
	Sea bream (*Sparus aurata*)	The protein, fat, moisture, and ash were 19.9 %, 6.2 %, 72.4 %, and 1.4 %, respectively. The initial pH, TVB-N, TMA-N, and TBA of bream were 6.12, 15.9 mg N/100 g, 0.31 N/100 g, and mg of malondialdehyde/kg, respectively. During storage at 4 °C, the all parameters increased	
	Sea bass (*Dicentrarchus labrax*)	Fat, moisture, total nitrogen, nonprotein nitrogen, and ash were 4.81, 76.72, 3.11, 0.28, and 1.23 (w/w), respectively The initial pH, TMAO, TMA-N, TVB-N, free fatty acid (FFA), and TBA values were 6.39, 22.08 mg N/100 g flesh, 0.20 mg N/100 g flesh, 0.20 mg N/100 g flesh, 1.78 g oleic acid/100 g lipid, and 0.37 mg malondialdehyde/kg fresh. The pH, TMA-N, TVB-N, FFA, and TBA values increased during storage. TMAO decreased during storage.	Kyrana and Lougovois (2002)
	Sardines (*Sardina pilchardus*)	The chemical composition values were determined as follows: moisture 63.63 %, ash 2.62 %, total protein 21.82 %, total fat 2.29 %, and total carbohydrate 1 %. pH, TVB-N, TMA-N, histamine, and TBA were 6.02, 10.18 mg/100 of muscle, 2.6 mg/100 of muscle, 12.30 mg/kg, and 2.7 mg MDA equivalents per kilogram, respectively	Erkan and Özden (2008)
	Atlantic salmon (*Salmo salar*)	The fat content, astaxanthin, liquid loss, water loss, and fat loss were 22.2 %, 8.0 (mg/kg muscle), 3.7 %, 1.4 %, and 2.3 % Lightness (L^*), redness (a^*), yellowness (b^*), chromaticity (C^*_{ab}), and Hue (H^*_{ab}) were 44.2, 13.2, 16.7, 21.3, and 51.7, respectively	Veiseth-Kent et al. (2010)
	Turbot (*Scophthalmus maximus*)	The protein, fat, moisture, and ash were 19.6 %, 2.7 %, 76.3 %, and 1.3 %, respectively pH value was 5.9 TVB-N value was 20.04 mg TVB-N/100 g muscle Instrumental texture of hardness was 148.6 N Emulsify capacity was 1481.6 g emulsified oil/g total proteins	Abugoch et al. (2011)

	Silver carp (*Hypophthalmichthys molitrix*)	pH, TBA, and TVB-N were 6.0, 1–2 mgMDA/kg, and 35–40 mgTVB-N/100 g	Fan et al. (2009)
	Horse mackerel (*Trachurus trachurus*)	Water contents ranged between 75 % and 80 %, and the lipid contents ranged between 1.20 and 2.70 FFA content was 1.39 g FFA/100 g lipids TBA value was 0.10 mg MDA/kg TBV-N and TMA values were 25 mg TVB-N/100 g muscle and 0.1, respectively	Aubourg (2001)
	Chilean jack mackerel (*Trachurus murphyi*)	pH, FFA, TVB-N, peroxide value (pv), and TBA were 5.9, 0.25 g FFA/100 lipids, 4.0 mg TVB-N/100 g, 0.01 meq active oxygen/kg lipids, and 0.5 mg MDA/kg muscle, respectively	Quitral et al. (2009)
	Gray mullet, sardine, Atlantic bonito, and bluefish	The TBA content from gray mullet (small and large), sardine (small and large), Atlantic bonito, and bluefish were between 1.967 and 2.474, 0.624 and 1119, 1.590 and 2.574, 2.180 and 8.210, 0.553 and 6.490, and 0.329 and 1.19 mg MDA/kg meat	Tokur et al. (2006)
	Ray fish (*Dasyatis brevis*)	TVB-N: 21.18 mg TVB-N/100 g muscle TMA: 2.9 mg TMA/100 g muscle pH: 6.78 WHC: 16.18 % L^*, 51.54; a^*, 19.33; and b^* 13.71	Higuera-Ocaño et al. (2011)
	Jack mackerel (*Trachurus symmetricus murphyi*)	Moisture, fat, protein, and ash in sample were between 74 % and 78 %, 3.0 and 5.0 %, 17 and 19 %, and 1.0 and 1.4 % TVB-N, FFA, and PV were 10 mg N/100 g of flesh, 9 mg FFA/100 g lipids, and 3.5 meq/kg lipids	Aranda et al. (2006)
Autolyzed	Seafoods' muscle	Partly hydrolyzed proteins, nonprotein-nitrogenous compounds, pH above 7, soft, sticky	Connell (1980) Leten et al. (2006)
Reported changes in fresh seafoods' muscle	Trout (*Oncorhynchus mykiss*)/storage 3.5 and 12°.	Putrescine, cadaverine, tyramine, spermidine, and spermine contents during 7 days in storage were 5.7, 8.8, 2.4, 2.4, and 3.3 mg/kg in meat trout, respectively. The tryptamine and phenylethylamine were not detected The amine content was increased during storage	Matejková et al. (2013)

(continued)

Table 27.1 (continued)

Changes in seafoods' muscle: fresh and HP treated	Sample/treatment	Achievements	References
Pre-rigor mortis with HP treatment	Sea bass (*Dicentrarchus labrax L.*) fillet/100, 200, 300, 400, and 500 MPa, storage for 0, 7, and 14 days at 4 °C	Application of pressure on fillet induces an increase in L^* value and chroma; a^* value decreases; b^* and hue change diverse ways around its initial value. All parameters changed significantly with storage time High-pressure treatment induced a decrease of exudation and water-holding capacity Pressure treatment above 300 MPa provoked higher fish hardness after storage than in untreated sample, proving the ability of high pressure to improve textural quality of chilled, stored fish fillet (see Fig. 27.6, *letter d*). These assessments were corroborated with microstructure (see Fig. 27.5a, b, c, d, and e)	Chéret et al. (2005)
Post-rigor mortis/autolyzed with HP treatment	Red mullet (*Mullus surmuletus*) Pressurized at 220, 250, and 330 MPa at 3, 7, 15, and 25 °C for 5 and 10 min	Color: an increased in L^* and b^* values and a decreased in a^* value as pressure is increased The TMA value was not affected after treatments The TBA value in the pressurized samples at 3 and 25 °C for 5 and 10 min regularly increased with pressure. The TBA values of pressurized samples at 7 and 15 °C for 5 and 10 min were significantly higher than those found in unpressurized samples pH values of HP treated exceeded the limit after 17 days TVB-N after 7 days of storage remained higher than the rejection limit (35 mg/100 g) for all samples	Erkan et al. (2010)

	Red abalone (*Haliotis rufescens*)/500 MPa for 8 min and 550 MPa for 3 and 5 min	Significant increases in moisture content relative untreated sample were apparent for HP-treated abalone. In parallel, the protein and crude fat content was significantly lower in treated abalones with high pressure The pH of abalone was significantly lower in fresh samples than in treated samples TVB-N values of fresh exceeded this limit after 15 days and HP-treated samples 45 days TMA-N values of fresh exceeded the limit after 21 days and HP-treated samples not exceeded the limit during experiment Color: abalone tissue HP treated at 500 MPa/3 min did not differ significantly in lightness, redness, or yellowness compared to untreated abalone, but there are significant differences in L^*, a^*, and b^* values in treated abalone tissue at 500 MPa/8 min and 550 MPa/3 min Texture profile analysis (TPA) control samples were not significantly different in terms of hardness and springiness in samples treated with 500 and 550 MPa In the samples treated with HP and control samples in terms of cohesiveness and chewiness, there was a significant difference The microstructure analysis in abalone showed that a more compact structure was identified as the pressure was higher. Thus, it was concluded that holes in muscle fibers were often due to protein gelation, whenever pressure and protein concentration are high enough, conforming that the structure of abalone muscle treated with high pressure differed significantly from that of raw abalone meat (see Fig. 27.5i, m, n, o, and p)	Briones-Labarca et al. (2012)

(continued)

Table 27.1 (continued)

Changes in seafoods' muscle: fresh and HP treated	Sample/treatment	Achievements	References
	Atlantic salmon (*Salmo salar*)/300 MPa for 10 min at two temperature levels (5 °C and 40 °C)	Color: increased L^* and b^* values and a decreased a^* value as pressure is increased, compared with the raw muscle High-pressure treatment (300 MPa for 10 min at 5 and 40 °C) produced ~40 % decrease in salt-soluble protein All processed samples showed with high pressure a dramatic reduction in viscosity compared to the raw fish, and this can be attributed to extensive protein aggregation FTIR spectroscopy analysis showed changes in loop structures in samples treated compared to raw salmon muscle; nevertheless, these structures tend to decrease slightly in comparison with raw muscle when high pressure was applied at 5 °C, whereas the opposite was found to be the case at 40 °C Carbonyl group content was lowest in the fresh raw sample, but protein oxidation increased significantly in all treated samples, reaching maximum value in the muscle pressurized at 40 °C The total volatile basic nitrogen in the lots treated with high pressure barely changed the TVB-N values	Ojagh et al. (2011)
	Gilthead sea bream (*Sparus aurata*)/200, 300, and 400 MPa for 10 min were stored at 3 °C	The application of 300 and 400 MPa pressures appeared to enable preservation of elasticity and stiffness of fish muscle during storage. On the contrary, samples at 200 MPa underwent a decrease in elasticity during storage The water-holding capacity of dorsal muscle was also assessed, and it was found to decrease with increasing pressures (see Fig. 27.6b). Immunoblot studies performed on the main structural proteins revealed that a pronounced time-dependent degradation of desmin, observed in untreated samples, could be prevented by treatment at 400 MPa	Campus et al. (2010)

	Cold-smoked salmon (*Salmo salar*)/400, 500, 600, 700, 800, and 900 MPa for 10, 20, 30, and 60 s	All the smoked fillet samples were slightly higher TBARS values than the fresh fillet samples (see Fig. 27.6c) The effect of HP on the redness of the product was not observed; however, immediate effect on the lightness was noticed and salmon becomes lighter in color as function of both time and pressure The effects on the microstructure increased with both time and pressure and were most significant at 900 MPa and 60 s (see Fig. 27.5f, g, h, i, j, and k)	Gudbjornsdottir et al. (2010)
	Cold-smoked salmon (*Salmo salar*)/220, 250 and 330 MPa for 5 and 10 min at 7, 15, and 25 °C	The only treatments that gave significantly different (lower) L^* values compared to control were 220 MPa/15 °C/5 min, 220 MPa/15 °C/10 min, and 220 MPa/25 °C/5 min. a^* values, the only treatments which were significantly different, were 220 MPa/15 °C/10 min and 250 MPa/25 °C/10. b^* values, the only treatments which were significantly different, were 330 MPa/15 °C/10 min and 330 MPa/25 °C/10. Looking at the delta E scores, the lowest score (least change) was obtained with a treatment of 220 MPa/3 °C/5 min TBA values for HP treatments at 3 °C/5 min, 15 °C/5 min, 15 °C/10 min, and 25 °C/10 min did not differ significantly from untreated sample, irrespective of pressure level. Other treatment conditions gave significantly higher TBA values The TMA-N values obtained at 220 MPa/7 °C/5 min, 330 MPa/7 °C/5 min, and 250 MPa/25 °C/5 min were lower than the untreated sample. Otherwise, all the other treatment combinations gave similar or higher values than the untreated sample HP-treated cold-smoked salmon fillets showed significant changes in free amino acid content	Erkan et al. (2011a)

(continued)

Table 27.1 (continued)

Changes in seafoods' muscle: fresh and HP treated	Sample/treatment	Achievements	References
	Cold-smoked salmon (*Salmo salar*)/100,150, and 200 MPa for 10 and 20 min at room temperature	Application of high pressure caused increase in moisture content Cold-smoked salmon was found to contain higher water-holding capacity (WHC) than fresh Atlantic salmon. Application of high pressure to fresh salmon reduced the WHC by 5 % at 200 MPa for 10 and 20 min, whereas HP did not cause much change in relation to WHC and cold-smoked salmon Proton relaxation values were affected at all pressure levels used in this study; there was more effect on cold-smoked salmon, indicating possible removal of water due to protein denaturation	Lakshmanan et al. (2005b)
	Squid (*Todarodes pacificus*)/300 MPa at 20 °C for 5, 10, and 20 min. The high-pressure-treated samples were stored at 4 °C	Dimethylamine (DMA) contents were increased with increasing storage time, except for treated samples for 20 min, and the DMA contents were significantly decreased with increasing pressure holding time. The result indicates that the DMA formation was retarded by HP treatment Trimethylamine (TMA) contents at HP for 5, 10, and 20 min were not significantly increased throughout the refrigerated storage No significant differences in pH were observed with HP treatments throughout the refrigerated storage period	Gou et al. (2010)
	Atlantic mackerel (*Scomber scombrus*)/150, 300, and 450 MPa with holding times 0, 2.5, and 5 min, prior freezing	A marked inhibition of free fatty acids and tertiary lipid oxidation compound formation during storage was observed when increasing the pressure level or the pressure holding time of HP treatment. However, only minor differences in the polyene index and no effect in the content of primary and secondary oxidation compounds were observed	Vázquez et al. (2013)
	Silver carp (*Hypophthalmichthys molitrix*)/200, 300, 400, and 500 MPa between 0 and 30	High-pressure processing could be utilized to improve and maintain fish texture due to its ability to inactivate the endogenous proteases implicated in texture deterioration	Qiu et al. (2013)

	Seafood/HP	HP denatures the adductor muscle and induces the shell to open spontaneously HP shucking reduces the need for manual shucking and increases the quantity of meat removed from the shell HP is successfully employed to treat different types of seafood, such as lobsters, at pressures between 250 and 500 MPa, thereby improving the microbiological quality and product yields Texturizing effects of HP have been used to increase the gel strength of uncooked surimi by two- to threefold by making protein substrates more accessible to transglutaminase, which increases intermolecular cross-link formation and gel strength (see Fig. 27.6a)	Campus et al. (2010)
	Trout (*Oncorhynchus mykiss*)/300 and 500 MPa for 10 min at 20 °C in storage 3.5 and 12 °C	The effect of pressurization in amines (putrescine, cadaverine, and tyramine) on the stability of samples was substantial and it was evident at both pressure levels Samples treated with 300 MPa and 500 MPa did not show signs of decay up to the 28th and 42th days of storage, respectively, at 3.5 °C Faster decomposition processes at 12 °C gave rise to higher levels of amines in less time The polyamines (spermidine and spermine) of trout flesh treated with HP were not clearly observed in this experiment The histamine, tryptamine, and phenylethylamine were found only in samples kept at 12 °C HP increased L* values of horse mackerel. The a* and b* of treated horse mackerel did not change significantly after HP applications After, HP, TBA-I, TMA, and free amino acid values of all HP-treated horse mackerel samples remained unchanged than those of untreated samples	Matejková et al. (2013)

(continued)

Table 27.1 (continued)

Changes in seafoods' muscle: fresh and HP treated	Sample/treatment	Achievements	References
	Horse mackerel (*Trachurus trachurus*)/220, 250, and 330 MPa for 5 and 10 min at 7, 15, and 25 °C	Significant differences were found ($p > 0.05$) during pressure treatment, suggesting that increased moisture content may be due to increased water. Moreover, significant reduction was found after pressure treatment Fracturability was affected by pressure treatment at 400 MPa; however, no changes were observed at 200 MPa, regardless of step-pulsed or continuous treatment	Erkan et al. (2011b)
	Bay scallop adductor muscle (*Aequipecten irradians*)	The behavior observed in scallop adductor muscle under pressurization (400 MPa at 20 °C) indicated a decrease in hardness, a softening of muscle due to changes in the connective tissue surrounding the muscle fibers The delta E values showed significant decreases in this parameter at 400 MPa Pressure treatment compressed the muscle fibers generating a rearrangement of perimysium, with a size reduction of the endomysium, changing the honeycomb structure	Pérez-Won et al. (2005)
	Carp (*Cyprinus carpio*)/100, 140,180, and 200 MPa for 10 and 30 min	HP treatment on TBA values increased significantly with pressure and also with pressurization time versus the untreated for all the pressure levels tested, except for the samples pressurized at 100 MPa for 15 min Free fatty acids released from HP-treated samples increased as pressure increased The L^*, a^*, and b^* values increased of treated samples	Sequeira-Muñoz et al. (2006)
	Indian white prawn (*Fenneropenaeus indicus*)/100, 270, 435, and 600 MPa at holding time of 5 min at temperature of 25 °C	pH and TBA values significantly increased after HP treatment and storage. Significant reduction of TMA and TVB-N values after high-pressure treatment was observed during storage. There was gradual increase in all samples. Hardness, whiteness (L^* value), and yellowness (b^*) increased with increasing pressure and redness (a^* value) was found to decrease	Bindu et al. (2013)
	Jack mackerel (*Trachurus murphyi*) (untreated and HP treatment)	The basic unit of the sarcomere muscle fiber loses its structure when it is subjected to HP treatment. Z lines and A and I bands disappear completely after HP (see Fig. 27.2 and 27.3)	Briones-Labarca V, Roco-Bugueño T, Tabilo-Munizaga G, Perez-Won M (unpublished data)

induces many changes in the bacterial cell, including inhibition of key enzymes and protein synthesis and alterations in cell morphology and membrane, along with the disruption of transcription, translation, and other cellular functions responsible for survival and reproduction (Corbo et al. 2009). Several studies have shown that HP treatment causes a number of changes in the cell morphology, cell wall, thermotropic phase in cell membrane lipids, ribosome dissociation, and biochemical reactions, and it results in the loss of genetic function in the responsible microorganisms. These observations are all proposed as possible reasons and mechanisms contributing to microbial inactivation due to HP treatment (Rendueles et al. 2011). Apparently, no damage is caused to any cellular structure or function of the microorganisms; cell death occurs due to damage to multiple parts in the cell. When the accumulated damage exceeds the cell's ability to repair, death occurs. Under several circumstances, the damaged cell can recover if posttreatment conditions are favorable (Wang et al. 2013).

Seafood's microflora from temperate waters is dominated by psychrotrophic, aerobic, or facultative anaerobic Gram-negative, rod-shaped bacteria and, in particular, by *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Shewanella putrefaciens*, *Flavobacterium*, *Cytophaga*, *Vibrio*, *Photobacterium*, and *Aeromonas* (Hubbs 1991; Lalitha and Surendran 2006; Pantazi et al. 2008). Since seafoods are sterile at the time of slaughtering/catch, they become quickly contaminated by surface and intestinal bacteria and from equipment and humans during handling and processing. Psychrotrophic *Pseudomonas* and *Shewanella* dominate the microflora after 1–2 weeks of chilled storage (Sivertsvik et al. 2002). *Shewanella putrefaciens* is the specific spoilage bacteria of marine temperate-water fish stored aerobically in ice. *Pseudomonas* sp. is the specific spoiler of ice-stored tropical freshwater fish and, together with *S. putrefaciens*, is the spoiler of marine tropical fish stored in ice (Gram and Melchiorsen 1996). However, postmortem biochemical changes in fish muscle are strongly influenced by the post-catch handling practices. These postmortem changes that directly and strongly affect its quality and shelf life are associated with protein and ATP degradation, drop of pH, lipid oxidation, undesirable compound production as trimethylamine (TMA-N), and the low-molecular-weight volatile bases (TVB-N), which are produced by bacterial action (Ocaño-Higuera et al. 2011). The bacterial degradation of fish constituents, particularly nonprotein-nitrogen (NPN) compounds, leads to the development of the off-odors typically associated with fish spoilage of which the TVB-N and TMA-N are particularly important (Campos et al. 2005). TMA-N has a typical unpleasant fish spoilage odor and is ultimately responsible for the sensory rejection of fish by consumers. Besides *Shewanella* species, *Enterobacteriaceae*, *Alteromonas, Photobacterium, Pseudomonas*, and *Vibrio* can all reduce TMAO to TMA-N. The TMA-N content of fresh fish is usually less than 3 mg N/100 g and spoilage is considered to have started when it exceeds 15 mg N/100 g (Karim et al. 2011).

At first, the main objective of the high-pressure treatment was to eliminate *Vibrio* spp. from oysters, which are often eaten raw or only lightly cooked. *Vibrio* spp. are relatively sensitive to high pressure, and typical treatments of 250–350 MPa for 1–3 min at ambient temperatures are used commercially without significantly affect-

ing sensory quality (Patterson 2005). Nowadays, there are several studies that are focused on exploration of HP as alternative to the traditional depuration process, such as the effect of HP on the survival of *Vibrio parahaemolyticus* in live clams (Mootian et al. 2013) and bacterial culture (Wang et al. 2013), and they found that *V. parahaemolyticus* was reduced to nondetectable level ($<10^1$ CFU/g) achieving >5 log reduction at 450 MPa for 4 min and 350 MPa for 6 min in live clams and 300 MPa for 10 min on bacterial culture where HP was good to inactivate *V. parahaemolyticus* by inducing morphological changes in internal and external structures in the cell, as well as by causing cell membrane damage, cell wall rupture, and membrane protein degradation (Wang et al. 2013). Moreover, infestation of marine fish and cephalopods with *Anisakis* sp. has increased worldwide affecting most commercially valuable species and many fishing grounds and causing consumer health problems related to infestation with live larvae (anisakidosis) when fish are consumed raw or undercooked (Vidacek et al. 2009). Pressure-treated mackerel confirms that fatty fish submitted to 300 MPa for 5 min can be cleared of vital nematode larvae of the *Anisakis* genus. The fact that at the pressure applied the mackerel did not undergo any degradation of their sensory properties such as to compromise their salability leads us to hope for similar results in fatty fish such as sardines and anchovies, in particular for their use in the preparation of marinated products (Brutti et al. 2010). This practice involves a considerable loss of color of the product, which could, however, be marketed without recourse to freezing or particular marinating treatments with high percentages of salt or vinegar, which are necessary to devitalize *Anisakis* larvae (Brutti et al. 2010).

On the other hand, the use of predictive microbiology to model microbial growth during storage of seafood processed by high-pressure processing plays an important role in accessing the effects of this technology on the product shelf life (Briones et al. 2010). However, few studies using predictive models for this purpose have been carried out (Slongo et al. 2009). Moreover, Chen et al. (2012) have been working on a kinetic model that describes microbial inactivation under HP. They developed an optimization algorithm to estimate survival parameters through finding the best fitting curves to nonisobaric survival data. On this sense growth parameters of microorganisms (the initial cell number (N_0), maximum cell number (N_{max}), maximum specific growth rate (μ_{max}), lag phase (λ)) have been used to characterize microbial growth on food (Corbo et al., 2006; Liu et al. 2006; Briones et al. 2010).

27.1.2.3 Effect of HP on Chemical Constituents and Nutritional Value

From a physical point of view, an increase in pressure has an important effect on the molecules, for they get closer to each other. HP treatment has been reported to follow Le Chatelier's principle (Campus 2010). Accordingly, any phenomenon in equilibrium (chemical reaction, phase transition, change in molecular configuration), accompanied by a decrease in volume, can be enhanced by HP treatment.

From a chemical point of view, HP treatment can be considered as softer than a thermal process. Thus, the covalent bonds are not broken, but the weak energy bonds like hydrogen and hydrophobic ones can be irreversibly modified, which means that low-molecular-weight food components (responsible for nutritional and sensory characteristics) would not be affected, whereas high-molecular-weight components (proteins whose tertiary structure is important for functionality determination) are sensitive.

Concerning HP application to seafood, previous research accounts for a wide range of studies focused on protein denaturation analysis, especially concerning the activity inhibition of deteriorative endogenous and bacterial enzymes (Murchie et al. 2005). This research topic has been related to all kinds of marine products, both from wild and cultivated sources, and assessing the straight relationship with sensory and nutritional value. Concerning the lipid fraction, changes as a result of HP treatment of marine foods have also been studied, although not in a so exhaustive way than for the protein fraction; thus, lipid oxidation and hydrolysis development have been analyzed taking into account its negative effect on quality. The effect of HP treatment on other kinds of chemical components strongly related to quality, and nutritional losses can be considered scarce (nucleotides) or even inexistent (vitamins and minerals).

Protein-Type Components

The breaking of covalent bonds is associated with an increase in volume, and so covalent bonds would not be disrupted by HP treatment. Accordingly, primary structure of proteins (amino acids in a polypeptide chain joined by covalent bonding) would not be modified (Hendrickx et al. 1998; Knorr 1999). However, HP processing has shown to affect non-covalent bonds (hydrogen, ionic, and hydrophobic bonds) substantially as most of them are very sensitive to pressure. As a result, marked changes are likely to be produced in quaternary (namely, dissociation of oligomers), tertiary (namely, modification of hydrophobic and ionic interactions), and secondary (namely, modification of hydrogen bonds) protein structures.

Most research concerning the application of HP treatment on marine foods has been basically focused on the ability for protein denaturation, being the intensity and reversibility of this effect strongly dependent on the strength of HP conditions. Consequently, deteriorative molecules such as hydrolytic and oxidative microbial and endogenous enzymes can be inactivated for a subsequent storage/processing of the fish product. Because of their greater presence in marine species, the greatest attention has been accorded to changes produced in the myofibrillar fraction. However, sarcoplasmic proteins have shown to be more sensitive to the HP treatment than any other protein group.

27.1.2.4 Chemical Modifications of Proteins

Direct Effect of HP Treatment

The stability of isolated extracts of black marlin (*Makaira mazara*) and jack mackerel (*Trachurus japonicus*) myosins to HP treatment (100–500 MPa) was examined by Ishizaki et al. (1995a); a solubility decrease in both myosin species was observed above 300 MPa, being S-1 the pressure-sensitive myosin fragment. Later on, HP treatment (500–2000 atm for 0–60 min) was applied to determine the aggregation and viscoelastic properties of tilapia (*Oreochromis niloticus*) myosin (Hsu and Ko 2001); it could be observed that native myosins unfolded at 500 atm and aggregated at 1000 atm, and both pressures formed a precipitate and gel. The same research group (Ko et al. 2003) showed that after a 50-MPa treatment for 0–60 min, tilapia myosins slightly decreased their total sulfhydryl contents and exposed their hydrophobic residues largely; results indicated that 100 and 150 MPa applications caused the formation of intermolecular disulfide bonds and increased the myosin surface hydrophobicity.

Similarly, actomyosin extracted from tilapia (*Oreochromis niloticus*) muscle was subjected to HP treatment (50–300 MPa for 10–60 min) to investigate the changes produced in sulfhydryl groups and on conformation (Hsu et al. 2007). Transmission electron microscopy showed that the structure of actomyosin was aggregated and disrupted above 100 MPa, and more regular network aggregates were attained as pressure increased. Analysis by SDS-PAGE showed that molecules larger than the myosin heavy chain were observed when actomyosin was treated at and above 200 MPa; below this pressure, actomyosin formed aggregates, mainly with hydrogen bonds. Additionally, total sulfhydryl group content of actomyosin decreased with increased pressure and pressure holding time. According to this study, 200 MPa would be the critical pressure that induced actomyosin to form regular network structures.

Protein stability was found to be influenced by the pressure level and the pressure holding time when turbot (*Scophthalmus maximus*) fillets were subjected to HP treatment (100–200 MPa for 15 and 30 min) (Chevalier et al. 2001); as a result, differential scanning calorimetry (DSC) indicated that from 180 MPa the modifications induced on proteins were more important, showing a full denaturation of myosin at 200 MPa. A similar behavior had been obtained on cod (*Gadus morhua*) muscle myosin processed at 100–200 MPa for 20 min (Angsupanich and Ledward 1998).

Fine myofibrillar structure of pressurized (50–500 MPa for 10 min) carp (*Cyprinus carpio*) muscle was examined by electron microscopy (Yoshioka and Yamamoto 1998). It could be observed that at pressures over 300 MPa, the striated structure of the myofibrils disappeared; additionally, at 500 MPa the contractile elements of the muscle fibers disintegrated, thus resulting in an appearance of irregular electron-opaque bodies. It was concluded that high pressurization clearly affected contractile proteins, particularly actin.

Concerning extractable sarcoplasmic proteins, its content showed to decrease after HP treatment (2000, 4000, and 6000 atm for 30 min) on cod (*Gadus macro-*

cephalus) and mackerel (*Scomber japonicus*) muscle (Ohshima et al. 1992). For both fish species, the SDS-PAGE analysis showed that most of the sarcoplasmic components in control samples were not detected in the pressurized samples when 4000 and 6000 atm conditions were applied. For mackerel, a marked content decrease could also be observed for 94, 50, and 43 kDa components when pressurized at 2000 atm. Rather than being degraded, such disappeared components were expected to be covalently linked together and be thus resistant to extraction with SDS.

Pressure-assisted thawing was applied to frozen whiting (*Gadus merlangus*) fillets (Chevalier et al. 1999). The influence of different factors such as pressure applied (0–200 MPa), freezing rate, pressurization rate, and pressure holding time (15–60 min) was studied in comparison with thawing process at atmospheric pressure. Related to chemical changes, the electrophoresis analysis of sarcoplasmic protein fraction showed a modification of the 48 kDa band, whose intensity decreased when applying a pressure higher than 150 MPa. A marked decrease in sarcoplasmic protein content was also evident in proteins of coho salmon (*Oncorhynchus kisutch*) subjected to HP treatment (135, 170, and 200 MPa for 30 s) (Ortea et al. 2010); additionally, the SDS-PAGE analysis of such protein fraction showed a partial loss of a band corresponding to 29 kDa that was identified as phosphoglycerate mutase.

Contrary to the abovementioned results, extraction of water-soluble proteins was barely affected by HP treatment (50–300 MPa for 1–12 h) when applied to tilapia (*Oreochromis niloticus*) fillets (Ko et al. 2006); however, extraction of salt-soluble proteins decreased 60 % after HP treatment at 250 MPa for 1 h, being actomyosin extracted below 10 % for the meat treated beyond 200 MPa.

Effects of pressure-shift freezing and/or pressure-assisted thawing on the quality of sea bass (*Dicentrarchus labrax*) muscle proteins were evaluated and compared with conventional (air-blast) frozen and thawed samples (Tironi et al. 2007). Microstructural analysis showed a marked decrease of muscle cell damage for pressure-assisted frozen samples. According to DSC analysis, protein extractability, and SDS-PAGE results, HP treatment (200 MPa for 20 min) produced a partial denaturation with aggregation and insolubilization of the myosin, as well as alterations of the sarcoplasmic proteins. HP-treated systems led to a decrease of water-holding capacity, but differences between HP and conventional methods were found to disappear after a cooking process.

Effect of HP Treatment Followed by a Subsequent Processing

Ashie and Simpson (1998) analyzed the protein modifications in refrigerated bluefish (*Pomatomus saltatrix*) that was previously submitted to HP treatment (1000–3000 atm for 30 min); disintegration of myofibrillar structure was evident as a result of HP treatment, although no observable changes in myofibrillar structure were observed during the subsequent 21-day storage period under refrigerated conditions (4–7 °C). According to this, the electrophoresis profiles of HP-treated (100–300 MPa for 5 min) sea bass (*Dicentrarchus labrax*) muscle showed that sarcoplasmic

proteins were modified as a result of the HP treatment and subsequent refrigeration (4 °C up to 7 days) (Chéret et al. 2006); for the myofibrillar proteins, the only changes were due to the HP treatment. Meantime, sea bream (*Sparus aurata*) was subjected to HP treatment (200–400 MPa for 10 min) and subsequently stored under refrigerated (3 °C up to 13 days) conditions (Campus et al. 2010); SDS and immunoblot analyses revealed that a refrigeration time-dependent degradation of desmin took place that could be prevented if the pressure applied was 400 MPa.

Changes during a subsequent chilling storage were studied by Ortea et al. (2010). The effects of a previous HP treatment (135, 170, and 200 MPa for 30 s) on breakdown and aggregation events in proteins of coho salmon (*Oncorhynchus kisutch*) were studied during a 20-day chilling storage. A marked decrease in sarcoplasmic protein content was evident in samples corresponding to 170 and 200 MPa treatments throughout the chilling storage; additionally, the SDS-PAGE analysis of such protein fraction showed a partial loss of a band corresponding to 29 kDa, which after excision, digestion, and mass spectrometry analysis was identified as phosphoglycerate mutase.

Related to changes resulting from freezing and a frozen storage period, turbot (*Scophthalmus maximus*) fillets were frozen either by pressure release (i.e., pressure-shift freezing, 140 MPa, −14 °C) or by air-blast freezing (−20 °C) and were then stored at −20 °C for 75 days (Chevalier et al. 2000). Important changes in extractability of salt-soluble proteins and toughness increase were observed 2 days after freezing for pressure-shift freezing samples as compared to nonfrozen samples. These modifications were correlated with a decrease in the apparent viscosity of the salt-soluble protein extract of pressure-shift freezing samples and with a significant decrease in the intensity of myosin heavy chains showed by electrophoresis analysis. Air-blast freezing induced lower changes in protein extractability, but the toughness of fillets frozen by this process increased with storage time and was significantly higher than that of pressure-shift freezing samples after 75 days of storage.

The quality of frozen sea bass (*Dicentrarchus labrax*) muscle stored at −15 and −25 °C for 1, 3, and 5 months after a pressure-shift freezing process (200 MPa; −18 °C) and pressure-assisted thawing process (200 MPa for 20 min) was evaluated in comparison with samples frozen and thawed using conventional methods (Tironi et al. 2010). Frozen storage of HP-treated samples did not affect the initial quality of frozen muscle; thus, parameters related to protein denaturation, extractability, and water-holding capacity presented similar values than those obtained for non-stored samples. On the other hand, conventionally treated samples experienced changes during frozen storage, such as protein denaturation and water-holding capacity modifications.

Enzymatic Activity Modifications

Enzymatic degradation has been reported to be responsible for the postmortem softening of fish muscle and allows a proliferation of bacterial flora. The contribution of proteolytic systems to the fish muscle degradation has been focused on the action of two groups of endogenous enzymes: calpains and cathepsins.

Calpains are intracellular endopeptidases requiring Ca for their enzymatic activity, being described as heterodimers composed of a large subunit and a small subunit with a molecular weight of about 80 and 28 kDa, respectively. The large subunit has a catalytic role and the small subunit a regulatory role, being both regulated by an endogenous specific inhibitor (calpastatin) (Chéret et al. 2007). Cathepsins are known to hydrolyze myofibrillar proteins during *postmortem* storage of fish muscle and are widely distributed in muscles and organs. After fish death and during the muscle storage, cathepsins B (a cysteine protease) and D (an aspartic acidic protease) may be released from the lysosomal matrix into both the cytoplasm and the intracellular spaces as a consequence of lysosome breakdown (Chéret et al. 2006).

HP treatment has shown the possibility of inhibiting the activity of hydrolytic enzymes. However, effects on fish muscle would depend on several factors causing activation or inactivation of muscle enzymes, according to HP conditions applied. Thus, HP treatment conditions can lead to reversible or irreversible damages in proteases, this leading to a marked inhibition of their action; in contrary, HP treatment can also disrupt lysosomal membranes and cause the release into the fish muscle of proteases and provoking a subsequent hydrolytic activity increase.

Direct Effect of HP Treatment

The effect of HP treatment (1–5000 atm for 15–60 min) on flying fish (*Gypselurus opisthopus*) and sardine (*Sardinops melanostictus*) actomyosin ATPases was studied from the standpoint of the interaction between myosin and actin (Ko et al. 1991). The activities of actomyosin Mg-ATPase markedly decreased and those of the EDTA-ATPases rapidly increased with prolonged pressure treatment at 3000 and 5000 atm. Changes of activities in F-actin plus HP-treated myosin Ca-ATPases showed similar results to those of pressure-treated actomyosin ATPases, while myosin plus pressure-treated F-actin resulted in decreased Mg-ATPase activity and increased EDTA-ATPase activity.

A marked inactivation of Mg-ATPase activity in lecithin-added yellowfin tuna (*Thunnus albacares*) actomyosin was evident by pressurizing up to 100 MPa for 10 min (Ishizaki et al. 1995b); by pressurization, lecithin was supposed to play a significant role in the activation of myosin Mg-ATPase up to 100 MPa and in the dissociation into myosin and F-actin under 100–200 MPa condition. Effects of combination treatments involving HP (1000–3000 atm for 30 min), pH, and crude α_2-macroglobulin on bluefish (*Pomatomus saltatrix*) muscle protease activity were investigated (Ashie et al. 1996); proteolytic activity was found to be reduced with increasing pressure and inhibitor (α_2-macroglobulin) concentration.

Tilapia (*Oreochromis niloticus*) myosin was HP treated (50–300 MPa for 0–60 min) to determine the inactivation of myosin Ca-ATPase (Jao et al. 2007); 150 MPa was found as the pressure value that caused apparent myosin denaturation and the typical network structure formation beyond 50 % decrease of myosin Ca-ATPase activity. HP processing of sea bass (*Dicentrarchus labrax*) white muscle up to 500 MPa for 5 min was found to enhance the activity of cathepsins B, H, and

L, whereas the activity of cathepsin D increased up to 300 MPa and decreased above 300 MPa (Chéret et al. 2005); with regard to calpain activity, HP processing led to a decrease of activity, which was negligible above 400 MPa.

The effects of HP (300 MPa for 20 min) treatment on the proteolytic degradation in the myofibrillar proteins of sardine (*Sardina pilchardus*) and blue whiting (*Micromesistius poutassou*) muscles were compared (Hernández-Andrés et al. 2008). It could be observed that the peak activity pH was 3 for sardine and 8 for blue whiting, the main enzyme families being aspartic proteases and alkaline serine proteases, respectively. Pressurization lowered the activity levels in the fish muscle (by 30.8 % in sardine and by 9.5 % in blue whiting) in agreement to protein degradation shown in the electrophoretic profiles. Overall, the observed changes in proteolytic activity could be attributed not only to the effect of HP treatment on enzymes, but also and mainly to the HP effect on other muscle proteins, in particular the myofibrillar proteins.

Effect of HP Treatment Followed by a Subsequent Processing

Effects of combination treatments involving HP (1000–3000 atm for 30 min), pH, and crude α_2-macroglobulin on bluefish (*Pomatomus saltatrix*) muscle protease activity were investigated (Ashie et al. 1996); proteolytic activity was shown to be reduced with increasing pressure and was not regained during a 3-week storage at 4 °C. Later on (Ashie et al. 1997), tissue enzymes (cathepsin C, collagenase, and chymotrypsin- and trypsin-like enzymes) from the same fish species were inactivated by HP treatment (1000–3000 atm for 30 min); however, enzyme activity showed to be reactivated to various extents depending on the level of pressurization over a 3-week storage (4–7 °C); meantime, scanning electron microscopy of the tissues revealed that at 1000 atm, there were no significant changes in myofibers, while pressurization at 2000 and 3000 atm resulted in breakdown of myofibers and connective tissue networks.

Modifications of sea bass (*Dicentrarchus labrax*) muscle enzymes after HP treatment (100–300 MPa for 5 min) and refrigerated storage (4 °C up to 7 days) were analyzed by Chéret et al. (2006). Calpain activity decreased with HP treatment and evolved differently during the refrigerated storage, depending on the level of pressurization. Its inhibitor (calpastatin) was not affected by HP treatment, but its inhibiting potential decreased during the refrigerated storage. The activities of cathepsins were modified by HP treatment and the storage time, this modification being dependent on the cathepsin class.

Chéret et al. (2007) evaluated the changes encountered by the calpain system during the postmortem storage (0–7-day period at 4 °C) of sea bass (*Dicentrarchus labrax*) muscle after HP treatment (0–500 MPa for 5 min). From 100 MPa, HP treatment of purified calpains resulted in a loss of their activity as well as in the dissociation of the heterodimeric form. In muscle, HP processing showed to decrease the initial activity of calpain. This activity loss was explained as an inactivation by a structural change. Initial calpastatin activity was not modified by the HP treatment,

but it decreased during the storage from the beginning for a treatment at 300 MPa after which calpastatin was stable during 2 days.

The effects of HP processing (100–300 MPa for 20 min) on the activity of proteolytic enzymes in cold-smoked salmon (*Salmo salar*) and enzyme extracts were analyzed by Lakshmanan et al. (2005a). The activities of three enzymes (cathepsin B-like, cathepsin B + L-like, and calpains) were reduced at all pressure levels tested in crude enzyme extracts prepared from cold-smoked salmon. Calpain almost completely inactivated at 300 MPa. HP treatment did not influence the general proteolytic activity but activated the enzymes in muscles at higher-pressure levels studied until 18 days of refrigerated storage at 4–5 °C. An increase in the activity of cathepsin B + L-like and calpain was seen after 12 days of refrigeration, which was attributed to disruption of lysosomic membranes by pressure.

27.1.3 Lipid Fraction

Lipid damage has shown an important effect on quality loss during storage and processing of marine species. Previous research accounting for the effect of HP treatment on the hydrolysis and oxidation events of this constituent fraction is now reviewed. Studies focused on the corresponding nutritional value modifications produced are also mentioned.

27.1.3.1 Effect of HP Treatment on Lipid Hydrolysis

FFA has been reported to be produced as a result of hydrolysis of high-molecular-weight lipid molecules (namely, TG and PL). Accumulation of FFA in fish muscle has no nutritional significance, but is found undesirable due to secondary reactions, such as muscle texture changes, lipid oxidation enhancement, and interrelation with off-odor development (Pigott and Tucker 1990; Aubourg 2008). Hydrolysis development in processed fish has shown to be produced as a result of endogenous enzyme activity, microbial enzyme activity, and thermal breakdown. Concerning HP treatment, research has been carried out to assess the direct effect on FFA formation and its influence on the lipid hydrolysis development during a subsequent storage/processing.

Concerning the direct effect of HP treatment on FFA formation, contradictory results have been observed. Thus, Chevalier et al. (2001) subjected turbot (*Scophthalmus maximus*) fillets to different HP conditions (100, 140, 180, and 200 MPa for 15 and 30 min); it appeared that no effect on the FFA content was observed. The same conclusion was obtained by Pal Kaur et al. (2013) when applying different HP conditions (100, 270, and 435 for 5 min) to black tiger shrimp (*Penaeus monodon*). Contrary, Sequeira-Muñoz et al. (2006) subjected carp (*Cyprinus carpio*) fillets to HP treatment (100, 140, 180, and 200 MPa for 15 and 20 min), this leading to increasing FFA values with pressure and holding pressure

conditions applied. An FFA content increase was also obtained in coho salmon (*Oncorhynchus kisutch*) muscle after HP treatment (Ortea et al. 2010); in it, low-pressure conditions were also applied (135, 170, and 200 MPa for 30 s).

Related to the HP effect during a subsequent storage, He et al. (2002) did not observe inhibition of hydrolysis development in refrigerated (4 °C up to 27 days) oyster (*Crassostrea gigas*) previously pressurized at 207–310 MPa for 1–2 min. A similar observation was made by Gómez-Estaca et al. (2007) when studying the storage (5 °C up to 21 days) of cold-smoked sardine (*Sardina pilchardus*) previously treated at 300 MPa for 15 min.

Contrary results were obtained when considering a chilling storage. Thus, Ortea et al. (2010) subjected coho salmon to HP treatment (135, 170, and 200 MPa for 30 s) followed by a 20-day chilling storage; an inhibitory effect was concluded at the end of the storage (15–20-day period) when the highest pressure was applied. Similarly, Pal Kaur et al. (2013) studied the effect of HP treatment on quality and shelf life of black tiger shrimp (*Penaeus monodon*); an inhibitory effect was observed, especially in the case of the highest pressure applied.

Ohshima et al. (1992) found that enzymatic degradation of phospholipids in cod muscle was successfully inhibited during storage at −2 °C for 6 days when previously treated at pressures above 400 MPa applied for 15 and 30 min; however, no effect was observed when applying a pressure of 200 MPa. Later on, freezing by pressure release (i.e., pressure-shift freezing, 140 MPa) was compared to air-blast freezing (−20 °C) in turbot (*Scophthalmus maximus*) fillets (Chevalier et al. 2000); analysis of FFA formation during subsequent storage at −20 °C for 75 days did not provide differences. Finally, Atlantic mackerel (*Scomber scombrus*) was subjected to HP treatment (150, 300, and 450 MPa with pressure holding times of 0.0, 2.5, and 5.0 min) prior to freezing (−20 °C) and frozen storage (−10 °C for 3 months) (Vázquez et al. 2013); a marked reduction of FFA content was observed in all kinds of HP-treated fish, this increasing with the previous pressure and pressure holding time employed.

27.1.3.2 Effect of HP Treatment on Lipid Oxidation

Lipid oxidation is one of the most important reactions responsible for sensory (namely, off-odor formation, browning development) and nutritional (namely, polyene content loss) quality loss in stored and processed marine products. Lipid oxidation development is recognized as a complex process where different kinds of molecules are produced, most of them unstable and susceptible to breakdown and originate from lower-weight compounds or react with other molecules (nucleophilic type, mostly) present in the fish muscle. This means that in order to achieve a correct and complete analysis of its development, assessment of oxidation compounds corresponding to the different steps of its mechanism development is necessary.

Previous research shows that lipid oxidation studies carried out concerning the HP treatment effect on marine products can be considered scarce when compared to their counterparts focused on the inhibition of microbial and endogenous enzyme activities; additionally, a single lipid oxidation index has been considered in most cases to assess its development. Literature data on the effect of HP treatment on lipid oxidation development in fish can be considered somehow controversial. Although most research has shown an increase in lipid oxidation as a result of the HP treatment, extracted lipids have shown to be relatively stable against oxidation under HP conditions and during subsequent storage (Tanaka et al. 1991). Additionally, the possible prooxidant effect of HP treatment on muscle lipids was shown to be eliminated if a previous water washing of the muscle was applied or if a complexation compound (e.g., EDTA) was added (Gudmundsson and Hafsteinsson 2002; Lakshmanan et al. 2003). Consequently, iron-bound protein denaturation during HP treatment has been reported to facilitate a free metal ion content increase which would be responsible for lipid oxidation in fish meat after HP treatment.

Concerning the effect of HP treatment on lipid oxidation development, controversial results have been obtained. Thus, an increase in the TBARS formation was found by Angsupanich and Ledward (1998) after HP treatment (100–800 MPa for 20 min) of cod (*Gadus morhua*) muscle; the same results were obtained after HP treatment of carp (*Cyprinus carpio*) (100, 140, 180, and 200 MPa for 15 and 20 min) (Sequeira-Muñoz et al. 2006) and turbot (*Scophthalmus maximus*) (100, 140, 180, and 200 MPa for 15 and 30 min) (Chevalier et al. 2001). However, no differences in TBARS formation were observed in Atlantic salmon (Amanatidou et al. 2000) after applying 50–200 MPa treatment for 15 min and in horse mackerel when applying HP treatment (220, 250, and 330 MPa treatments for 5 and 10 min) (Erkan et al. 2011). A definite effect on lipid oxidation development was not obtained when applying different and complementary lipid oxidation analyses. Thus, Aubourg et al. (2010) subjected coho salmon (*Oncorhynchus kisutch*) to different HP conditions (135, 170, and 200 MPA for 30 s); as a result, no effect on peroxide and fluorescent compound formation could be observed, although a higher TBARS formation was obtained when applying the highest pressure.

Controversial results have also been obtained when considering the effects of HP treatment followed by a refrigerated (4–5 °C) storage. HP treatment (100–800 MPa for 20 min) has been reported to increase TBARS formation during the subsequent storage (4 °C up to 8 days) of cod (*Gadus morhua*) muscle (Angsupanich and Ledward 1998). Similarly, HP treatment (150, 300, 450, and 600 MPa for 15 min) led to TBA value increase in rainbow trout (*Oncorhynchus mykiss*) during cold (4 °C) storage (Yagiz et al. 2007). Furthermore, the effect of HP treatment (400, 500, and 600 for 5 and 10 min) on vacuum-packaged cold-smoked cod refrigerated at 5 °C for 60 day showed no differences in lipid oxidation (TBARS assessment) after pressurization and during storage (Montiel et al. (2012). On the other hand, an inhibitory effect has been reported in other refrigeration studies. Thus, a lower TBARS formation was found in minced albacore (*Thunnus alalunga*)

muscle previously HP treated (275 and 310 MPa, 2–6 min) and then refrigerated at 4 °C (Ramírez-Suárez and Morrisey 2006); additionally, an inhibitory effect on TBA-I score was also attained in red mullet (*Mullus surmuletus*) muscle during storage at 4 °C when previously HP treated (220 and 330 MPa for 5 min) (Erkan et al. 2010).

Related to the effect of HP treatment on a subsequent chilling storage, Aubourg et al. (2010) subjected coho salmon (*Oncorhynchus kisutch*) to different HP conditions (135, 170, and 200 MPa for 30 s) and analyzed the lipid oxidation development during a 20-day chilling storage; as a result, higher peroxide values were found in control samples when compared to their counterparts corresponding to 170 and 200 MPa; contrarily, quantification of TBARS and fluorescent interaction compounds showed higher levels in fish samples corresponding to such two pressure conditions. Changes in physicochemical characteristics of Indian white prawn (*Fenneropenaeus indicus*) subjected to different HP conditions (100, 270, 435, and 600 MPa for 5 min) and kept chilled up to 1 month were analyzed by Bindu et al. (2013); TBA values increased during storage with pressure previously applied.

Concerning the effect of HP treatment on lipid oxidation during a subsequent frozen storage, Ohshima et al. 1992 found a TBARS formation increase in frozen (−2 °C for six days) cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) muscle that had been subjected to HP treatment (616, 408, and 204 MPa for 15 and 30 min). Later on, freezing by pressure release (i.e., pressure-shift freezing; 140 MPa) was compared to air-blast freezing (−20 °C) in turbot (*Scophthalmus maximus*) fillets (Chevalier et al. 2000); analysis of TBARS formation during subsequent storage at −20 °C for 75 days did not provide a definite effect of the previous HP freezing. Contrarily, Vázquez et al. (2013) obtained a marked inhibition of tertiary lipid oxidation compound formation in frozen (−10 °C up to 3 months) mackerel (*Scomber scombrus*) by HP treatment (150, 300, and 450 MPa for 0.0, 2.5, and 5.0 min); this effect was greater when increasing the pressure level or the pressure holding time.

27.1.3.3 Effect of HP Treatment on Lipid Components Related to Nutritional Value

An important detrimental effect of lipid oxidation on the polyunsaturated fatty acid (PUFA) content, i.e., a decrease in the polyene index, would be likely to be produced (Aubourg 2008). However, previous researches concerning different HP conditions and subsequent storage conditions have shown no effect on fatty acid composition of lipid material.

Thus, Ohshima et al. (1992) did not find differences in saturated, monounsaturated, and polyunsaturated fatty acid contents in cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) muscle after 6 days of storage at −2 °C when previously treated under HP conditions (200, 400, and 600 MPa for 15 min). Similarly, Yagiz et al. (2009) analyzed the effect of HP treatment (150 MPa and 300 MPa for 15 min) and

cooking on the quality of Atlantic salmon (*Salmo salar*) during 6 days of storage (4 °C); as a result, no significant differences between control and HP treatment samples were found in terms of total saturated, monounsaturated, and ω3 and ω6 fatty acids. Additionally, Aubourg et al. (2010) did not find differences in coho salmon (*Oncorhynchus kisutch*) muscle polyene index as a result of HP treatment (135, 170, and 200 MPa for 30 s) followed by chilled storage for up to 20 days. Finally, Vázquez et al. (2013) did not find polyene content differences in frozen (−10 °C up to 3 months) mackerel (*Scomber scombrus*) by HP treatment (150, 300, and 450 MPa for 0.0, 2.5, and 5.0 min).

Related to the effect of HP treatment on vitamin content in marine food, Aubourg et al. (2010) subjected coho salmon (*Oncorhynchus kisutch*) to different HP conditions (135, 170, and 200 MPa for 30 s) and analyzed α- (vitamin E) and γ-tocopherol contents in the muscle during a 20-day chilling storage. As a result, no differences were obtained in any tocopherol compound both as a result of HP treatment or subsequent chilling storage.

27.1.4 Nucleotides

Nucleotides are known as the basic components of nucleic acids. However, during the fish living period, a small portion of nucleotides are present in free form. Furthermore, during postmortem fish storage, muscle nucleotides are known to degrade in a series of stages as a result of endogenous biochemical changes; the level of major adenine nucleotides and their related compounds (K-value assessment) has been utilized extensively as an index of freshness of fish muscle.

Different experiments account for a K-value decrease by means of the HP inactivation of enzymes that catalyze adenosine 5′-triphosphate (ATP) degradation. Thus, when carp (*Cyprinus carpio*) muscle was treated at various pressure values (200, 350, and 500 MPa for 30 min) and subsequently stored at 5 °C, suppression of the inosine 5′-monophosphate (IMP) decomposition was observed when employing 350 and 500 MPa values (Shoji and Saeki 1989); in such cases, a decreased K-value was obtained and an increasing umami taste development was evident, this being the result of an increased IMP presence. A lower K-value was also found in pressurized fresh tilapia (*Oreochromis niloticus*) fillets (50–300 MPa for 2–12 h), being this decrease higher with pressure increase (Ko and Hsu 2001). The effect of different HP treatments (100–300 MPa for 15 min) on K-value of yellowfin tuna (*Thunnus albacares*) chunks packed in ethyl vinyl alcohol films during chilling storage was studied (Kamalakanth et al. 2011); the K-value was found to decrease with pressure when compared to the control, this lower value being maintained throughout the chilled storage. Finally, Ginson et al. (2013) found a K-value reduction after HP treatment (100–600 MPa for 5 min) and throughout chilling storage of Indian white prawn (*Fenneropenaeus indicus*). In all cases, results could be explained as a result of protein denaturation and deactivation of enzymes

involved in the degradation of ATP and related compounds (namely, dephosphorylases) during HP treatment.

Contrary to such results, HP treatment (135, 170, and 200 MPa for 30 s) of farmed coho salmon (*Oncorhynchus kisutch*) did not lead to a significant K-value decrease after treatment neither throughout a subsequent 20-day chilling storage (Aubourg et al. 2010). Both for HP-treated and control samples, nucleotide degradation was found relevant during the chilled storage. It could be argued that deactivation of endogenous enzyme activity failed to occur probably as a consequence of employing relatively low-pressure and pressure holding time conditions.

27.2 Development of Patents in Fish Industry

Patents related to the use of high pressure in seafood seem to be increasing (De Boevere 2012) and could be grouped in the following three main areas:

(a) The first are focuses on food security, food quality, and increasing the shelf life of seafood.
(b) The second area is related to the application of high pressure with the inclusion of seafood.
(c) The third area is related to the use of high pressure to improving seafood processes.

27.2.1 Food Security, Food Quality, and Increasing the Shelf Life of Marine Seafoods

27.2.1.1 A Process of Elimination of Bacteria in Shellfish and of Shucking Shellfish

The purpose of this patent is to use high-pressure processing for eliminating pathogenic organisms and other bacteria from raw food products, such as raw shellfish. This product is exposed to high pressure, for example, between 20,000 psi (137.9 MPa) and 150,000 psi (1034.2 MPa) for 1–15 min. The process temperature is between ambient temperature and 150 DEG F (55.5 °C) leaving the raw shellfish substantially unaffected, in its desired raw state, such that the pathogenic organisms are destroyed, while sensory characteristics of the raw shellfish remain high. The same process can be used for shucking bivalve mollusks without any mechanical force, with the pressure ranging from 10,000 psi (478 MPa) to 100,000 psi (689.4 MPa). Taste of raw seafood is enhanced by adding flavor-enhancing agents to the pressure vessel before application of pressure (Voisin 2000, 2001, 2003).

27.2.1.2 Method for Shelf-Life Extension of Oyster by High-Pressure Treatment

A method of increasing oyster shelf life by ultrahigh-pressure treatment is provided to increase the shelf life of the oyster maximally while maintaining the quality by sterilizing *Vibrio parahaemolyticus* and *Escherichia coli* in pathogenic microorganisms or putrefactive microorganisms. A live oyster containing *Vibrio parahaemolyticus* and *Escherichia coli* is subjected to ultrahigh-pressure treatment at 0–22 °C and 100–400 MPa for 5–45 min, preferably at 22 °C and 350 MPa for 15 min or 10 °C and 350 MPa for 15 min. In the process, a pressure-rising rate is 2.5 MPa per sec and the pressure reduction time is 15 s (Lim et al. 2007).

27.2.2 General Application of High Pressure with the Inclusion of Seafood

27.2.2.1 Seafood Product and Process

A process for preparing ready-to-cook seafood is described. The process comprises (a) locating the seafood in a ready-to-cook container; (b) sealing the container under partial, substantial, or full vacuum; and (c) allowing the container and seafood to undergo high pressure. Thus, the seafood remains raw, i.e., uncooked, but is at least partially sterilized from infectious agents. The seafood also retains its spores and enzymes, hence its taste and quality. The effect of at least partial sterilization of the product within the container during the pressure and any added gas mixture provides a product with a significantly extended shelf life, from several days to possibly several weeks, facilitating easier subsequent storage, transportation, and sales (Van-Seldijk 2003).

27.2.2.2 Method for Manufacturing High-Pressure-Processed Food Products

A method for processing food products, for example, beef, chicken, pork, lamb, and seafood, such as cakes and crab cakes, vegetables, sauces, and starches in order to extend the shelf life and eliminate bacteria and microorganisms comprises: (a) vacuum sealing a food product within a plastic material which has an oxygen transmission rate greater than 10,000 cc/m.sup. 2/24 h. to form a vacuum-sealed package; (b) placing said vacuum-sealed package into a pressurizing apparatus, (c) loading water into said pressurizing apparatus, and (d) pressurizing said water inside said pressurizing apparatus to exert high pressure on said vacuum-sealed package in a range from 1 min to 30 min at a pressure ranging from 25,000 psi to 120,000 psi (172.3–827.3 MPa) (Love et al. 2007).

27.2.3 The Use of High Pressure to Improving Processes

Once it was shown that the high-pressure processing had a positive effect on the control of microorganisms and shelf life of seafood, with the purpose of improving seafood processing, especially for crustaceans and shellfishes, this HP technology was first used industrially.

27.2.3.1 Apparatus for Pressure Treating Shellfish

The same process using high pressure between 20,000 psi and 50,000 psi (137.9–344.7 MPa) for 1–15 min can be used for shucking oysters without any mechanical force. Also, pathogenic organisms from raw food products, such as raw shellfish, and molluscan shellfish such as oyster are eliminated (Voisin 2002).

27.2.3.2 Method of Processing Crustaceans

The invention relates to a method of processing crustaceans, live and cooked to cause detachment of shells from crustacean meat. According to the method, live crustaceans are exposed to high pressure, for example, between 20,000 psi (137.9 MPa) and 60,000 psi (413.7 MPa) for 2–5 min. The process is conducted at minimal elevated temperatures, in the range of above ambient temperature of 40° to about 110° Fahrenheit, leaving the raw crustacean meat substantially unaffected, in its desired raw state, while the connective tissue deteriorates and the shell detaches from the meat. As a result separating the edible meat from the shell becomes much easier and less time-consuming. Cooked crustacean meat can also be processed under high pressure from 30,000 psi (206.8 MPa) to 60,000 psi (413.7 MPa) to eliminate or substantially reduce bacteria in the meat, thereby extending shelf life of the product (Voisin 2008).

High-pressure processing had a positive effect on the control of microorganisms and shelf life in seafood, and other applications using high pressure were developed to improve the processing of seafood, especially crustaceans and mollusks.

Lobster, crab, and shrimp are invertebrates belonging to the class known as "Crustacea." The skeletons of crustaceans are located on the exterior surface of their bodies and are known as exoskeletons, which form a hard shell. This shell is attached to the underlying muscle tissue by a continuous series of intracuticle fibers that extend across the entire exterior surface of the crustacean. These intracuticle fibers extend from the surface of the muscle tissue to the outer surface of the shell via pore canals in the shell. This continuous attachment renders it difficult to remove the shell by manual cutting. Several processing means have been developed to weaken the linkages affixing the shell to the body in order to facilitate removal of the shells and retrieval of the meat underneath.

Traditional methods for removing the shells of crustacean shellfish, such as shrimp and lobster, involve cooking the animals prior to peeling. Heat denatures the linkages attaching the exoskeleton to the shellfish meat, thus facilitating removal of the shells. However, following the application of heat, additional means must be employed to separate the meat from the shell. Manual extraction of the meat is time-consuming and laborious, while mechanical means often cause the meat to be minced or flaked, thus limiting its potential applications. A further difficulty with using heat to facilitate loosening of the shell is that the shellfish meat underneath inevitably becomes cooked during the process. Cooking of the shellfish meat affects both its flavor and its texture.

A method applying high pressure for a period of time sufficient to effect detachment of the meat from the shell of a lobster, crab, or shrimp has been developed. Following pressurization, the shell can be easily removed using any available means to provide de-shelled raw lobster, crab, or shrimp meat.

27.2.3.3 Method for Shucking Lobster, Crab, or Shrimp

The method developed is to apply pressure of at least about 25,000–100,000 psi (172.4–689.4 MPa) for a period of at least between 15 and 180 s at a temperature of between about 10 °C and about 30 °C. With the application of this method, the total yield of lobster meat (total weight of claw, tail, and leg meat/total green weight of lobster) is at least about 29 % (Jabbour et al. 2011).

27.2.3.4 Preparation of Eviscerated Raw Whole Lobster

Another patent also related to crustaceans is to produce a seafood product, for example, a lobster with an intact shell; the said method involves applying high pressure of at least 25,000 psi (172.3 MPa) for a period of time sufficient to effect detachment of the meat from the shell, removing the internal organs through an aperture in the abdomen by applying vacuum pressure from about 5 to about 25 in. of Hg through the wand, and removing the wand providing a whole lobster with meat completely detached from its shell and was not subjected to degradation by digestive enzymes (Jabbour et al. 2009).

27.2.3.5 High-Pressure Seafood Processing Method

The method of processing crustacean or shellfish to obtain a shelled crustacean or shellfish with a significantly open shell comprises the following steps: Controlled depressurization for 65–120 s to reduce the applied pressure from 50 % to 20 % of the predetermined pressure, exposing the crustacean or shellfish to heat and/or osmotic stress to obtain a prepacked crustacean or shellfish, and applying a

predetermined pressure of 2700 bars (270 MPa) for 35–45 s to obtain a shelled crustacean or shellfish having an open shell. The depressurization step is released instantly when it reaches a predefined threshold of 300–700 bars (30–70 MPa). The exposure of crustacean involves heating the shellfish at a preconditioning temperature of 12 °C and immersing the crustacean or shellfish in brine bath having a temperature lower than the preconditioning temperature and a salt concentration of greater than 38 g/l to produce osmotic stress. The prepacked crustacean or shellfish is introduced, in bulk, in a bag containing micro-filtered seawater before applying the predetermined pressure (Tauge 2011a, b).

27.2.3.6 Method for Processing of Seafood with a High Pressure

Two new patents established by Tauge (2011a, b) through the company Cinq Degres Ouest relates to a method of transforming a crustacean or shellfish by applying a predetermined pressure between 2000 and 4000 bars (200 and 400 MPa), preferably between 2300 and 3200 bars (230 and 320 MPa), to obtain a crustacean or shellfish husked shell being substantially open. According to the invention, such a method of processing comprises depressurizing wherein the duration controlled to reduce the pressure applied to 50 % and 20 % of the said predetermined pressure is at least 20 s, respectively, of at least 40 s.

27.2.3.7 Process for Providing a Frozen Fish Product

A process for obtaining a frozen fish product comprises treating frozen or nonfrozen fish with high pressure within the range of 50–250 MPa and frozen at temperature of less than 40 °C. This process would obtain a frozen fish product having at least some resistance to frozen deterioration by increasing the water-holding capacity on thawing and cooking step. And the treated fish product has an increased resistance to frozen deterioration of texture.

This process is based in regulate the conformational change of the head region of the molecule of myosin which changes irreversibly with the application of high pressure, but the conformation of the molecule of actin does not change completely and irreversibly. These biochemical changes in these proteins lead to a fish with improved functional conditions after frozen storage (Goodband et al. 1999).

27.2.3.8 Other Patents with New Trends in the Use of HP

When someone is allergic to fish, the body's immune system, which normally fights infections, overreacts to proteins in the fish. Recently a patent related to the use of high-pressure treatment on proteins to reduced immunogenicity has been published, wherein the protein composition comprises an isolated protein and a pharmaceutically acceptable carrier, where the immune response to the therapeutic

protein composition treated by high pressure is reduced by at least about 50 % as compared to the immune response to the composition of the same protein prior to treatment by high pressure in an animal with induced tolerance to the protein. HP causes several conformational changes in proteins that could be studied from the therapeutic and medical point of view (Randolph et al. 2007).

Another line of research based on the published patents is also related to protein modification but with the purpose of changing some biochemical properties which eventually can lead to development of new food ingredients with controlled functional properties. In line with this, it is possible to mention an invention which describes a method for increasing the digestibility of a food protein by subjecting the food protein to a single cycle of ultrahigh pressure. These food proteins find applications in nutraceuticals, food and nutrition, or dietary supplements (Lands et al. 2007) and in protein recovery using HP, which can in the same degree control the folding and unfolding states of the protein by maintaining the native state of the protein and in some case using the unfolded region to add some reactants that could change some protein properties (Randolph et al. 2010; Robinson et al. 2005). In summary, they report an effective method for the refolding of denatured proteins in solution so that when properly folded, biologically active protein in solution is recovered in high yield. The refolding takes place at pressures between about 0.25 kbar and about 3.5 kbar, advantageously at about 1.5 kbar to about 3 kbar.

From a technological point of view, the most promising is the use of high pressure and high temperature for the purpose of obtaining sterilized fish products, and some general patents have been published related to this topic.

In high-temperature/ultrahigh-pressure sterilization of foods, the process involves heating a food to a pre-pressurized temperature (75–105 °C), subjecting the food to pressure treatment (50,000–250,000 MPa), which instantaneously raises the temperature of the food and then releasing the pressure so that the temperature returns to the original pre-pressurized temperature. The method leverages the adiabatic temperature rise which occurs when the food is pressurized, coupled with the lethality of the pressure, to achieve appropriate sterilization conditions (Baker and Wilson 2000).

27.3 Final Remarks and Future Trends

During the last decades, consumer choice has been transformed by developments in the production, distribution, and retailing of food, which with improvements in the design and equipment of the domestic kitchen have facilitated a major change in our lifestyle. From the point of view of the quality and safety of marine food products, current and future applications of advanced systems should allow a more automated and hygienic handling and storage of such foods. Care would be required at every stage in the chain, from primary raw material production through manufacture, distribution, retail, and consumer use.

Up to now, HP technology has shown a wide potential application in the seafood industry for surimi and kamaboko production and cold-smoked fish preparation, as in assisting freezing, thawing, and thermal processing and as a previous treatment to refrigerated, chilled, and frozen storage. In all cases, the establishment of the shelf life of marine foods would require a full appreciation of the microbial, chemical, physical, and biochemical aspects which influence the sensory acceptability of products and have to be considered in relation to the safety and nutritional value. According to these needs, further and complementary research related to the above HP issues would undoubtedly contribute to continued development and innovation in this technology. It is considered that a special stress ought to be provided to the following aspects:

1. Optimization of HP conditions to each kind of product
   According to most research carried out up to now, a different response is to be produced according to the kind of product (whole, fillet, mince, etc.), kind of species (size, fat content, general composition, skin resistance, wild or cultivated, etc.), and other biological aspects (capture season, maturity, sex, eating state, etc.). HP conditions (pressure, pressure holding time, and temperature) ought to be optimized in each case in order to enhance sensory and nutritional values, rather than extrapolating the findings made with any one species for all others.
2. Study of nutritional parameter retention related to HP treatment
   Concerning chemical constituents, most research related to HP treatment has been focused on protein and lipid fractions, especially on the former one. However, researches concerning nutritional aspects such as essential amino acid (lysine, methionine, etc.) contents, protein digestibility, and PUFA and ω3-PUFA contents have been very scarce.

   Scarce or even inexistent information is available concerning the effect of HP treatment on minor components such as vitamins and minerals in marine foods. In the case of vitamins, the biggest attention should be accorded to the lipid-soluble ones (namely, A and D). Meantime, no direct effect of HP treatment on the mineral content should be expected. However, no previous research related to their content changes in processed marine foods is available nowadays. It is worth pointing out that protein denaturation and water-holding capacity loss can lead to important dripping and exudate losses, these implying a possible loss of water-soluble components, such as minerals, vitamins, and proteins.
3. Elaboration of ready-to-eat and minimally processed products
   A recent strategy to increase marine product distribution according to new trends in food consumption and lifestyle is represented by the increasing commercialization of ready-to-eat (RTE) food products, which include a myriad of refrigerated, frozen, cured, and canned seafood products. Thus, novel and attractive RTE products, most of them minimally processed, are increasingly available today in the market and restorer sectors. In this sense, HP treatment can provide a profitable tool in order to contribute to the elaboration of such products and allow them to meet the corresponding strict safety controls and attempt to satisfy the consumer's expectations for taste, flavor, and healthiness.

4. Elaboration of pressure-assisted thermal products
   The development of a novel pressure-assisted thermal product (PATP) technology to improve the sensory quality, nutritional content, and commercial value of fish products can provide a profitable issue for HP processing. This would include the search for PATP conditions (pressure, pressure holding time, and temperature) yielding a sensory quality and nutrition retention superior to that of conventional processes and a minimum alteration of the physicochemical properties in marine products. The PATP treatment conditions selected must ensure also the absence of microbial risks based on a study of the inactivation of spores from thermophile bacteria and the absence of toxicological risks.

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Chapter 28
Egg-Based Product Development for High-Pressure Processes at Low and High Temperature

Pablo Juliano

Abstract This chapter reviews the current scientific knowledge and future outlook of high pressure processing of egg products. Overall product quality improvement through modification of ingredients, formulation, processing, and packaging has been highlighted. Factors influencing microbial safety of the egg products are highlighted. Consumer acceptance of pressure-treated precooked egg-based products is presented.

Keywords High pressure processing • Egg • Functionality • Microbial safety

28.1 High Pressure Processing of Egg Products

High pressure processing (HPP) is a post-packaging technology applied for safety assurance and nutrient preservation of packaged foods. It is an industrially tested technology that offers a more natural, environmentally friendly alternative for pasteurization or shelf life extension of a wide range of food products. Commercial high-pressure low-temperature (HPLT) methods achieve inactivation of vegetative microorganisms by subjecting packaged food in flexible packaging to treatment at hydrostatic pressures of 600 MPa (or less) and initial temperatures lower than 40 °C generally for 1–15 min depending upon the product and use. Eggs and egg products contain heat-sensitive materials and the high-pressure treatment at lower temperatures can enable better retention of sensory attribute characteristic of "just prepared" as well as food nutritional components. Furthermore, similar quality levels can be reached when processing large volumes or larger samples.

About one-third of the eggs produced are further processed into egg products for the foodservice and food-manufacturing industries. The egg product sector in the

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USA was 15 % of the total egg production in 1984, whereas in the last two decades it has increased to about 30 %, or more than 61 million cases of shell eggs broken into egg products (American Egg Board 2012). In fact, tremendous growth of the use of precooked frozen egg products, such as scrambled egg patties, has occurred in foodservice venues, ranging from gas stations to fast-food restaurants, especially as breakfast menu items (Turner 2003).

As described earlier in this book (Chaps. 24–27), HPP has demonstrated strong potential for the delivery of a wide range of high-quality chilled products with extended shelf life. Among these, rare and cooked meats, fruits, vegetables, seafood, fresh herbs, and a variety of products prepared with these ingredients can be mentioned. Some of the products include ready-to-eat (or heat and serve) meat/meals. The food industry has shown interest in exploring HPP technology for the development of modified egg-based items including custards and flans. Several precooked egg-based products are currently available in markets around the world. These products include scrambled eggs, omelets, cook-in-bag scrambled eggs, egg pizza, French toast, breakfast sandwiches, crepes, and quiche. Even though such products are mainly sold in frozen form to fast-food outlet chains in the food service industry, there is potential for HPP stabilization into chilled products or reformulation from new egg protein functionalities obtained via HPP. In fact, the production of chilled (extended shelf life) and shelf-stable precooked egg patties is finding a new niche in the ready-to-eat meal market, especially as military/humanitarian rations and outdoor food items.

Only a few companies offer ready-to-eat scrambled eggs and whole hard-cooked/peeled eggs with longevity of 6–12 weeks at refrigerated conditions (American Egg Board 2012). The main challenge is to assure product safety during cooling, packaging, and post-packaging stages. This requires either additional thermal treatment or an alternative treatment that has minimal effects on the product's final quality. Furthermore, egg patties and omelets are often vacuum packaged in multiple amounts. If a thermal post-packaging treatment is applied, these products will need to handle long periods of heat exposure to reach the target process temperature throughout the entire package volume.

Manufacturing of acceptable commercially sterilized egg products using conventional thermal processing is not yet feasible, as retort processing yields undesirable flavors, greenish-black discoloration, and detrimental changes in texture and syneresis (Luechapattanaporn et al. 2005). In 2003, the US Army was obliged to stop the production of retorted scrambled eggs in plastic institutional trays (net weight 2.7 kg) due to the dissatisfaction found by military consumers with respect to the quality of this benchmark product (Dunne 2005). The current practice is to supply a freeze dried scrambled egg product, which requires on-site preparation.

The advantage of using rapid compression heating through high-pressure high-temperature processing (HPHT) to sterilize precooked scrambled egg products was addressed in a project granted by the Combat Rations Network (CORANET, US Defense Logistics Agency). This short-term project surged from a need identified among the US Army and Marine Corps for acceptable group or individual shelf-stable egg-based breakfast rations. Three academic institutions, Washington State

University, the Ohio State University, and the National Center for Food Safety and Technology, coordinated a number of tasks to evaluate various aspects of HPHT development, including egg-based product and microbial validation studies. The project was carried out with the collaboration of partners from industry and government, namely, Michael Foods Egg Products Company, Avure Technologies, the US Army Soldier Systems Center (Natick), shelf-stable ration manufacturers, and the American Egg Board. This chapter will highlight part of the output of this project, mainly focusing on quality, process design aspects, and microbial spore inactivation and product stability. Other aspects important for egg product development and stabilization via HPLT are also highlighted.

28.1.1 High-Pressure Low-Temperature Processing

Different from heat penetration, hydrostatic pressurization allows "instant" pressure transmission in fluids and semisolids within the pressure vessel, thereby achieving reduced product damage from pressurization at temperatures below 50 °C. Moreover, HPLT can add a significant shelf life to an existing refrigerated product (Hjelmqwist 2005). In fact, it has the potential to deliver chemical- or additive-free products, with minimum impact on shelf life. The theory and other practical aspects about the combined pressure-thermal process including product packaging options are discussed in detail in Chaps. 1, 5, 11, and 14.

Thermal pasteurization of liquid eggs, designed especially to control *salmonellae*, can denature and even coagulate egg proteins depending on the temperature and time history of the process. The US minimum treatment for controlling *salmonella* species in whole liquid egg and egg white is 60 °C for 3.5 min. A slightly longer treatment can impair the nutritional value, flavor, and functional properties (foaming and emulsifying ability, foam and emulsion stability, coagulation, texture, and color) by forming or destroying covalent bonds. HPLT has been applied to inactivate target microorganisms inoculated in liquid whole egg. The results obtained depend on the type of microorganism and the conditions of the treatment. *Salmonella enteritidis*, *Escherichia coli*, and *Listeria innocua* counts can be reduced more or less efficiently (8-, 7-, or 4-log units, respectively) after application of pressures higher than 400 MPa combined with a temperature of 50 °C (Guamis et al. 2005). In raw liquid whole egg, the initial bacterial count was totally reduced, and after 15 days of storage at 4 °C, only 10 cfu/mL was detected. The synergistic combination of HPP with nisin, trietil citrate, or subsequent heat treatment allows enhancing shelf life at refrigerated conditions while maintaining the biophysical properties (Ponce et al. 1998; Monfort et al. 2012). A more extensive review on the effects of HPLT on vegetative microorganisms has been compiled by Guamis et al. (2005).

HPLT is known for its potential to manufacture novel value-added foods with retained heat-labile nutrients, flavors, and aromas, from individual to institutional size packages (e.g., 6 kg sliced turkey breast package). The use of industrial scale HPLT equipment has been suggested for stabilizing precooked egg-based products,

such as individual and family size "Spanish tortillas" (Hiperbaric 2012), among other foods such as stabilized scrambled egg patties, egg-based sticks, and egg in pasta wraps or pockets (Ting 2005; Barbosa-Cánovas and Juliano 2008).

Like any other food preservation process, HPP is product specific, making shelf life extension dependent on food composition, on the presence of enzymes, and on the actual bacterial species/strains present in a given food factory. As shown later on, another use of HPP is in texture modification of foods with high protein content. Modified egg-based ingredients of varied functionality are some examples of the benefits observed in texture modification (Montero and Gómez-Guillén 2005; Guamis et al. 2005).

28.1.2 High-Pressure High-Temperature Processing

HPLT technology is unable to produce low-acid shelf-stable products, since bacterial spore inactivation requires high pressures of at least 800–1700 MPa at room temperature, far in excess of what is commercially feasible (Farkas and Hoover 2000; Leadley 2005). Even foods with pH lower than 4.5 require refrigerated storage and other preservation hurdles to prevent enzymatic degradation reactions and to inhibit spore germination.

High-pressure high-temperature (HPHT) processing, or pressure-assisted thermal processing (PATP), involves the use of moderate initial chamber temperatures between 60 and 90 °C, which through internal compression heating at pressures of 600 MPa or greater can reach pressurization temperatures between 90 and 130 °C (see also Chaps. 11, 14, and 29). The process has been proposed as a high-temperature short-time process, where both pressure and compression heat contribute to the process's lethality. In this case, heat developed through pressurization allows instantaneous and volumetric temperature increase, which, in combination with high pressure, accelerates spore inactivation in low-acid media like egg and most egg-based products.

The compression heating rate of whole raw egg, egg white, and egg yolk at an initial temperature of 25 °C was found to be 3.3 °C/100 MPa, 2.9 °C/100 MPa, and 4.3 °C/100 MPa, respectively; the latter being higher due to the higher compression heating rate obtained in fats (Patazca et al. 2007). At higher initial temperature, compression heating rate is expected to increase and will also depend on the composition of the egg product formulation (Barbosa-Cánovas and Juliano 2008). In general, compression heating properties of egg mixtures are similar to water. For example, the compression heating rate of precooked scrambled egg patties has been determined to be 3.3 °C/100 MPa or 4.8 °C/100 MPa at initial pressurization temperature of 25 °C and 80 °C, respectively.

Several patents show a number of approaches for the attainment of commercial food sterility in selected low-acid foods (Meyer et al. 2000; Wilson and Baker 2000; van Schepdael et al. 2002; März 2002, 2003; Wilson and Baker 2003; Cooper et al. 2004). In 2009, the US FDA approved a petition for the commercial use of a

pressure-assisted thermal sterilization (PATS) process for application in the production of mashed potato in a 35 L high-pressure sterilization vessel. The application of a single pulse above 600 MPa for 5 min or less, combined with initial temperatures above 60 °C, has proven to be cost-effective and safe for industrial purposes (de Heij et al. 2005). Success of this processing approach depends on the efficient use of the compression heat generated while achieving nearly adiabatic conditions. Products stabilized using HPHT processing can be categorized as long life, chill stable, and shelf stable. The chill stable category includes meat snacks, vegetables, and ready-to-eat meals or heat and serve meats among many products (Franceschini et al. 2005; de Heij 2012). The quality, acceptability, and nutritional value of these products will not only depend on the developed formulation but also on the design of the process, i.e., the preheating equipment, the high-pressure system, and the packaging material chosen.

A number of high-pressure systems specified for high pressure (600–1000 MPa) and high temperature (130 °C) have been developed. There are several designs for vessel volumes ranging from micro/laboratory scale (0.02–2 L) to pilot scale/semi-commercial scale (10–50 L). However, not all systems fulfill equal requirements in terms of pumping speed, compression heat retention, and type of compression fluid used (Balasubramaniam et al. 2004). In most cases, vessels are heated to the required initial temperature by means of an internal heater (jacket or coils), which also controls the temperature. However, this is not enough to retain compression heat generated during pressurization. Modern systems are required to use several features for heat loss prevention by mainly (a) adapting a dense polymeric insulating liner with a free moving piston at the bottom or valve to allow adequate pressure transmission, (b) preheating the inflowing pressurization fluid and pipes, and (c) preheating the vessel at a temperature higher than the initial fluid/sample temperature. Successful installation of these features can make the system close to adiabatic and, in this way, maximize preservation efficacy at chosen HPHT conditions. High-pressure vessels insulated from the interior by means of a cylindrical liner can prevent heat losses through the steel structure. In this case, a material with low thermal conductivity (less than 1 W/m/K) is required as part of the vessel design (de Heij et al. 2003). This product container (5 mm or more in wall thickness) can be made of polymeric materials with compression heating such as dense polypropylene, polyoxymethylene, polyetheretherketone, or ultra-high-molecular-weight polyethylene to provide intended heat retention (de Heij et al. 2003; Knoerzer et al. 2010).

A single pulse HPHT process involves six main process time intervals: (1) sample vacuum packaging and product loading, (2) preheating to target temperature inside the product, (3) product equilibration to initial temperature, (4) product temperature increase to pressurization temperature by means of compression heating, (5) product temperature decrease during decompression, and (6) product cooling to ambient temperature. Each of these steps marks the temperature evolution of the process. However, reaching preheating target temperature inside the food, maintaining it up to the pressure pump starts, achieving constant target pressure, and retaining heat inside the product during pressure come-up and holding time are all critical to achieving consistent product sterility.

A number of publications prove the bactericidal effectiveness of 700 MPa and process temperature of at least 105 °C for the accelerated inactivation of selected spores (*Clostridium sporogenes*, *Bacillus stearothermophilus*, *Bacillus licheniformis*, *Bacillus cereus*, and *Bacillus subtilis*) in selected matrices like phosphate buffer, beef, vegetable cream, and tomato puree (Gola et al. 1996; Rovere et al. 1998; Raso et al. 1998; Krebbers et al. 2003; Balasubramanian and Balasubramaniam 2003).

More recent publications in other food matrices (Bolognese and cream sauces) support the fact that HPHT is synergistic to inactivate spoilage organisms and selected *Clostridium botulinum* strains at 121 °C (Bull et al. 2009; Olivier et al. 2011). Olivier et al. (2011) found that across a range of low-acid food spoilage microorganisms, *Bacillus coagulans* and *Bacillus sporothermodurans* were the most HPHT-resistant isolates in laboratory and pilot-scale studies. Ahn et al. (2007) proved that HPHT treatment at 700 MPa and 121 °C for less than 1 min was sufficient to completely inactivate up to 7–8-log spore/mL from *C. sporogenes*, *Clostridium tyrobutylicum*, *Bacillus amyloliquefaciens*, and *Bacillus sphaericus* suspended in deionized water. In comparison to thermal treatment alone, the combined pressure-thermal conditions accelerates the inactivation of the spores tested.

28.2 HPP Effects on Liquid Egg and Egg Component Functionality

Higher hydrostatic pressure levels and longer pressurization times of egg protein solutions can increase apparent viscosity and affect other rheological properties. Hayashi et al. (1989) obtained egg white gels at pressures from 400 to 600 MPa for 30 min at 25 °C, with no destruction of vitamins and amino acid residues and greater digestibility than heat-induced gels. Previous studies on high-pressure formation of gels from whole liquid eggs, egg white, egg yolk, and egg yolk/white (Ma et al. 2001; Ahmed et al. 2003; Lee et al. 1999) have shown that pressures greater than 600 MPa not only increase apparent viscosity but also provide instantaneous gelation of egg yolk and egg white. Lee et al. (1999) studied the degree of egg protein coagulation as a result of pressure treatment. With increasing pressure under isothermal conditions, the starting point of coagulation was shortened and the rate of coagulation was increased. At pressures exceeding 250 MPa, coagulation occurred almost instantly. Complete and instantaneous gelation of egg yolks and egg whites occur above 600 MPa and 25 °C (Palou et al. 1999). Only pressures higher than 500 MPa and room temperature were shown to mainly affect the conformation of ovomacroglobulin and γ-livetins in liquid eggs (Ma et al. 2001). However, temperatures higher than 70 °C have been reported to affect conformation of the egg proteins livetins, conalbumins, globulins, and ovomacroglobulin (Ma et al. 2001) in both liquid whole eggs and cooked-frozen-thawed-reheated egg products.

Gels from egg white and egg yolk induced by pressures above 500 MPa have been found to give a more lustrous surface than heat-induced gels (Hayashi et al. 1989). Gels obtained by pressure did not present the typical cooked flavor of boiled eggs. There are no significant differences between heat and pressure (500 MPa) processing of egg yolk in oxidation (Beltran et al. 2001; Guamis et al. 2005).

Functional properties of egg components are also influenced by HPP in different ways: foam and emulsion stability is increased (Anton et al. 2001), and native color is maintained in comparison with heat-treated yolk even if treated above 800 MPa (Hayashi et al. 1989). Van der Plancken et al. (2007) found that pressure treatments (400–700 MPa at 10–60 °C, 20 min) gave foam solutions that were moist and creamy, showing smaller bubble size and little or no sensitivity to foam collapse (10 % v/v or 9.64 mg protein/mL), particularly at pH 7.6, where most stable, denser foams were obtained.

28.3 HPP Effects on Sensory and Other Physical Descriptors of Precooked Eggs

Recent research has explored the impact of HPHT on color, texture, water retention, and sensory descriptors on precooked egg patties (Juliano et al. 2006a, b, Juliano et al. 2012). A six-member descriptive sensory panel was trained to use 29 terms for appearance, texture, and flavor attributes. No previous work has considered formulating precooked egg-based products to suit HPLT and HPHT processing. Precooked scrambled egg products were developed in collaboration with Michael Foods Egg Products Company (Gaylord, MN) following the production process explained in more detail elsewhere (Knipper et al. 2002). Whole eggs were mixed with dry and liquid ingredients (basic formulation: whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid), after which the mixture was pumped into a mold within a flat cooking belt. Egg mixture portions were cooked (or formed) in a convection oven at 180–250 °C for a predetermined time, then frozen, and packaged. The initial formulation exploration work was carried out in a 1.7 L chamber with a polymeric liner to maximize heat retention during pressurization (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA).

In general, after HPLT treatment (30 °C/675 MPa/5 min), precooked egg patty formulations maintained their overall physical characteristics. These included color (*chroma*) texture profile analysis (TPA) descriptors, water retention, and sensory descriptors (appearance, texture, and flavor). Hence, high-pressure processing technology has definitely potential for post-packaging pasteurization of commercial egg-based patties.

Conversely, Juliano et al. (2006a) showed that HPHT treatments (50–90 °C/675 MPa/5 min) mostly decreased the *chroma* values representing yellowness and affected the egg product texture. Even though the patty was formulated with an acidifying agent, citric acid, as well as iron chelator to prevent discoloration, the

combination of high pressure and temperatures greater than 70 °C yielded green compounds. Texture was initially identified as one of the most challenging problems in the quality of the egg products after HPHT pressurization. At higher preheating temperatures, the hardness (TPA) and panel descriptors firmness, density, particle size, mouthfeel roughness, syneresis, and dryness of HPHT-treated egg patties were significantly higher than untreated (preheated and non-pressurized) egg patties. The basic egg patty formulation treated at 90 °C/675 MPa/5 min was perceived with similar flavor tones to retort treated scrambled eggs.

Besides preheating temperature, pressure magnitude also affected TPA descriptors at initial chamber temperature of 90 °C; even at the lowest pressure level of 300 MPa, hardness, gumminess, and chewiness increased significantly with respect to the preheated (non-pressurized) egg patty. Even though egg proteins coagulated during the cooking process, high-pressure conditions at high temperatures might have induced further protein aggregation, providing a firmer egg structure. These physical changes were attributed to the collapse of internal voids during pressurization, which might have led to further egg protein gelation, resulting in a firmer structure. As a consequence of increased firmness, the egg coagulum could squeeze liquid out of the protein matrix, thereby increasing syneresis.

The basic formulation was then modified, adding xanthan gum as a plasticizing agent with the aim of improving water retention and setting the egg/water ratio higher. Furthermore, EDTA was added to improve color retention as well as natural and artificial flavors to evaluate whether flavor profiles are maintained after thermal pressurization and sterilization conditions. A 675 MPa/105 °C/5 min, treatment showed higher tones of butter flavor as well as lower tones of rancid, unclean, and retort flavor than the basic formula after pressure (Juliano et al. 2006a). Chelator EDTA was probably effective in binding iron, preventing formation of iron green compounds after HPHT treatment. This modified formulation had lower hardness values (41 % and 25 %; lower after 675 MPa and initial chamber temperature of 70 °C and 90 °C, respectively) than the HPHT-treated basic formulation.

The above-mentioned basic and xanthan gum-modified formulations and an additional formulation prepared with processed cheese were then treated in the DUST 35 L machine (QUINTUS Food Autoclave Type 35L-600, Avure Technologies, Kent, WA) at HPHT conditions (700 MPa/105 °C/5 min or 700 MPa/121 °C/5 min). Both HPHT-treated egg patty formulations with xanthan gum and processed cheese showed a 33 % in hardness improvement with respect to the basic HPHT-treated formulation. Contrary to the first two formulations, the level of syneresis of the cheese-added egg patty formulation after HPHT treatment did not differ from the non-pressurized basic formulation. This was probably due to increase of casein-water interactions in cheese at high temperatures, which led to higher water absorption. This research (Juliano et al. 2006b) also found reduced hardness after HPHT treatment when using medium vacuum level (medium 400 mbar vs. high 10 mbar) in xanthan gum-based egg patties with more gel-like form and reduced initial porosity. High-vacuum packaging level of 10 mbar gave highest hardness and syneresis in a highly porous formulation with added xanthan, due to an additive effect of vacuum and HPHT treatments on patty structure.

Therefore, attention should be paid to establishing an optimum level of vacuum that does not affect texture after HPP. The authors also proved that adding up to 15 % of water on the surface of the basic formulation prior to packaging can decrease hardness after HPHT treatment at processing temperatures 105 °C and 121 °C while also increasing water holding capacity in the egg matrix.

The preheating method established to reach the initial temperature before pressurization inside the package can have an important role in the texture of precooked egg products after HPHT treatment. A fast preheating enabled via steam injection or microwave can prevent further cooking on the surface of the egg product while heat penetrates into the center of the package. This is of particular interest for the preheating of large (institutional size) products. The same would apply when a high number of pouches placed into the carriers are touching. Reduction of preheating time, by means of a higher preheating rate, in a larger sized egg patty would provide less exposure to heat, especially at the food's surface, and possibly improved texture.

28.4 Consumer Evaluation of High-Pressure-Treated Precooked Egg-Based Products

Other than the work carried out under the CORANET project, little or none has been reported on consumer acceptability of HPP products and particularly HPP egg products. Consumer evaluation has been performed on the HPHT-treated egg patty formulations and their non-pressurized controls described in Sect. 28.4 (Juliano et al. 2007). Three sessions, with 40 untrained consumer panelists, were run simultaneously with the trained descriptive panel, which verified previous studies on the effects of process temperature on product quality after HPHT processing. Consumer panelists evaluated control and treated egg patties for overall acceptability, appearance, aroma/flavor, and texture using a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely). Egg patties were treated in the DUST 35L machine (QUINTUS Food Autoclave Type 35L-600, Avure Technologies, Kent, WA) at HPHT conditions (700 MPa/105 °C/5 min or 700 MPa/121 °C/5 min).

HPHT-treated egg patties with xanthan gum and no processed cheese showed lower acceptability (≤5) than HPHT-treated egg patties with processed cheese and the non-pressurized controls (6 or more), making the HPHT-treated patties with cheese the preferred formulation. The HPHT-treated (700 MPa/105 °C/5 min) egg patty formulation with cheese was clearly acceptable (scoring 6.0). Degradation of yellow color and flavors and a firmer texture induced by the thermal pressurization process at 105 °C or 121 °C were the main causes for lower overall acceptability values obtained in formulation with added xanthan gum. Even though 5–15 % water addition to egg patty added with xanthan gum improved water holding capacity after HPHT treatment patty (Juliano et al. 2006b), consumer panelists detected no difference with the overall acceptability of the HPHT-treated patties with 5 % water added (Juliano et al. 2007).

Consumers slightly disliked the texture of HPHT-treated patty added with xanthan gum. In particular, a higher pressurization temperature that simulated thermal sterilization conditions (700 MPa/121 °C/3; $F_0 = 3.3$ min; scoring 3.8) decreased the acceptability scores of the egg patty with added xanthan gum in comparison to a milder treatment (700 MPa/105 °C/5 min; scoring 4.8). Therefore, demonstrating the minimum achievable pressurization temperature to inactivate target pathogenic and spoilage sporeforming bacteria is key for identifying more appealing egg product formulations for HPHT processes.

28.5 Microbial Validation of High-Pressure Sterilization Processes on Egg Products

As mentioned before, the effectiveness in the inactivation of microorganisms will be influenced, among other factors, by the product matrix. Microbial challenge studies have been performed on potential surrogates in egg matrices to validate the HPHT inactivation of microbial spores. *B. amyloliquefaciens* in buffer media has proven to be more resistant to the HPHT process than *C. botulinum* strains and was suggested as a potential surrogate for sterilization studies due to its non-toxigenic nature (Margosch et al. 2004). Ahn et al. (2005) obtained up to 7–8-log reduction of several *Clostridium* and *Bacillus* surrogate spores including *B. amyloliquefaciens* after subjecting them to a combination treatment at 700 MPa and 121 °C for less than 1 min. As a result, inactivation trials were performed in egg by suspending *B. amyloliquefaciens* and *B. stearothermophilus* spores (Rajan et al. 2006a, b), which confirmed that a process temperature of 105 °C at 700 MPa for 5 min can still accelerate spore inactivation. D-values of both species decreased considerably with increased process pressure. The same authors highlighted that the baking process of egg mix can contribute up to one log cycle reduction of *B. stearothermophilus* (Balasubramaniam 2005). Results are in agreement with Koutchma et al. (2005), who also determined a 6-log inactivation of *B. stearothermophilus* in spore strips located between two egg patties at 105 °C and 700 MPa for 5 min. Koutchma et al. (2005) also showed that pressurization at 110 °C and 700 MPa for 5 min is sufficient to achieve a 6-log reduction of *C. sporogenes* PA3679 in egg patties. The resistance of *B. stearothermophilus* in egg and other media, given by its D-values, also proved to be much lower when using pressure. Despite the fact that the egg patties tested had a fat content of approximately 11 g/100 g per patty, the product did not have any protective effect against *B. stearothermophilus* spores since there were no significant differences in the decimal reduction values in the egg patties and deionized water (Rajan et al. 2006b).

A remaining challenge is to determine the kinetics of inactivation of *C. botulinum* strains in egg patties by including the preheating step as a germination process (Juliano et al. 2012). It is possible that the thermal load received between preheating and pressurization in larger scale volumes facilitates spore inactivation. There is a need to explore how application of different thermal profiles within the product can affect spore inactivation after the pressure treatment.

Microbial shelf stability studies using the end-point method (i.e., package bulging detection at accelerated storage conditions) were also performed in selected egg patty formulations after HPHT (Juliano et al. 2007). Pouches of various egg formulations, controlled (non-pressurized) and processed at 688 MPa/ 105 °C–121 °C/5 min, were incubated at 37 °C for 3 and 6 months. Untreated egg patties degraded after at least 1 week of incubation, some of them producing gas and some undergoing proteolytic reactions probably due to spoilage bacteria. However, HPHT processed products did not produce gas or decompose for at least 6 months.

28.6 Final Remarks and Outlook

HPP has been presented as a promising technology for the transformation of egg materials to achieve desired functionalities. This can be advantageous at the time of formulating precooked egg products that can be stabilized by hydrostatic pressures at low temperature. Shelf life extension can also be enhanced by combining high pressure and heat, which will enable the manufacture of prepackaged shelf-stable egg products, not currently achievable by conventional heating techniques for food sterilization. This is of particular relevance for institutional size or multi-product type products that require instant heat penetration at near sterilization temperatures to avoid product degradation.

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Part VII
Regulatory and Consumer Acceptance

Chapter 29
Pressure-Assisted Thermal Sterilization Validation

Cynthia M. Stewart, C. Patrick Dunne, and Larry Keener

Abstract This chapters discusses the efforts by the US Army food industry academic consortium research to develop validation protocols and demonstrate efficacy of a pressure-assisted thermal sterilization process (PATS) for the production of a commercially sterile ambient stable, low-acid mashed potato product. Studies include qualification of the equipment, product, and package and process performance. Under the specified conditions of the validation study, it was concluded that the PATS process is capable of eliminating six $\log_{10}$ of heat- and pressure-resistant *C. botulinum* spores/145 g from the deliberately contaminated packs of mashed potatoes. Subsequently the consortium submitted a filing of a mashed potato product treated by PATS with FDA. FDA issued a letter of no objection to the consortium by 2009.

Keywords Pressure-assisted thermal sterilization • Validation • *C. botulinum* • Commercial sterility

29.1 Background

The first collaborative research programs in high pressure processing (HPP), were conducted at the University of Delaware and Oregon State University with leadership by Dallas Hoover and Dan Farkas, respectively. After 6 years of research, the feasibility of using HPP to produce a range of safe and long shelf-life acid foods was established. Subsequently, Flow International was added to the team.

All scientific work related to this chapter was completed by Cynthia M. Stewart while in residence at the National Center for Food Safety & Technology. The views expressed in this chapter are those of the author and do not necessarily reflect the position or policy of DuPont Nutrition and Health

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V.M. Balasubramaniam et al. (eds.), *High Pressure Processing of Food*,
Food Engineering Series, DOI 10.1007/978-1-4939-3234-4_29

Flow provided the framework for improved food processing equipment using high pressure. Ed Ting and Errol Raghubeer provided technical leadership for Flow. The next step was to address the key barriers in applying high pressure to produce shelf-stable low-acid foods and obtaining the requisite regulatory approvals. This initiative was established by the US Department of Defense under its Dual Use Science and Technology (DUST)program. This program was established in 1997 to fund competitive projects to establish an industrial base for new technologies for joint military and civilian use. The program required compulsory cost matching from the industrial partners established through a Cooperative Agreement with the US Army in 2000. From 2000 to 2009 Flow International (currently Avure Technologies) was the lead contractor of the high pressure sterilization research consortium. Ed Ting was the project's original principal investigator. Ultimately, the consortium built and operated a 35-L high pressure sterilization unit and located it at the National Center for Food Safety and Technology (NCFST; currently the Institute for Food Safety and Health) operated by the Illinois Institute of Technology in Chicago, a project subcontractor. Several key industrial partners contributed both funding and technical expertise to help drive the accomplishments of the consortium. The original Consortium members were Hormel, Unilever, Basic American Foods, Kraft Foods, ConAgra, and Washington Farms; they were later joined by Mars, Baxter International, and General Mills while Kraft, Washington Farms, and ConAgra dropped from the membership.

In conjunction with and co-funded by the US Army Natick Soldier RDEC and Avure Technologies, Inc., the National Center for Food Safety and Technology (NCFST; currently named the Institute for Food Safety & Health) was contracted by a food industry consortium, the Dual Use Science and Technology program, to investigate the use of heat and high pressure for delivering a conventional thermal process for low-acid canned foods (LACF), with the benefit of improving food quality as compared to conventional retort or HTST processing methods. The envisioned thermal process would take place in a 35-L high pressure-high temperature processing vessel (Avure Technologies) located at the NCFST's facilities in Summit-Argo, IL. Owing to this novel processing approach, NCFST, International Product Safety Consultants (served as process authority), Avure Technologies, and its DUST industry members (Basic American Foods, Baxter Healthcare, ConAgra, General Mills, Hormel, Mars, Unilever and formed a team of scientists and engineers to evaluate and validate the efficacy of the process. The primary goal of the high pressure sterilization consortium was to validate the capability of the process and its ability to deliver (thermal) lethality sufficient to inactivate the spores of *Clostridium botulinum* in a low-acid food product. The two objectives of this consortium were:

1. To demonstrate with a high degree of assurance, the efficacy of a pressure-assisted thermal sterilization (PATS) process when used in the production of ambient stable, low-acid mashed potatoes in a hermetically sealed package to produce a commercially sterile product that complies with all existing FDA regulatory requirements as codified at 21 *CFR* 108.35(c)(2) and 21 *CFR* 113.
2. To submit a successful process filing to the US Food and Drug Administration (U.S. FDA) for a mashed potato product in MRE pouches thermally processed in the 35-L high pressure-high temperature unit located at NCFST.

Key initial steps were undertaken by Flow International to build equipment capable of operating at 100,000 psi (690 MPa) at initial temperatures of 100 °C. vThese operational conditions were necessary for food processing. This project demanded innovations in design of the 35 L pressure vessel, pumps and intensifiers, and thermocouples. Likewise, the product carrier, loading system, and a product preheating system were required elements of the overall process. There was also a need to select and qualify pressure- and temperature-resistant strains of *Clostridium botulinum* and also to test the microbial inactivation kinetics of nonpathogenic species of bacterial spores in a search for possible surrogates. Flow International built a specialized small high pressure kinetics unit called a PT-1 that was used in some of these microbial studies as well as in the determination of parameters for compression heating of both food materials and polymers that could be used to fabricate product carriers (Rasanayagam et al. 2003; Schwhecker et al. 2002; Ting et al. 2002). Criteria for validation of low-acid foods by pressure-assisted thermal sterilization were proposed (Sizer et al. 2002). Collaborative studies were done on microbial inactivation at the National Food Laboratory in Dublin, CA, and by FDA researchers at NCFST after they acquired their own PT-1 unit. Australia's Commonwealth Sciences Industry Research Organization (CSIRO) and Mars also contributed to data confirming thermal and pressure resistance of bacterial spores that would ultimately be used in the bio-validation phase of the project.

The original team at NCFST was under the Center Director Charles Sizer with V.M. (Bala) Balasubramaniam as the lead engineer and lead project investigator. In 2004 NCFST management changed with Martin Cole becoming the Center Director and Cindy Stewart the project manager with Eduardo Patazca taking over NCFST engineering duties. Larry Keener of International Product Safety Consultants then joined the project as the process authority. The new team under the leadership of Martin Cole and Cindy Stewart embraced the path to regulatory approval using a multiphase Food Safety Objective approach as detailed in the paper by Stewart et al. (2011). Representatives of the industrial partners took active roles in all steps from experimental design to active participation in trial production runs to help streamline the process.

The official process was originally filed with LACF in September 2008 and subsequently refiled, after review and consultation with FDA, in early February 2009. The DUST Consortium's objectives were achieved when it received a "letter of no objection" from the FDA on February 13, 2009 (Fig. 29.1).

Fig. 29.1 Letter of No Objection Received from Food Drug Administration for DUST Consortium filing of a mashed potato product treated by pressure-assisted thermal processing

Fig. 29.1 (continued)

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration

February 13, 2009

Larry Keener
National Center for Food Safety and Technology
6502 S. Archer Rd.
Summit-Argo, IL 60501

RE: FCE 02930

Dear Mr. Keener:

We have completed our review of the supplemental information and supporting documentation submitted (Master File) by your firm for the use of a pressure assisted thermal process (PATS) utilizing an Avure QFP-35-600-S high pressure vessel to thermally process mashed potatoes in flexible MRE-type pouch.

At this time, we have no additional questions on the Master File information provided with your filing of SID 2009-02-06/001.

The Master File information should be referenced by your Food Canning Establishment (FCE) when filing scheduled processes which use this system. By referring to this information, your firm will have complied with the filing requirements of 21 CFR 108.35(c)2 pertaining to process filing.

If you have any additional questions, feel free to contact us.

Sincerely,

Susan J. Brecher

Susan Brecher

Food and Drug Administration
Office of Food Safety
Food Processing Evaluation Team

cc:
HFS-302 (Brecher, Zink, Mignogna, Kamara)
HFC-450 (Larkin, Anderson)
P:\LACF Information\Process Review\PATS\FCE 020930

29.2 Introduction

29.2.1 High Pressure "Pasteurization" of Foods

The reduction of microbial contamination to improve the safety and extend the shelf life of food products is one of the oldest manufacturing practices in existence. While thermal food processing treatments have been the most studied and widely used technologies, a vast array of today's technologies are utilized to improve shelf life, nutritional quality, taste, and texture, while ensuring the microbial safety of food products. Over the past 10 years, the use of high pressure processing (HPP) has gained commercial viability as an alternative preservation method for refrigerated food products (microbial safety of pressure-treated products is reviewed in Chaps. 14–17).

For food "pasteurization," HPP at chill or ambient temperatures utilizes hydrostatic pressure in the range of 300–700 MPa for a few seconds to a few minutes to inactivate vegetative pathogens and spoilage microorganisms and therefore can be thought of as a "cold pasteurization" process (Hoover 1997). Microorganisms are variable with regard to their sensitivity to HPP. Results from experiments conducted by Shigehisa et al. (1991) suggest that the order of sensitivity to HPP is Gram-negative bacteria > yeast > Gram-positive bacteria > bacterial spores. Tens of millions of pounds of pressure-treated food products have, in fact, been safely available on the commercial market for over a decade, with the first jams and jellies being introduced in Japan in 1991. These refrigerated pasteurized products include many fruit-based and, to a lesser extent, vegetable-based refrigerated food products currently on the international market, including a range of fruit smoothies, jams, juices, applesauce, fruit preparations as ingredients for yogurts, fruit blends, guacamole and other avocado products, tomato-based salsa, and meal kits containing acidified sliced bell peppers and onions. Additionally meal kits containing heat-and-serve beef or chicken slices, ready-to-eat meat products, and seafood, including oysters, are in the market in the USA, Europe, Australia, New Zealand, and Asia (Stewart et al. 2007). This process has been successful commercially and, when properly utilized, has less deleterious impact on the quality of food products as compared to conventional processes, while ensuring or enhancing the microbiological safety of these foods.

29.3 High Pressure Inactivation of Bacterial Spores

Even at the beginning of the pioneering work of applying pressure to food processing, it was indicated that spores would be difficult to eradicate with HPP at ambient initial temperatures (Black et al. 2007). In the nineteenth century, Bert Hite subjected milk to high hydrostatic pressure, as opposed to high temperature, in order to prevent it from turning sour (Hite 1899). By using pressures ranging from 400 to

700 megapascal (MPa) at room temperature, a 4-$\log_{10}$ reduction in microbial counts was achieved, while maintaining product freshness. Hite had some success in creating shelf-stable pressure-treated fruit products with a low pH (the pH prevented the outgrowth of spores), but he never could achieve shelf-stability of pressure-treated milk due to its neutral pH and presence of spores (Hite et al. 1914).

The ability to make low-acid pressure-treated products as safe as foods subjected to a botulinum cook (i.e., thermal sterilization equivalent to treatment at 121 °C for 3 min) by pressure alone has not yet been possible. For example, an early study by Timson and Short (1965) showed that viable spores of *Bacillus subtilis* and *B. alvei* survived in milk subjected to a treatment at 1034 MPa for 90 min at 35 °C.

Therefore, a number of coactive preservative factors (hurdle technology) to enhance or accompany the effects of high pressure on spores to achieve inactivation have been studied; these include pressure cycling and combinations of pressure, high and low temperatures, and antimicrobial agents (Black et al. 2007).

29.3.1 Combining High Initial Temperatures with Pressure Treatments

Whereas food products (mainly refrigerated) produced using high pressure pasteurization are increasingly available on the international market, the commercial production of low-acid shelf-stable foods has yet to become a reality. Although attempts have been made to formulate a Tyndallization process applying pressures of 50–400 MPa to germinate bacterial spores followed by pasteurization by high pressure or by high temperature, it is unlikely that this process will become a commercial reality as typically a small fraction of the spore population does not germinate and survives the pasteurization treatment (Black et al. 2007). A more promising route seems to be the use of pressure-assisted thermal sterilization (PATS) (Meyer et al. 2000; Wilson and Baker 1997). In the literature, the process is also refereed as pressure-assisted thermal processing (PATP).

PATS is the process whereby high pressure (250–800 MPa) and mild heat (80–95 °C) are combined to achieve processing temperatures that are customarily used in the preservation (canning) of ambient stable, low-acid food products (Stewart et al. 2008). Initial testing using high pressure techniques found that pressure alone had a remarkable ability to inactivate vegetative forms of microorganisms; however, sole use of high pressure has been found to have little effect on bacterial spores and various enzymes. Of course, spores are a great concern to the food industry, specifically with regard to *Bacillus* species and *Clostridium* species, especially *C. botulinum*. In the production of shelf-stable, low-acid foods, microbiological safety is the single most important prerequisite, with spores of *C. botulinum* being the critical target for elimination. By combining heat treatment of the product with pressure application, inactivation of spores and enzymes can be achieved (Rovere et al. 1998). This dual process application can no longer be considered nonthermal, but the overall

processing times are significantly shorter as compared with traditional thermal retorting processes; therefore, the PATS process can effectively eliminate microbial presence, both vegetative and spore, while producing a product quality that is superior to that of conventional thermal preservation techniques commonly used today.

29.3.2 Principles of Pressure and Heat Treatment

Pressure-assisted thermal sterilization combines the sterilization effects of both pressure and temperature. As reviewed in Chap. 2, two general principles underlie the effects of high pressure. First, Le Chatelier's principle states that any phenomenon (phase transition, change in molecular configuration, chemical reaction) accompanied by a decrease in volume is enhanced by pressure (Rovere et al. 1998). Thus, pressure shifts the system to that of lowest volume. Second, the isostatic principle states that pressure is uniformly distributed throughout the entire sample, whether in direct contact or in a flexible container (Rovere et al. 1998). Pressurization process time is therefore independent of sample size. This is in contrast with thermal processing.

Heat can be integrated into high pressure processing through adiabatic heating (compression heating) and initial product temperature. Adiabatic compression is an isentropic process, where pressure alone can increase or decrease the product temperature (see Chap. 6 for more discussion). PATS starts the high pressure treatment at elevated temperatures (e.g., 60–90 °C) and uses thermodynamically induced adiabatic heating to raise the temperature of products rapidly and homogeneously. The process temperature for high pressure processing technology is dependent not only on the initial temperature of the product but also on the temperature increase/decrease due to adiabatic compression (utilizing maximum pressure achieved). The temperature increase from adiabatic compression is reversible, so pressure-release brings instantaneous cooling. PATS' clear advantage over traditional heat processing is the theoretical rapid and homogeneous temperature change (heating and cooling) of the product without dependence on product size. In real practice, potential heat transfer effects may alter this homogeneous temperature change due to property differences between the food product and the processing environment.

Patazca et al. (2007) have postulated the following mathematical relationship (Eq. 29.1) to explain the quasi-adiabatic temperature increase (δs, °C/100 MPa) in food during high pressure processing:

$$\delta s \sim (T_f - T_i) / \Delta P[^{\circ}C / 100MPa] \tag{29.1}$$

where T_f is the temperature of the sample at the applied pressure (°C), T_i is the initial temperature (°C), and ΔP is the applied pressure, MPa. These workers reported the phenomenon of temperature increase in foods due to compression plays a critical role in establishment of a high pressure process. It was found that the magnitude of temperature increase in relation to the pressure increase per 100 MPa was

dependent on product composition and initial temperature. Among the food products tested, the high fat content foods such as mayonnaise and cream cheese resulted in a higher temperature rise during HPP compared to those foods with high water content. These workers reported finding, for example, that the effect of initial temperature on δs for vegetable oil decreased with increasing pressure and did not depend on initial temperature compared with foods with no or lower fat content. They also reported that water, honey, and cream cheese heated better at higher initial temperatures but the magnitude of δs did not vary significantly at the final pressures tested. Readers can find additional details about heat of compression and other relevant thermo/physical properties of food materials in Chap. 6.

*29.3.3 Pressure and Heat Inactivation of Bacterial Spores (*Bacillus *spp. and* Clostridium *spp.)*

Impact of combined pressure-thermal treatment on different vegetative bacteria, spores, yeast, mold, and viruses are discussed in Chaps. 15 and 16. The nonpathogenic types of *Clostridium* spores appear more sensitive to inactivation than spores of *C. botulinum.* Ahn et al. (2007) used 121 °C and 700 MPa for 1 min to inactivate 7- to 8-$\log_{10}$ CFU/mL spores of *Clostridium sporogenes* and *Clostridium tyrobutyricum* suspended in water. Kouchma et al. (2005) inactivated spore strips of *C. sporogenes* embedded in egg patties using 110 °C and 690 MPa for 4 min. Ahn et al. (2007) evaluated spore suspensions of *Thermoanaerobacterium thermosaccharolyticum* (formerly *Clostridium thermosaccharolyticum*) and *Bacillus amyloliquefaciens* in water and found 121 °C and 700 MPa for 5 min inactivated 4.5-$\log_{10}$ CFU/mL.

Scurrah et al. (2006) surveyed spores of *Bacillus* species from dairy sources. *Bacillus sphaericus* proved most resistant to PATS inactivation in skim milk at 600 MPa and 75, 85, and 95 °C. When pressure-treated for 1 min at 72 °C and 600 MPa, the range of responses of the 40 isolates of bacilli ranged from no inactivation to 6-$\log_{10}$ spores/mL. Gao et al. (2006a) found optimal process parameters to eliminate *B. subtilis* spore populations of 6-$\log_{10}$ to be 87 °C and 579 MPa for 13 min in milk buffer. For spores of *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*), Gao et al. (2006b) reported 86 °C and 625 MPa for 14 min gave 6-$\log_{10}$ reductions in a milk buffer. In water, Patazca et al. (2006) found thermal resistances of spores (expressed as *D*-values) of *G. stearothermophilus* varied according to the pressures used.

The observation that pressure can protect or stabilize spores to inactivation from heat has been reported. For example, Rajan et al. (2006) found application of 700 MPa reduced the *D*-values for PATS treatments from 95 to 110 °C, but treatment at 121 °C at this pressure resulted in a *D*-value increase. Margosch et al. (2006) examined spores of *C. botulinum* and *B. amyloliquefaciens* and found a heat exposure to 100 °C alone inactivated more spores than treatment at 600 or 800 MPa and 100 °C (and above).

The phenomenon of tailing has been reported in PATS studies (Margosch et al. 2006; Ahn et al. 2007). Tailing could be the result of pressure stabilization of spores; however, this could also be due, all or in part, to spore super-dormancy or nonlinear inactivation kinetics naturally inherent in these studies involving large populations of spores or cells (Black et al. 2011). Tailing might also be an artifact attributed to other aspects of equipment design. Additionally, when interpreting the data and conclusions presented in these papers, one should consider that there is no data presented on the thermal characterization of the spore crops used in these studies, which makes conclusions difficult. It is also difficult to determine the exact process that has been delivered in these studies, as a full processing profile is not reported in these publications that indicates the exact pressure-temperature-time course of the process. In many instances, especially when using small, lab-scale equipment, the loss of heat from the sample during the processing hold time is significant and can lead to misinterpretation of the resulting microbiological data (Stewart and Szabo 2004).

29.3.4 Pressure and Heat Inactivation of C. botulinum *Spores*

Much of the food industry regulations and sterilizing techniques revolve around the elimination of spores of *C. botulinum*. Unfortunately, spores of *C. botulinum* are extremely tolerant to combinations of high pressure and heat. Effective temperature and pressure levels for $\log_{10}$ reductions of spores can range from 60 to 121 °C and approximately 800 MPa (Hendrickx and Knorr 2001). Reddy et al. (2003) found treatment at 827 MPa and 75 °C for 15 min to reduce 3.2 $\log_{10}$ CFU/mL spores of *C. botulinum* type A. Rodriguez et al. (2004) treated spores of *C. botulinum* for 15 min at 80 °C and 101 MPa for a reduction of 4-$\log_{10}$ CFU/mL. Reduction of *C. botulinum* spores after treatment at 600 MPa and 80 °C ranged from a 5.5-$\log_{10}$ reduction to no reduction at all (Margosch et al. 2004). Heat resistance of specific spores also did not seem to correlate with pressure resistance. It was also observed that on combined heat/pressure treatments of *C. botulinum* where 99.999 % of spores were inactivated and dipicolinic acid was released; retention of dipicolinic acid was directly correlated to resistance of *C. botulinum* spores. Overall, inactivation data of *C. botulinum* spores support the potential of pressure-assisted thermal sterilization, although varying resistances among different spore populations seem to skew inactivation results. Survival curves seem to depend largely on which strain and specific microorganism is targeted, as well as the pressure equipment being used in the study. Due to a lack of inactivation kinetics data for *C. botulinum* spores, currently, only a biological validation of a PATS process for LACF food is sufficient to ensure that these products can be manufactured safely. In order to fully reach its commercial potential, a kinetics-based process delivery calculation procedure will be critical moving forward.

29.4 Outline of Project Plan

The objectives of the project plan were to qualify those elements of the PATS process, as delineated in the following process flow diagram (Fig. 29.2), which were considered likely sources of variation that may adversely affect the expected outcomes of applying the process with the intent of producing a commercially sterile, low-acid food product. The validation protocol was established with the view of determining those conditions and process parameters that define a common cause PATS process. Ultimately, it was the intent of this initiative to demonstrate, using objective scientific methods, that the PATS process when properly applied and controlled is capable, with a high degree of confidence of the verifiable and reproducible elimination of *Clostridium botulinum* spores from ambient, stable low-acid foods that are intended for use as human food. The basis for demonstrating proof-of-process efficacy was an inoculated pack study using a multi-strain cocktail of *C. botulinum* spores and mashed potatoes.

The validation procedures used in this work adhered to the recommendations in the Process Validation Guidelines of the Global Harmonization Task Force, Study Group #3 (Hojo 2004), as well as to the process validation guidelines of the US Food and Drug Administration (Shaw 1987).

The processes to validate the efficacy of a PATS process as reported in this document involve scientific scrutiny of the following elements of the manufacturing and sterilization processes:

1. Equipment installation qualification
2. Product performance qualification
3. Packaging performance qualification
4. Process performance qualification

29.4.1 Generalized Process Flow Diagram

The major elements of the process are represented in the following process flow diagram (Fig. 29.2). Included are activities associated with the formulation, standardization, and packaging of the mashed potatoes, as well as those key activities associated with preheating the packaged product in order to achieve initial product temperature and high pressure thermal sterilization using the PATS process.

29.4.2 Equipment Installation Qualification

Over a period of approximately 2 years, the equipment used in the delivery of the PATS process were evaluated in an attempt to validate its suitability and reliability for the production of commercially sterile, low-acid food products. The assessments

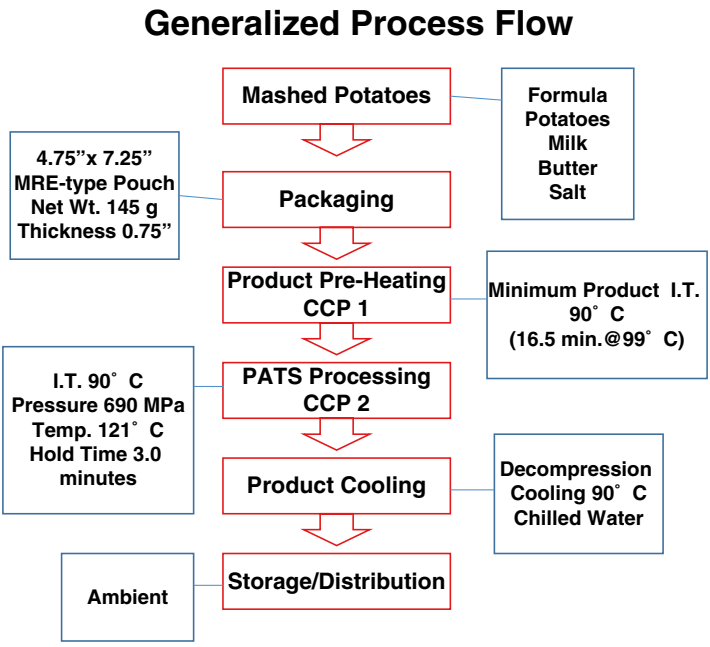

Fig. 29.2 General process flow diagram employed for DUST validation studies

of the equipment involved more than 1500 production cycles. During this interval, mashed potatoes in MRE-type pouches were produced and processed using the equipment. The items of equipment listed and discussed in this section are considered critical for process delivery, in that they impact directly whether or not the desired outcomes—commercially sterile product—will be achieved with the requisite high degree of confidence. The equipment essential for delivering the PATS process and/or monitoring critical performance characteristics of the process was evaluated. The following items were considered critical processing and monitoring equipment: product preheating tank; product cage and carrier assembly; type K thermocouples and assembly; high pressure vessel and its appurtenances; high pressure pump and associated water heaters; process water divert valve; and pressure transducers (Fig. 29.3). The general operational characteristic of the equipment is summarized here:

- Product preheating tank—Free standing water-filled vessel used in raising the temperature of the product to specified initial temperature prior to transfer to the high pressure vessel. Product heating is accomplished by immersing the loaded product carrier assembly into boiling water (steam injected) circulating within the tank with a centrifugal pump.
- Carrier and cage assembly—Cylindrical vessel and closure used for holding product during both preheating and pressure treatment. The carrier is designed to

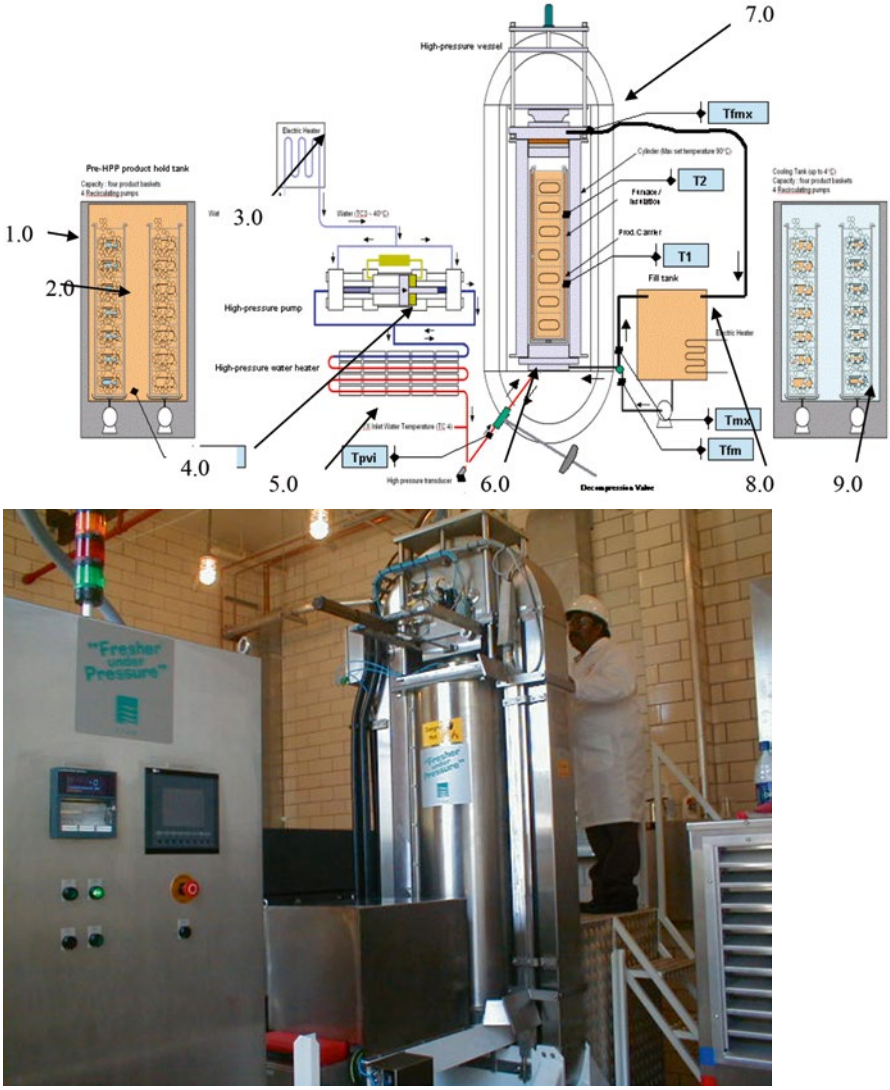

Fig. 29.3 (**a**) Equipment Installation Diagram. The components highlighted in this diagram are critical for the safe and effective delivery of a pressure-assisted thermal sterilization process: (*1.0*) preheating tank; (*2.0*) product cage and carrier; (*3.0*) intensifier preheater: (*4.0*) intensifier pump; (*5.0*) high pressure water heater; (*6.0*) high pressure water divert valve; (*7.0*) high pressure vessel; (*8.0*) process water source tank and heater; and (*9.0*) cooling tank (Avure). Tables 29.1 and 29.2 summarize details about temperature and pressure sensors. (**b**) DUST 35-L pilot scale high pressure equipment used in the study

Table 29.1 Identification and location of temperature sensors corresponding with equipment installation diagram (Fig. 29.3a)

Critical temperatures sensors	Location
Tfmx	Temperature of pre-fill medium exiting from pressure vessel before cycle
Tfm	Temperature of the pressure vessel pre-fill fluid (medium) as measured at the entrance to the pressure vessel
Tht	Temperature of the product hold tank as measured by submersed TC and MIG device
Tpvi	Temperature of high pressure water entering into pressure vessel
Tmx	Temperature of pressure medium exiting from pressure vessel after cycle
T1	Temperature of ID product carrier located on insulating (Location 1)
T2	Temperature of ID product carrier located on insulating (Location 2)

contain the product cage and its closure to accommodate K-type thermocouple mounting. The carrier assembly features a locking valve that is open during preheating to allow the constant flow of water across the expanse of the product cage contained within. The valve is locked or closed before the carrier is removed from the preheating tank. The hot water-filled assembly is then transferred directly to the high pressure vessel.

- High pressure vessel—The wire-wound vessel is designed to achieve and operate at pressures up to 700 MPa. The unit is filled with processing water at a specified processing temperature. The unit is pressurized using hot water (hydrostatic pressure) derived from the pressure transducers. Pressurization results in the compression heating of the vessel and the fluid contained within it.
- Intensifier—Pumping system used to increase the hydrostatic pressure within the pressure vessel.
- K-type thermocouples—Specially designed thermocouples for monitoring process temperature within the confines of the closed and pressurized high pressure vessel. The thermocouple assembly is designed to be integrated into the carrier closure assembly.
- Pressure transducer—Monitor the pressure change within the high pressure vessel during pressurization and decompression.
- Process water divert valve—Valve located at the interface of the intensifier and the pressure vessel. The valve will divert water emitted from the intensifier and prevent it from entering the pressure vessel until it has reached a temperature that is equal to or greater than the temperature of the process water resident in the pressure vessel (Tables 29.1 and 29.2).

When used collectively in a deliberate, purposeful manner for delivering a PATS process, these items of equipment were shown through objective analysis to perform in a manner that would allow them to be used with a high degree of confidence in the production of commercially sterile low-acid food products. All associate

Table 29.2 Identification of pressure sensors and locations to correspond with equipment installation diagram (Fig. 29.3a)

Critical pressure sensors	Location
P1	Static pressure measured of the pressure vessel based on average of two pressure transducers of different make

process controls and process monitoring equipment were calibrated against known standards and shown to provide the degree of accuracy in measurement that are required for verifying that the process is operating within the specified control limits. When process deviations or other abnormal situations were observed, efforts were made to assign cause and to identify and apply effective countermeasures. For example, based on data resulting from heat penetration testing conducted within the high pressure vessel, the product cage was modified from its original design. This action was taken due to noted inconsistencies in product heating at the upper and lower aspects of the original cage. Likewise, when the testing data showed that the furnace, originally contained within the confines of the pressure vessel, was superfluous, it too was removed.

In summary, the results obtained from more than 2 years of equipment qualification studies demonstrated that the equipment as it was configured and used in the final process is reliable and, further, that the observed variability in the delivery of that process, as attributable to the equipment, are within limits that are consistent with food safety.

29.4.3 Heat Distribution and Heat Penetration Studies to Qualify the Product

The primary processing equipment used in the study included a preheating tank and an Avure Model 600 35-L Quintus high hydrostatic pressure press and its appurtenances (Avure Technologies, Kent, WA). Both the preheating tank and hydrostatic press were evaluated to determine their respective thermal distribution and heat penetration characteristics when using mashed potatoes held in 4.75 × 7.25 in. polymeric MRE-type pouches fitted with either T-type or K-type thermocouples. T-type thermocouple wires were used for characterizing thermal conditions within the preheating tank.

K-type thermocouples, rated operational at 150 °C at a pressure of 100,000 psi (Omega Engineering, Stamford, CT), were used in thermal characterization studies within the high pressure vessel. Using these methods, both heating and heat loss rates were established for the pressure vessel and the food matrix. Moreover, the data acquired from these experiments were used to calculate accumulated lethality for the processed packs.

29.4.4 Preheating Tank

Prior to data collection, the thermocouples for environmental temperature measurements were calibrated at 200 °F (93.30 °C) against a model 6120 Micro-Bath (Fluke Co., American Fork, UT) at the Hormel R&D laboratory (Austin, MN). The obtained correction factors were later applied to the collected data.

T-type flexible thermocouples were used for heat penetration data collection. 0.75″ (19 mm) height plastic spacers were used to secure the thermocouples at the geometric center (slowest heating spot) of the tested pouches. The temperature sensing tip of each thermocouple was at least 0.5″ (12.7 mm) away from the spacer to minimize any potential effect of the spacer on the product heating rate. Time-temperature data were collected every 30 s using a 32-channel CALPlex datalogger (TechniCAL Inc., New Orleans, LA) coupled with a laptop computer. The collected data were analyzed using a NumeriCAL software version 3.000 (FMC FoodTech, Madera, CA).

The pouches were located inside the cage following the standard location as described in Fig. 29.4. Eleven (11) thermocouples were placed in various locations in the cage around the product, while ten thermocouples were placed at the geometric center (GC) of the ten individual pouches.

The "cold-spot" (zone of slowest heating) locations in the carrier were found to be at the top of the cage. Product initial temperatures (IT) varied from 60 to 70 °F (15 to 21 °C). It took 16.5 min for the product center to reach 90 °C (Fig. 29.5). Based on the collected temperature distribution and heat penetration data, the following scheduled processes were calculated using the heating factors of $j = 0.92$ and $f_h = 17.19$ min, which were determined from the slowest heating thermocouple #24:

- 18.5 min with IT = 40 °F (4.40 °C)
- 18.0 min with IT = 50 °F (100 °C)
- 17.5 min with IT = 60 °F (15.50 °C)

These scheduled processes are designed to deliver the product center temperature of 194 °F (90 °C), the minimum product temperature used for subsequent product sterilization in the HPP unit at NCFST. The maximum pouch thickness is 0.75″ (19.0 mm). Similar data was generated in replicate experimental runs (data not shown).

29.4.5 Thermal Mapping and Heat Distribution Studies to Qualify the High Pressure Vessel

The results obtained from heat distribution studies and thermal mapping within the confines of the high pressure press indicated that the upper and lower aspects of the vessel were the slowest heating areas in relationship to the product carrier and cage

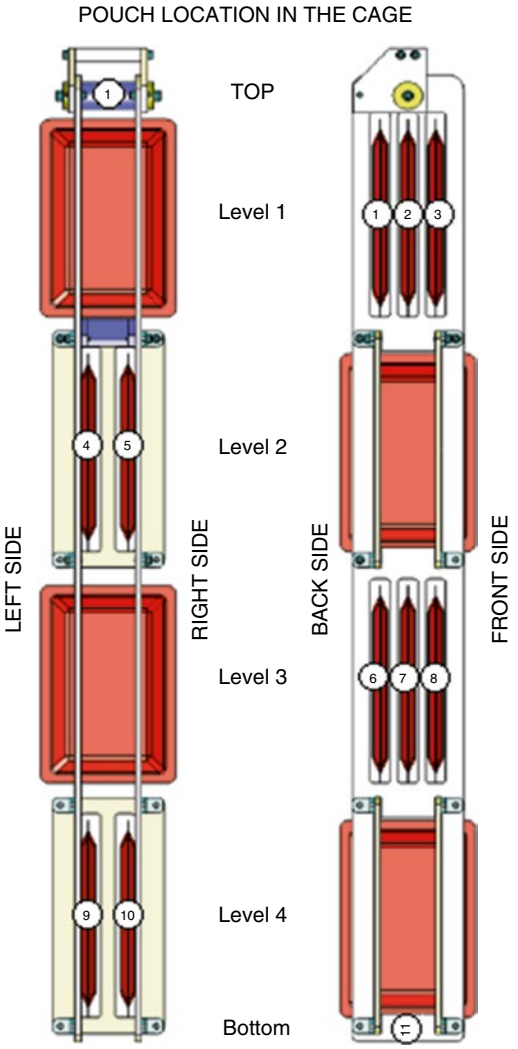

Fig. 29.4 Location of the product in the cage

assembly. Based on the data from the thermal mapping studies, the product cage was reconfigured so as to minimize the potential negative impact of the slowest heating zones on the overall performance of the PATS process. The product cage redesign ultimately redefined the working zone, or processing space, within the pressure vessel. Thermal mapping studies were completed with the new configuration to confirm that the adverse impact of the slowest heating areas on the thermal distribution within the product cage had been mitigated and did not represent a potential failure mode.

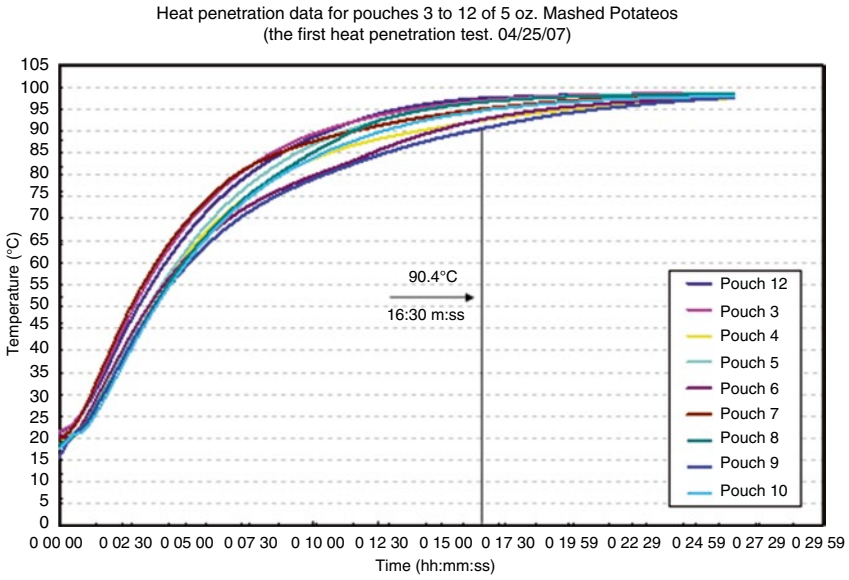

Fig. 29.5 Heat penetration data for 3–12 of 5 oz mashed potato pouches

29.4.6 Product Qualification

The mashed potato product that was the subject of this qualification was formulated in accordance with a proprietary recipe provided by Basic American Foods Company (Blackfoot, ID). The final food is formulated using commercially available components to include Russet potatoes, whole milk, unsalted butter, and salt. Over the course of approximately 2 years, numerous batches of mashed potatoes were produced and analyzed to investigate and identify those product-specific attributes likely to impact the outcomes of a PATS process.

Studies were undertaken to quantify normal microbial loading on the potatoes as well as to identify the various types of bacteria that were associated with the raw potatoes and also the unprocessed mashed potatoes. Levels of spore-forming bacteria, both anaerobic and aerobic, were also established. In addition to microbiological analysis, chemical and physical studies were conducted to elucidate mashed potato properties that might impact the delivery, both adversely and positively, of the PATS process, e.g., pH, water activity, consistency, moisture, thermal conductivity, density, fat content, and defect content level (Table 29.3).

It was concluded that, of all the attributes studied, none were found to be critical in terms of limiting the effectiveness of the proposed PATS process. However, it was also concluded that the confirmatory testing to establish physical properties was an important indicator of batch consistency, i.e., while there is no conclusive evidence that the measured physical attributes have an impact on process efficacy, they are a

Table 29.3 Summary of mashed potatoes physical property testing

Mashed potatoes					
Measurement made by	NCFST		Hormel		
	Before PATS	After PATS	Before PATS	After PATS	
Property					
pH[a]	5.56	5.54	6.21	6.08	5.99
Water activity[b]	0.985	0.985	0.99[a]	0.99[a]	0.99[a]
Density, g/mL	1.08	1.08			
Specific Heat, mJ/m^3 K	3.917	3.805			
Thermal conductivity, W/mK	0.553	0.538			
Thermal diffusivity, mm^2/s	0.14	0.148			
Consistency (cm)	0.5	0.5			
Composition %					
Protein			2.34	2.26	2.25
Fat			5.07	5.01	5.53
Ash			1.64	1.49	1.51
Salt			0.88	0.82	0.89
Moisture	75.24	74.3	77.34	74.76	76.57

[a]pH difference by lab is attributed to difference in methods of analysis

[b]A_w values rounded to the nearest hundredth of a unit

good indicator that the batch-to-batch variation is within acceptable limits. The product formula was considered important for predicting the overall performance of the PATS process.

29.5 Thermal Product/Pouch Penetration Study

A comparative study of the temperature inside MRE pouches filled with mashed potatoes and probed at the middle and outer locations was conducted using the PATS cycle. The information generated in this study was intended to support the corresponding regulatory submission documenting the correctness of the probing procedure to be used to perform further temperature penetration studies.

From the standpoint of heat transfer, the most significant failure mode during the high pressure exposure dwell affecting the accumulation of F_0 is related to a cooling process, apparently induced by the relatively lower temperature of the metallic components of the QFP-35 L-S unit. Temperature of the steel components is significantly lower during a PATS cycle because their temperature increment due to compression is minimal when compared to that of the water. Thus, the corresponding probing location for the penetration units should be very close to the wall of the container where the cooling effect of the internal environment of the sterilization chamber will lead to lower temperatures and correspondingly lower values for the accumulated F_0.

Table 29.4 Accumulated lethality (F_0) during the exposure dwell at the worst-case probing location and at the middle probing location

Data file name	Probing location	Position within MPC	Accumulated F_0, min
TempDataExport_04_11_2008_Pressurize.txt	WCL	A1	11.3
TempDataExport_04_11_2008_Pressurize.txt	Middle	B1	19.0
TempDataExport_04_11_2008_Pressurize.txt	Middle	C3	20.8
TempDataExport_04_03_2008_Pressurize.txt	WCL	A1	13
TempDataExport_04_03_2008_Pressurize.txt	Middle	B1	21.3
TempDataExport_04_03_2008_Pressurize.txt	Middle	C3	24.3
TempDataExport_04_10_2008_Pressurize.txt	WCL	A1	11.9
TempDataExport_04_10_2008_Pressurize.txt	Middle	B1	19.1
TempDataExport_04_10_2008_Pressurize.txt	Middle	C3	20.2

The middle of the product unit is most probably sheltered to a great extent during the exposure dwell from the cooling effect by the mashed potatoes potentially acting as a thermal insulation. This situation is drastically different to what is found in steam sterilization of conduction-heated products where the central region is the worst case for the accumulation of F_0 during exposure.

This study verified the assumption that the selected probing location was indeed the worst-case location from the standpoint of the accumulation of F_0. The thermocouple probes to be used to acquire penetration temperature values in high pressure sterilization studies of the MRE pouch should be placed approximately at the estimated worst-case probing location near the wall of the MRE pouch. An example of the test results is given in Table 29.4.

The mean accumulated F_0 values after the 3.0 min pressurized holding time were 12.1 min at the estimated worst-case location and 20.8 min at the middle location. The sample standard deviations of the accumulated F_0 values after the pressurized holding time were 0.9 min at the estimated worst-case location and 2.0 min at the middle location (Table 29.4).

The mean accumulated F_0 was clearly larger at the middle probing location (by more than 50 %), verifying the idea that the region where the accumulation of F_0 is expected to be minimal corresponds to the estimated worst-case probing location. At the 95 % confidence level, the difference between the mean was found to be significant using a Student's *t* unpaired comparison of means test.

29.6 Quantification of Process Hold Time and Accumulated Lethality

This study estimated the exposure dwell time needed to accumulate a minimum of 3 min of F_0 inside MRE bags filled with mashed potatoes from penetration temperature data. The most significant failure mode during the exposure dwell

affecting the accumulation of F_0 is related with a cooling process apparently induced by the relatively lower temperature of the metallic components of the HP sterilization vessel. Thus, the corresponding probing location for the penetration units is very close to the wall of the container where the cooling effect of the internal environment of the sterilization chamber will lead to lower temperatures and correspondingly lower values for the accumulated F_0.

The accumulated F_0 value was calculated from the penetration temperature data using (Eq. 29.2) the following formula:

$$F_0 = \int_{\text{time}}^{0} 10^{\frac{T(t)-250}{18}} \, \mathrm{d}t \tag{29.2}$$

where $T(t)$ is the temperature in °F as a function of time (t). If °C are used, the value 18 (z) must be changed to 10 and the reference temperature from 250 to 121.1.

The time needed to accumulate 3 min of F_0 was estimated at the 99 % confidence limit for 99 % of the population.

At the 99 % confidence level, more than 99 % of the values determined experimentally required less than 0.7 min to reach the value of 3.0 min of accumulated F_0 at the worst-case probing location.

The PATS cycle has a dwell time of 3 min. More than 99.9992 % of the values are estimated to be within this range.

The exposure dwell at high pressure of the PATS cycle may be shortened from 3 min to 1 min, as long as no other modifications are performed and the goal remains to accumulate a minimum of 3.0 min of F_0.

29.6.1 Packaging and Fill Weight Qualification

This study was designed to determine, by way of investigating both its chemical and physical properties, the compatibility of the packaging film for use with the proposed PATS process. Additionally, it was designed to establish the fill weight tolerance for mashed potato filled pouches to be processed by this method.

The packaging material (5 oz MRE Retort Pouches-Quad "Desert") that was the subject of this qualification is a four-ply laminate that is reported by its manufacturer, Smurfit Flexible Packaging, to combine the heat- and chemical-resistant properties of PET film with the excellent barrier properties of foil. In addition, it includes a layer of Biax Nylon for superior strength and abuse resistance. The structure is completed with a polypropylene sealant. It is targeted for MRE, retort, and other thermal cook-in type applications. The MRE material has been used in numerous production trials, using the PATS process, for a period of not less than 2 years.

The pouches used in this work have been shown to withstand the harsh environment created by the combined effects of high temperatures at high pressures without damage or apparent alteration. The possible exception being the tendency to form small pinhead-like blisters on the external body panels of some pouches

corresponding with the canners end seal. This phenomenon was thoroughly investigated and shown not to compromise the pack's integrity or to place the food at risk of contamination. The qualification procedure included an oxygen transmission analysis of the film as well as a headspace analysis of the filled and sealed pouch. Neither was determined to be compromised by the PATS process.

Experiments were conducted to confirm the oxygen permeation rates for both processed and unprocessed pouches. The studies were performed by FDA scientists (Moffett Center, Summit-Argo, IL) using a Systech™ model 8001 Oxygen Transmission Rate Analyzer (Systech Instruments LTD). The testing was conducted using "High Barrier, Dry Test" settings and results reported as cc/m^2/24 h. The results reported from the analysis of three (3) unprocessed pouches were (separated testing dates) 0.382, 0.385, and 0.349 cc/m^2/24 h. The results obtained from three (3) previously PATS processed pouches show a slight increase in oxygen permeation. The reported values were 0.437, 0.467, and 0.421 cc/m^2/24 h. The increase in oxygen transmission rates between unprocessed and process pouches was considered insignificant.

Headspace gas analysis was conducted using both a "cylinder and bubble method" and a "water displacement method." Results obtained from both methods confirm headspace volume per pack at less than 0.1 cc for both unprocessed and PATS processed packs. The headspace volume, as reported, was considered insignificant in terms of its impact on the delivery of the PATS process.

During all production runs and experimental trials, the weights of the individual pouches were measured manually using a properly calibrated balance. Weights were recorded in notebooks or on production worksheets. Pouches found to exceed the upper or lower limits of the fill weight specification were removed from the study and the fill weight adjusted to meet specification requirements of 145.0 g, ± 3.0 g. Packages were vacuum sealed and visually inspected to confirm proper closure and seal integrity.

29.7 Process Qualification Utilizing Inoculated Pack Studies

In order to confirm the efficacy of the PATS process, inoculated pack studies were conducted to assess its lethality against a cocktail of heat- and pressure-resistant strains of *Clostridium botulinum* spores suspended in MRE-type packs of mashed potato.

MRE-type pouches were filled with mashed potatoes that contained nominally 10^4*C. botulinum* spores (approximately 145 g were used to demonstrate the effectiveness of the PATS process). The objective of the study was the complete inactivation of *C. botulinum* spores from the test packs. The spores used in this work were calibrated and known to have classical thermal resistance characteristics (D_{121} = approximately 0.2 min). The indicator organisms selected to be used in this work was a three-strain cocktail of *Clostridium botulinum* spores. The cocktail was

composed of roughly equal proportions of the following strains: 69A, 62A, and 7273B. The decision to use these microorganisms as opposed to *C. sporogenes*, the classical surrogate for *botulinum* spores, was predicated on reports in the literature, suggesting that *C. botulinum* spores are more resistant to the combined effects of heat and high pressure than are the spores of *C. sporogenes*. The *C. botulinum* strains used in the study have also been reported by workers at both Food Science Australia and the National Food Labs to be among the most resistant to the combined lethal effects of heat and high pressure.

The criteria used to determine that mashed potato packs inoculated with proteolytic *C. botulinum* spores and processed using PATS will result in a commercially sterile, shelf-stable product was based on the outcome of no processed packages (111 packages total) produced during any of the three production day swells (due to outgrowth of the challenge microorganisms) within the 30-day storage period at 35 °C. Additionally, subculturing of a statistically significant number of pouches (35 packs) after a minimum of 30 days of storage was conducted to verify the absence of viable microorganisms, including anaerobic spores.

The inoculated packs were treated with a pressure-assisted thermal sterilization process, using the previously qualified and calibrated processing and monitoring equipment. The process parameters used in this work were the following:

1. Product initial temperature = 90 °C minimum
2. Applied pressure = 690 MPa minimum
3. Process temperature = 121–130 °C (121 °C minimum)
4. Pressurized holding time at process temperature = 3 min

The processed packs were held at 35 °C for a minimum of 30 days and observed for evidence of spoilage (Table 29.5). Ultimately a statistically significant number (35/111) of the flat- and normal-appearing packs were opened and subcultured to detect the presence of viable, sublethally injured spores. While growth was reported in five of the 35 packs examined, subsequent testing by mouse bioassay confirmed that the positive-appearing cultures did not contain botulinal toxin. Hence, the observed growth in the subcultures was not initiated by the germination and outgrowth of *C. botulinum*. It is concluded that the microorganisms recovered from the subcultures resulted from post-processing recontamination of the sterile product during sample handling and preparation within the microbiology laboratory. Of the remaining 76 pouches produced for the inoculated pack study, none evidenced spoilage after more than 60 days of continuous incubation at 35 °C.

In addition, 2 days of production were conducted with various levels of intentional under-processing, in order to attempt to demonstrate where the process fails. In order to achieve intentionally under-processed products, pressure process hold times of 15 s (35 packages) and 5 s (35 packages) were used. Additionally, a “zero hold time” process (70 packages) was run in which 690 MPa was achieved followed by immediate decompression. Additional under-processing trials were conducted, where the samples were processed by preheating for 10.5 min in boiling water and pressure processed at 690 MPa for 90 s (14 packages), 45 s (14 packages), and “zero

Table 29.5 Summary of fully processed inoculated pack studies

Production date	Spore level CFU/pack	Cycle	Fully processed Preheat 16.5 min + 180 s at 690 MPa	Unprocessed No preheat step No UHP	Processing comments
			# Positive/total samples		
4/30/2008		1	0/7		OK
		2	0/7		OK
		3	0/7		OK
		4	0/7		OK
		5	0/7		Note 1
	3.6×10^4	Controls		3/3 (2 days)	
5/6/2008		1	0/7		OK
		2			Note 2
	4.1×10^4	Controls		3/3 (2 days)	
5/9/2008		1	0/7		Note 3
		2	0/7		OK
		3	0/7		OK
		4	0/6		OK
		5	0/7		OK
	2.5×10^4	Controls		3/3 (2 days)	
5/13/2008		1	0/7		OK
		2	0/7		Note 4
		3	0/7		OK
		4	0/7		OK
		5	0/7		OK
	3.5×10^4	Controls		3/3 (2 days)	
Total number of fully processed packs			111 packs		

Note 1—Cold fill tank temperature
Note 2—Cycle aborted during hold time; testing of cycles 3–5 aborted due to operational issues with equipment
Note 3—Sample test were preheated twice due to low temperature in cooking tank
Note 4—Cycle failed to start and samples were taken out and heated in boiling water for 5 more min. TC was changed; therefore heat penetration was not tested

hold time" (7 packages) cycles. The processed pouches were held in incubated storage at 35 °C for a period of more than 60 days. In that interval none of the under-processed packs succumbed to or evidenced spoilage (Table 29.6).

Finally, additional work was conducted using inoculated packs with higher spore counts (10^6 spores/145 g) but with shortened processing parameters. These studies were carried out with the intent of simulating worst-case process conditions and corresponding process failures. A total of 182 inoculated pouches were produced and processed using these failure modes. The processed pouches were held in incubated storage at 35 °C for a period of more than 60 days. In that interval none of the under-processed packs succumbed to or evidenced spoilage (Table 29.7).

Table 29.6 Summary of 16.5 min preheated/under-processed inoculated pack studies, May–June, 2008

Production date	Spore level CFU/pack	Cycle	Under-processed				Unprocessed	Processing comments
			Preheat 16.5 min 0 s at 690 MPa	Preheat 16.5 min 5 s at 690 MPa	Preheat 16.5 min 15 s at 690 MPa	Preheat 16.5 min 32 s at 690 MPa		
			Number swells/total samples					
5/6/2008	4.1×10^4	2				0/7		Note 1.0
5/16/2008		1	0/7					Note 2.0
		2	0/7					Note 3.0
		3	0/7					OK
		4	0/7					OK
		5	0/7					OK
	1.5×10^6	Controls					3/3 (2 days)	
5/20/2008		1	0/7					OK
		2	0/7					OK
		3	0/7					OK
		4	0/7					OK
		5	0/7					OK
	2.6×10^6	Controls					3/3 (2 days)	
5/23/2008		1		0/7				
		2		0/7				
		3		0/7				
		4		0/7				

Table 29.6 (continued)

Production date	Spore level CFU/pack	Cycle	Under-processed				Unprocessed	Processing comments
			Preheat 16.5 min 0 s at 690 MPa	Preheat 16.5 min 5 s at 690 MPa	Preheat 16.5 min 15 s at 690 MPa	Preheat 16.5 min 32 s at 690 MPa		
		5		0/7				
	2.2×10^6	Controls					3/3 (2 days)	
5/28/2008		1			0/7			
		2			0/7			
		3			0/7			
		4			0/7			
		5			0/7			
	2.4×10^6	Controls					3/3 (2 days)	
Total number of packs			70	35	35	7		

Note 1.0—Cycle aborted during hold time; testing of cycles 3–5 aborted due to operational issues with equipment
Note 2.0—No record of product temperature data

Note 3.0—No record of product temperature data

Table 29.7 Summary of 10.5 min preheated/under-processed inoculated packs

Date produced	Spores/ pack	Cycle	Under-processed	Under-processed	Under-processed	Not processed	Comments
			Preheat 10.5 min + 0 s at 690 MPa	Preheat 210.5 min + 45 s at 690 MPa	Preheat 10.5 min + 90 s at 690 MPa		
6/17/2008		1	0/7				
		2	0/7				
		3		0/7			
		4		0/7			
		5					Aborted run
		6			0/7		
	2.2×10^6	Controls				3/3 (2 days)	
Total number of processed packs		3	14	14	7		

29.7.1 Conclusions from the Inoculated Pack Studies

The objective of the fractional processing studies was to create processing modes whereby failures could be observed. A total of 182 packs were produced using the various fractional processing methods described above. The processed packs were held at 35 °C for a period of approximately 60 days and none evidenced spoilage. By contrast each of the 15 unprocessed (positive controls) packs that were produced to accompany these two studies spoiled within 72 h of storage.

A combined total of 293 inoculated samples were produced, processed (fully or fractionally), and investigated for evidence of spoilage by *C. botulinum*. Thirty-five flat, normal-appearing packs that had received the full PATS process were opened and subcultured. All were shown free of mesophilic, spore-forming bacteria consistent with *C. botulinum*. Moreover, the remaining 258 inoculated and processed (fully and fractionally) pouches held in incubation for more than 60 days, none evidenced spoilage. Based on the outcomes of these studies, especially those in which the pressurized holding time approximated 0 min, it is clear that there is sufficient lethality associated with coming-up-to pressure (pressurization) and the corresponding associated incremental increase in product temperature during this interval to provide for the inactivation of the challenged microorganism. Moreover, the results from the heat penetration studies conducted within the pressure vessel show that in 0.7 min, at process pressure (690 MPa) and processing temperature in excess of 121 °C, there is an accumulated lethality (F_0) equivalent to 3.0 min. Therefore, the attenuated pressurized holding time (approximating 0 min) would in reality provide sufficient lethality to inactivate the spore load (10^6 spores/145 g) used in this work. Hence, the lack of observed spoilage in the failure mode packs were analyzed. This observation also suggests a synergistic relationship between heat and pressure in contributing to the inactivation kinetics of the *C. botulinum* spores used in this work.

29.8 Summary

Based on the cumulative findings resulting from the implementation of this validation protocol, it is concluded that when using the processing equipment, procedures, packaging materials, and mashed potatoes that have been qualified in these studies, the PATS process is qualified and is capable (>99 % confidence interval) of eliminating 6 $\log_{10}$ of heat- and pressure-resistant *C. botulinum* spores/145 g from the deliberately contaminated packs of mashed potatoes.

This finding is also supported by thermal processing calculations. When accumulated lethality rates were calculated, it was determined that when using the PATS process, as it is defined in this report, 3 min of F_0 were accumulated in 0.7 min of exposure time. Furthermore, the data from the heat penetration studies conducted within the pressure vessel show at the worst-case probing location an accumulated

lethality in 3 min of exposure equal to 12. It is generally understood and accepted that a thermal process with an F_0 equivalent to 3 min is effective in the destruction of at least 10^{12} spores. The classical "Botulinum Cook" is based on 2.5 min at 121 °C and assumes a 12D reduction of *C. botulinum* spores. These findings confirm, with a high degree of confidence, the safety of the mashed potatoes as formulated, processed, and packaged in accordance with materials that have been the subject of these validation studies.

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Chapter 30
EU Regulatory Approach to High-Pressure Processing

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Abstract This chapter focuses on the application of the Novel Foods Regulation to high-pressure-treated foods. It highlights some other EU requirements applicable to HPP. The chapter first describes the general requirements for foodstuffs set out in the so-called EU General Food Law. We then analyze the provisions of the Novel Foods Regulation applicable to HPP in more detail. The relevant provisions regarding hygiene of high-pressure-treated foodstuffs and their labelling are discussed. Finally, the Directive concerning pressure equipment is presented.

Keywords High-pressure processing • European regulations • Novel foods • Hygiene

30.1 Introduction

Food products treated with high-pressure processing (HPP) must comply with all rules and regulations regarding food. A small survey among businesses engaged in the application of HPP revealed that business operators are particularly concerned about the possible application of the Novel Foods Regulation to HPP-treated foods (Cholewińska 2010).

High-pressure processing is a relatively new technology in Europe. Although the first high-pressure-treated product in the EU (orange juice) was produced in France in 1993, HPP was not commonly known before 1997. In 1997, EU Regulation 258/97 on novel foods (Novel Foods Regulation—NFR) went into effect.[1] This regulation introduced a premarket authorization requirement for all foodstuffs which were not used for human consumption to a significant degree before 15 May 1997 (the effective date of the regulation) and fell under one of a number of categories of foodstuffs among which the category of foodstuffs to which "has been applied a production process not currently used, where that

[1] OJ 1997L 43/1.

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process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism, or level of undesirable substances."[2]

This chapter focuses on the application of the Novel Foods Regulation to high-pressure-treated foodstuffs. In addition, it highlights some other EU requirements applicable to HPP. The chapter first describes the general requirements for foodstuffs set out in the so-called EU General Food Law. We then analyse the provisions of the Novel Foods Regulation applicable to HPP in more detail. We also discuss the relevant provisions regarding hygiene of high-pressure-treated foodstuffs and their labelling. Finally, the Directive concerning pressure equipment is presented. The last section contains our conclusions.

30.2 General Requirements of Food Safety

The general principles and requirements for all food and feed brought into the EU market, as well as procedures in matters of food and feed safety, are enshrined in the General Food Law (GFL)—Regulation 178/2002.[3] This regulation applies to "all stages of production, processing and distribution of food and feed," except for "primary production for private domestic use or to the domestic preparation, handling or storage of food for private domestic consumption."[4] As a regulation, the General Food Law is binding in its entirety and does not require any transposition into national legal systems; it is directly applicable in all EU member states.

The General Food Law places primary legal responsibility for ensuring food safety on the food business operator, broadly defined as the natural or legal persons having under their control "any undertaking, whether for profit or not, and whether public or private, carrying out any of the activities related to any stage of production, processing and distribution of food."[5]

The main requirement is set out in Article 14(1) GFL which states: "Food shall not be placed on the market if it is unsafe." The General Food Law does not provide a definition of food safety,[6] but it explains instead the concept of *un*safe food. According to the General Food Law, food is unsafe if it is either injurious to health or unfit for human consumption.[7] In determining whether a food is unsafe, account is taken of the normal conditions of use of the food by the consumer and by the food

[2] Art. 1(2)(f) NFR.

[3] Directive 97/23/EC of the European Parliament and of the Council of 29 May 1997 on the approximation of the laws of the Member States concerning pressure equipment, OJ 2002L 31/1.

[4] Art. 1(3) GFL.

[5] Art. 3(2 and 3) GFL.

[6] Food safety is defined by *Codex Alimentarius* as "assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use" (Codex General Principles of Food Hygiene, CAC/RCP 1-1969).

[7] Art. 14(2) GFL. For a detailed analysis, see van der Meulen (2012).

business operator at each stage of production or distribution and the information provided through the label or generally available information concerning certain adverse health effects. Furthermore, Article 14(4) GFL elaborates on the concept "injurious to health":

> In determining whether any food is injurious to health, regard shall be had:
>
> (a) not only to the probable immediate and/or short-term and/or long-term effects of that food on the health of a person consuming it, but also on subsequent generations;
> (b) to the probable cumulative toxic effects;
> (c) to the particular health sensitivities of a specific category of consumers where the food is intended for that category of consumers.

Food complying with EU or national provisions concerning food safety is presumed to be safe with regard to the aspects covered by the specific provisions contained therein. It must be highlighted, however, that even food complying with the EU or national legislation may be subject to restrictions if public authorities have reason to suspect that a food is unsafe despite such conformity. Similarly, under Article 19(3) GFL, food business operators are obliged to immediately notify competent authorities if they "consider or have reason to believe" that a food they have placed on the market may be injurious to health. This is a stronger requirement where only two conditions must be met: the food is on the market (or has left the immediate control of the initial food business) and it is considered unsafe by the food business operator. This obligation ensures that the public authorities receive early warnings about potential emerging risks.

If a food or feed which does not comply with food safety requirements is on the market (or has left the immediate control of the initial food business), the food business operator, according to Article 19 GFL, has the obligation to withdraw the product. This obligation arises when the food or feed has been identified as unsafe according to the criteria set out in Article 14 GFL. Depending on the results of the assessment of risks to human health, recall of the product from consumers may also be necessary. Such recall, however, must take place only if other measures are not sufficient to achieve a high level of health protection.[8]

The concept of unsafe food encompasses both the characteristics of categories of foodstuffs and the condition of specific foods. Some categories of products are generally unsafe, for example, because they are toxic. Other foods, while belonging to a category that as such is generally safe, may be unsafe due to certain conditions, such as contamination with pathogens. Ensuring the safe condition of individual foods is known as "hygiene." HPP is an instrument intended to further food hygiene by inactivating vegetative microorganisms.

Food categories with a history of safe use in the EU are presumed safe as long as no evidence to the contrary emerges. For several types of food products that do not have a history of safe use, this presumption of safety is replaced by an obligation to provide evidence of safety as a condition to market access. This is the case with novel foods.[9]

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[8] Art. 19(1) GFL.

[9] Other examples are food additives, food supplements, and genetically modified foods.

30.3 Novel Foods Regulation

All foods and food ingredients[10] that have not been used for human consumption to a significant degree in the EU before 1997 must undergo an authorization procedure before they can be placed on the market. Apart from the cut-off date of 15 May 1997, in order to be considered novel, food or food ingredients should fall under one of the following four categories:

Foods:

- With a new or intentionally modified primary molecular structure
- Consisting of or isolated from microorganisms, fungi, or algae
- Consisting of or isolated from plants and food ingredients isolated from animals, except for foods and food ingredients obtained by traditional propagating or breeding practices and having a history of safe food use
- To which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism, or level of undesirable substances[11]

The application procedure starts with a decision by the potential applicant. The applicant has to consider if the food product is novel and to collect the evidence supporting the case. The basic requirements for novel foods to be placed on the EU market are set out in Article 3(1) NFR, according to which novel food or novel food ingredients must not:

- Present a danger for the consumer
- Mislead the consumer
- Differ from foods or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer

If the food business operator is not sure whether the product is novel, he or she may consult the European Commission or authorities in the member states. In general, if the application is accepted by a member state, the food is considered to be novel. In situations when it is not clear to a member state whether a food falls under the Novel Foods Regulation, the Novel Foods Working Group may be consulted. This group is a platform where matters concerning novel foods are considered. It consists of experts from the member states and a chair, who is an officer from the Commission. If the Novel Foods Working Group is not able to form an opinion concerning the novelty of the food, the arbitration procedure of the Novel Foods Regulation (Article 13) should be followed (EC 2002).

[10] This separate reference to food ingredients shows that the Novel Foods Regulation predates the General Food Law and its definition of food (Art. 2 GFL). Food ingredients fully fulfil the GFL definition of a food. The additional reference to ingredients in the NFR therefore no longer has added value.

[11] Art. 1(2) NFR.

An application containing the safety assessment report is submitted to an EU member state authority for authorization. The competent authority decides whether additional assessment is necessary. If no additional assessment is necessary, and if the Commission or other member states do not object, an authorization decision is made. Once approved, the product can be marketed throughout the EU.

Apart from the authorization procedure, the Novel Foods Regulation also provides for a notification procedure for novel foods, which applies to foods that are substantially equivalent to foods existing on the market on the basis of the scientific evidence (Article 5 NFR). This simplified procedure, however, covers only two categories of novel foods, i.e., foods consisting of or isolated from microorganisms, fungi or algae, and foods consisting of or isolated from plants or isolated from animals. Therefore, this simplified procedure cannot be applied to foods to which a new production process has been applied.

Because food produced with HPP may fall under the last category, two factors are important in determining whether the Novel Foods Regulation applies:

1. Was HPP not used to a significant degree before 15 May 1997?
2. Does HPP give rise to significant changes in the composition or structure of the foods, affecting their nutritional value, metabolism, or level of undesirable substances?

The crucial aspect determining the novelty of a food product is whether it has been consumed to significant degree (or "significant quantity"[12]) in at least one member state. Consequently, proof regarding the consumption of a product coming from regions outside the European Union is not a relevant factor under the Novel Foods Regulation. Even the fact that the food was imported to Europe before the regulation went into force would not be sufficient if that country were not a member state of the EU (San Marino).[13] In a discussion paper on the implementation of the Novel Foods Regulation from 2002, the term "significant degree" was explained as a food that has been demonstrated as being generally available within the EU (EC 2002). Thus, if a food was sold only in pharmacies, it would not be considered proof that it was consumed to a significant degree. However, the availability of a food in food stores would constitute evidence that the food was generally available in the European Union.

30.4 First HPP Application Under Novel Foods Regulation

In December 1998, Groupe Danone submitted a novel food application to the French Food Safety Agency (AFSSA, now renamed French Agency for Food, Environmental and Occupational Health and Safety—ANSES) to place on the

[12] As referred to by the European Court in Joint Cases *HLH Warenvertriebs GmbH* (C-211/03) *and Orthica BV* (C-299/03 and C-316/03 to C-318/03) v *Germany*, [2005] ECR I-5141, para. 88.

[13] Case C-383/07, *M-K Europa GmbH & Co.* v *Stadt Regensburg*, [2009] ECR I-115.

market fruit-based preparations produced using high-pressure pasteurization. The process parameters proposed were 8 kbar (800 MPa) for 6 min at 20 °C. The application was supported by scientific studies, which included detailed physicochemical characterisation, cytotoxicity, mutagenicity, impact of high-pressure treatment on allergenic risk, microbial challenge tests, contact material migration tests, and HACCP plan.

The presented results revealed that the novel product was neither cytotoxic nor mutagenic. The inactivation of vegetative bacteria was equivalent to heat treatment. Moreover, the stability of vitamins C, B2, and B6 was equivalent or improved in comparison to traditionally produced products. However, bacterial spores were resistant to the employed pressure. Therefore, the applied risk management was based on prevention of spore germination and growth. Groupe Danone also defined the agronomic and manufacturing conditions (Food Standards Agency 2000).

The application was accepted by AFSSA and the initial assessment report was forwarded to the Commission, which forwarded it to all other member states in May 2000. During the time to review the dossier (60 days), several member states raised reasoned objections concerning the report, mainly concerning the safety of HPP with respect to bacteriological risks and allergenic potential.

This outcome shows that in assessing the safety of a food that is considered novel due to the application of a new hygiene technology such as HPP, the achieved level of decontamination is included.

Among the member states that raised objections was the UK. The UK Advisory Committee on Novel Foods and Processes was asked to comment on the report. The committee is an independent scientific body advising the UK Food Standards Agency on all matters relating to novel foods and novel processes. The committee required clarification on a number of points regarding the specifications of food preparation, quality assurance testing, and process controls. Also, with regard to the risk of botulism, the committee required that authorization for the use of high-pressured fruit preparations should be applicable only to final products. These products should also comply with the recommendations included in the Report on Vacuum Packaging and Associated Processes, published by the UK Advisory Committee on the Microbial Safety of Food.

In July 2000 the committee sent a letter to the European Commission, specifying the conditions relevant for ensuring the safety of high-pressured fruit preparations, in which it stated:

> In particular, in addition to chill temperatures, which should be maintained throughout the chill chain, the following controlling factors should be used singularly or in combination to prevent growth and toxin production by psychrotrophic *Clostridium botulinum* in prepared chilled foods with an assigned shelf-life of more than 10 days:
>
> – a heat treatment of 90 °C for 10 minutes or equivalent lethality;
> – a pH of 5 or less throughout the food and throughout all components of complex foods;
> – a minimum salt level of 3.5 % in the aqueous phase throughout the food and throughout all components of complex foods;
> – an a_w (water activity) of 0.97 or less throughout the food and throughout all components of complex foods (Food Standards Agency 2001a, 63–64).

These concerns did not relate to the safety of HPP as such, but rather to the outgrowth of spore formers (*Clostridium botulinum*), which can survive both thermal pasteurization and HPP pasteurization. In favorable conditions, spores may start to germinate and subsequently outgrowth can occur. Therefore, it is necessary to ensure that validated microbiological hurdles are in place.

Because objections at a national level were raised, the authorization decision was shifted to the EU level and taken in accordance with the so-called "comitology" procedure in Article 13(2) NFR (EC 2001).[14] On 23 May 2001, the Commission made a positive decision authorizing placement on the market of pasteurized fruit-based preparations using high-pressure pasteurization.[15] An annex to this decision specified the allowed HPP preparations, including types of fruit, conditions of fruit storage before HPP, percentage of added fruits to other ingredients, pH, dissolved sugar-to-water mass ratio of preparations (°Brix), water activity (a_w) and conditions of final storage.

Although the authorization was granted, Groupe Danone never brought the high-pressure fruit-based preparations to the market. The Danone case, however, shed some light on the application of the Novel Foods Regulation to HPP. As mentioned above, HPP was not common on the EU market before 1997. However, for a food to be considered novel under the last category, there must also be significant changes in its composition or structure resulting from the new production method applied.

30.5 Significant Changes in Food Composition or Structure

In July 2001, following the authorization granted to Groupe Danone for placement on the market of HPP fruit preparations, the competent authorities of the member states agreed that in the future national authorities should decide the legal status of foodstuffs produced using HPP based on information provided by the applicant. If the national authority concludes that authorization of the product is not needed under the Novel Foods Regulation, it should simply inform the other member states and the Commission (Eisenbrand 2005).

In the same year, the UK Advisory Committee on Novel Foods and Processes received two separate requests, from Italy and the UK, for an opinion in accordance with the NFR. Both applications concerned similar products: juices and smoothies treated with high pressure. After having provided relevant information concerning food safety, both companies received a positive scientific opinion. In its opinion, the advisory committee stated,

> As a successful application under Regulation 258/97 for HPP Fruit Based Products was made by Danone in 2000, High Pressure Processing per se is no longer considered a novel

[14] Art. 13(2) NFR refers to the old procedure set out in Articles 5 and 7 of Decision 1999/468/EC of 28 June 1999 laying down the procedures for the exercise of implementing powers conferred on the commission (OJ 1999L 184/23). This decision has been repealed by Regulation 182/2011 laying down the rules and general principles concerning mechanisms for control by Member States of the Commission's exercise of implementing powers, OJ 2011L 55/13.

[15] Decision 2001/424, OJ 2001L 151/42.

> process. However, any future use of HPP that used different operating conditions, or treated substantially different foodstuffs from those described in the Danone application must be able to demonstrate adequate kill of pathogenic bacteria, and have measures in place that prevent the germination of *Clostridium botulinum* spores (FSA 2001b).

This is a very interesting and important interpretation of the Novel Foods Regulation. A technology introduced after the cut-off date of 1997 can, at least to a certain extent, lose its "novel" status. Both this interpretation and the added requirement that future applications should be able to demonstrate adequate kill of pathogenic bacteria give pragmatism priority over the letter of the law.

Following the opinion issued by the UK Advisory Committee, the Spanish Food Safety and Nutrition Agency (Agencia Española de Seguridad Alimentaria y Nutrición—AESAN) notified that high-pressure pasteurized cooked ham was not considered a novel food and could be brought to the EU market without approval. In 2002 the UK Food Standards Agency (FSA) also informed that high-pressure-treated oysters were not considered novel foods.

During a meeting on 13 September 2001, the UK Advisory Committee on Novel Foods and Processes agreed on the following:

> [T]he Commission had concluded that High Pressure Processing was no longer considered to be a novel process. Nevertheless, some Member States were concerned that the foods treated in this way should still be assessed for their safety. A copy of the letter sent to the Commission asking that guidance be provided on the use of High Pressure Processing technology was tabled for Members' information. Members emphasised that the data required for a safety assessment would be determined on a case by case basis, but that in all cases microbiological data on products should be provided. They also noted that allergenicity and nutrition issues would need to be addressed (Food Standards Agency 2001c).

This quote again shows a struggle with the limits of the law: either a product is novel and subject to safety assessment under the Novel Foods Regulation or it is not. Desiring to have products assessed for safety that are considered not to be novel, the member states, referred to in the quote, overstep their statutory powers.

The view that HPP is as safe as existing processes such as pasteurization was further concluded during an open meeting of the advisory committee (FSA 2002). Moreover, on the ACNFP website, the following note was made public:

> [Although not mentioned at the meeting, the European Commission has discussed the status of HPP with representatives from the EU Member States. As a result, it has been accepted that the process does not produce any material change in the composition of the food and as a result does not require further assessment under the novel foods procedures (FSA 2002).

In the same way, in Germany, the then Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (BgVV) was asked to provide an opinion on the legal status of high-pressure preserved fruits and came to the conclusion that HPP does not cause significant changes in the composition or structure of fruits which would affect their nutritional value, metabolism, or level of undesirable substances (Eisenbrand 2005).

Many member states have decided on the legal status of foodstuffs produced with the use of HPP, concluding that this category of foodstuffs does not constitute

a novel process. These conclusions were based on evidence that HPP does not give rise to significant changes in the composition or structure of the foods or food ingredients. At the same time, however, some national authorities have expressed concerns about the safety of HPP, arguing that every application of HPP should be assessed on a case-by-case basis. Evaluation of the effects of HPP on food products or ingredients is seen as necessary to assess whether it produces any significant changes in the final product. Only if the assessment shows no significant changes in the final product, the premarket authorization procedure would not be needed.

This is probably the main cause of the ambiguity of the Novel Foods Regulation. In practice, products do not seem to be subjected to safety assessment under the Novel Foods Regulation if they meet the criteria of the definition of a novel food, but rather they are considered novel if member states believe their safety should be assessed.

In all cases, the responsibility to define "significant changes" is left with the person or agency bringing a product to the market. At the same time, however, in the current state of knowledge concerning HPP, it does not seem to be likely that an HPP product would fall under the scope of the Novel Foods Regulation. Theoretically, of course, there may be products that significantly change after HPP has been applied. In the vast majority of cases, however, the technology will have no such effect on the product.

The case-by-case approach has been recommended in the Opinion of the Senate Commission on Food Safety of the German Research Foundation, which states:

> [I]nvestigations of high-pressure treated foodstuffs have not revealed any evidence of any microbial, toxicological or allergenic risks as a consequence of high pressure treatment. However, these findings do not suffice for a general evaluation, because they derive from only a few already marketed products. At present it is necessary, when a new product category is involved, always to carry out an individual case-by-case examination of high pressure treated foodstuffs (Eisenbrand 2005, 1173).

The term "significant change" gives much room for multiple interpretations, especially when applications are evaluated by the authorities in all member states. For example, it is common sense that only changes that make a product worse should be considered in this context. Hence, reduction of a desirable substance would be a "significant" change, but the fact that the amount of vitamins in fruit preparations produced with the use of HPP is higher than in conventionally pasteurized foods would not cause the product to fall under the scope of the Novel Foods Regulation.

Finally, it should be noted that almost two decades have passed since the Novel Foods Regulation came into force in 1997. This period has definitely contributed to a better understanding of the area of application of the regulation. Initially, the Novel Foods Regulation also covered genetically modified (GM) foods[16] and, at that time, public attitudes towards GM food were very negative in the EU. Therefore, the Novel Foods Regulation was also perceived as quite strict.

[16] GM food and feed are now regulated separately in Reg. 1829/2003, OJ 2003L 268/1.

In 1997 the Commission indicated that the new production process might comprise, for example, "new types of heat processing, nonthermal preservation methods, new processes to chill or freeze products, to dehydrate products, and the application of new processes catalyzed by enzymes" (EC 1997). However, despite initial predictions concerning the application of the Novel Foods Regulation, the dossier submitted by Danone remains the only application for authorization of a food considered novel as a result of the application of a new process. So far the application of the Novel Foods Regulation has mainly concerned exotic fruits, bioactive substances, and carbohydrates with altered sources.

Experience has revealed that many foods and food ingredients theoretically falling under the Novel Foods Regulation are placed on the market without authorization. This especially applies to the category of "novel" products to which new processes have been applied. Because no standardized treatment conditions have been developed and many aspects of the application of HPP remain unknown, the use of HPP technology is still assessed on a case-by-case basis by many competent authorities. For this reason, food business operators perceive the regulation of HPP as a gray area (see Sprong et al. 2014). Increasing attention in research and progress in scientific studies concerning nonthermal preservation processes including HPP has been recently observed, which will definitely help reduce uncertainties concerning the legal status of HPP.

30.6 Hygiene, Microbiological Criteria, and HACCP

The inactivation of pathogens and/or extension of shelf life are considered the main objectives behind HPP (Fonberg-Broczek et al. 2005; Heinz and Buckow 2009). Microbial inactivation by high pressure is caused by a combination of different factors. One of the main targets of high pressure is the cell membrane. Changes, such as modification in permeability and ion exchange, may cause loss of resistance to selective chemical inhibitors. As a consequence, the inhibitors cannot be excluded from the cell since the cell membrane becomes damaged (Norton and Sun 2007). Another important factor in bacterial inactivation is disruption of the enzymatic systems controlling the metabolic actions (Heinz and Buckow 2009).

The impact of HPP on the presence of pathogenic microorganisms is of paramount importance for food business operators. As mentioned above, according to Article 14 of the General Food Law, food must not be placed on the market if it is unsafe. Food businesses have the primary responsibility to ensure that foodstuffs do not contain microorganisms or their toxins in quantities dangerous to human health.

In the EU, Regulation 2073/2005 on microbiological criteria for foodstuffs[17] sets out uniform food safety criteria concerning the acceptability of foods with regard to the presence of pathogenic microorganisms, defined as "bacteria, viruses, yeasts, molds, algae, parasitic protozoa, microscopic parasitic helminths, and their toxins

[17] OJ 2005L 338/1.

and metabolites."[18] This Regulation defines the acceptability of certain processes and sets a limit above which foodstuffs are considered unsafe.

Annex I to the Regulation lays down food safety criteria for food-borne bacteria, their toxins and metabolites, such as *Listeria monocytogenes*, *Salmonella*, staphylococcal enterotoxins, *Enterobacter sakazakii*, *E. coli*, and histamine for specific groups of foodstuffs (see Chaps. 14–17). The Regulation also sets process hygiene criteria to specify the correct production process.

These criteria are developed on the basis of Regulation 852/2004 on the hygiene of foodstuffs, which defines the food safety objectives to be achieved.[19] Due to limited sampling and uneven distribution of microorganisms, microbiological testing can never guarantee the safety of tested foods; the safety of foodstuffs is mainly ensured through a preventive approach, such as good hygiene practices or the implementation of procedures based on hazard analysis and critical control point (HACCP) or other hygiene control measures.

30.7 HACCP

All food business operators other than at the level of primary production must apply the procedure based on the HACCP principles. This procedure refers to identifying the critical points from a hygiene perspective and designing procedures that must be followed to keep these points under control. According to Article 5 of the Hygiene Regulation, requirements that must be met throughout the production, processing and distribution cycle consist of:

- Identifying any hazards that must be prevented, eliminated, or reduced to acceptable levels
- Identifying the critical control points at the step or steps at which control is essential
- Establishing critical limits at critical control points which separate acceptability from unacceptability, i.e., beyond which intervention takes place
- Establishing and implementing effective monitoring procedures at critical control points
- Establishing corrective actions when monitoring indicates that a critical control point is not under control
- Implementing own-check procedures
- Keeping records to demonstrate the application of these measures to facilitate official controls

[18] Art. 2(a) Reg. 2073/2005.

[19] OJ 2004L 226/3. This Regulation is part of the so-called "Hygiene Package"—a body of EU law laying down hygiene rules for foodstuffs in the EU. The other acts include Reg. 853/2004 laying down specific hygiene rules for food of animal origin (OJ 2004L 226/22) and Reg. 854/2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption (OJ 2004L 226/83).

The aim of the HACCP system is to ensure that food is produced safely. The Hygiene Regulation leaves food business operators responsible for adopting the safety measures necessary to guarantee food safety. By applying this system, food business operators must make sure that:

- The supply, handling, and processing of raw materials and foodstuffs under their control are carried out in such a way that the process hygiene criteria are met.
- The food safety criteria applicable throughout the shelf life of the products can be met under reasonable foreseeable conditions of distribution, storage, and use.[20]

Therefore, food business operators incorporating HPP in their HACCP plans must ensure that the stage of HPP either reduces or eliminates pathogens in foods in the same way as the use of other technologies. Good hygiene practices can be expected to be similar to heat-processed food. However, unlike heat treatments, for which internationally recognized standards exist according to different time–temperature combinations, to date no standardized treatment conditions have been developed for HPP (Rondueles et al. 2011). Similarly, no specific hygiene requirements have been issued for products packed in hermetically sealed containers to which treatment other than heat has been applied (cf. Chapter IX Regulation 852/2004).

So far, no guidelines concerning HPP have been issued by the competent authorities at EU or at the national level. In this context, a directive[21] concerning HPP and inspection program personnel verification responsibilities published by the US Department of Agriculture Food Safety and Inspection Service (FSIS), as well as the website of the US Food and Drug Administration, provide useful information on how HPP should be incorporated in the HACCP system (USDA 2012; FDA 2012). The directive contains instructions for FSIS personnel to perform HACCP verification tasks in establishments applying HPP antimicrobial treatment as a process step. To demonstrate that the HPP process adequately addresses hazards, the food business operator must maintain supporting documents, such as scientific journals, in-plant data, etc., in particular concerning log reduction achieved for the specific hazard and the critical operational parameters (pressure–time–temperature combinations) relevant to the actual process applied. The FDA study describes critical factors, their effects on inactivation levels, mechanisms for inactivation, as well as pathogens in high-pressure processing. The review includes methods to handle deviations and tentative flow charts for the application of the HACCP principles to HPP.

[20] Art. 3 Reg. 2073/2005.

[21] Directives issued by FSIS are not binding sources of law. They provide official communications and instruction to agency staff in carrying out their tasks.

30.8 Labelling

The General Food Law establishes a general principle of food law to provide a basis for consumers to make informed choices in relation to food they consume and to prevent any other practices which may mislead the consumer.[22] EU legislation on the labelling of foodstuffs consists of general provisions laid out in Regulation 1169/2011 on the provision of food information to consumers.[23]

Apart from the general provisions applying to all foodstuffs, specific legislations apply to some categories of foodstuffs. The Novel Foods Regulation also contains specific provisions concerning the labelling of novel foods. The general requirements for all novel foods are that they must not be dangerous to consumers or mislead them or differ from foods or food ingredients which they intend to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer (Article 3). In light of these general requirements, Article 8(1) states that the final consumer has to be informed of:

(a) Any characteristics or properties relating to composition, nutritional value or effects, or intended use of the food which render a novel food no longer equivalent to an existing conventional food
(b) The presence in the novel food or food ingredient of material which is not present in an existing equivalent foodstuff and which may have implications for the health of certain sections of the population
(c) The presence in the novel food or food ingredient of material which is not present in an existing equivalent foodstuff and which gives rise to ethical concerns

Further, the legislation explains that food is "no longer equivalent" if "scientific assessment, based upon an appropriate analysis of existing data, can demonstrate that the characteristics assessed are different in comparison with a conventional food or food ingredient, having regard to the accepted limits of natural variations for such characteristics."[24] All of the modified characteristics or properties must be included on the label, together with the method by which these changes were obtained.

Leaving aside the discussion of whether or not the HPP falls under the scope of the Novel Foods Regulation, as mentioned above, the application of HPP in most cases does not seem to produce any significant changes in food composition or structure.[25] One of the effects of the application of HPP is the extended shelf life of a product, of which the consumer can be adequately informed through the date of

[22] Art. 8 GFL.

[23] OJ 2011L 304/18. Food information in this context is a concept broader than labelling and encompasses all types of information to the final consumer, including modern technology or verbal communication (Art. 2(2)(a) Reg. 1169/2010).

[24] Art. 8(1)(a) NFR.

[25] HPP can cause both compositional and structural changes. For example, when used with oysters and lobsters, there are structural changes that cause the release of the organisms' shells. These structural changes are not deleterious, nor do they seem significant as understood in the NFR.

minimum durability or the "use by" date, which is a separate mandatory labelling particular.[26]

With regard to processes, the Regulation on the provision of food information to consumers stipulates that food treated with ionising radiation must be labelled as "irradiated" or "treated with ionising radiation." However, other specific treatments which foodstuffs have undergone, such as being freeze-dried, refrozen, quick-frozen, etc., should be indicated in the name of the products only where omission of such information could mislead the consumer.[27]

Therefore, given the facts above, it seems unlikely that omission of information about HPP on the label would mislead consumers or unable them to make an informed choice about whether to buy a product. Consequently, in the light of the general principles of food labelling, mentioning that the foodstuff has undergone HPP does not appear to be a mandatory requirement. It remains, however, an open question whether food business operators will decide to voluntarily include this information on the label to highlight the benefits of their products in comparison with those of their competitors.

30.9 High-Pressure Processing Equipment

Finally, specific provisions apply to high-pressure processing equipment itself. Only equipment that complies with Directive 97/23 concerning pressure equipment (Pressure Equipment Directive—PED)[28] can be used for HPP within the EU. This Directive has recently been recast, with a view to streamline and simplify the rules for putting pressure equipment on the market and to lower costs for businesses. Directive 2014/68/EU will repeal the existing one on 19 July 2016.

The Pressure Equipment Directive follows the so-called new approach to technical harmonisation and standards in the EU, outlined in the Council Resolution of 7 May 1985 (Council of the European Union 1985). In line with the "new approach," the directive sets out the essential safety requirements for pressure equipment, but does not contain any detailed technical solutions. Equipment complying with standards harmonised at the EU level drawn up by private bodies, such as the European Committee for Standardization (CEN) and the European Committee for Electrotechnical Standardization (Cenelec), is presumed to meet the essential safety requirements of the Pressure Equipment Directive. Products that comply with this Directive have undergone a conformity assessment and, those which carry the CE marking,[29] can circulate freely between all EU member states.

[26] Art. 9(1) Reg. 1169/2011.

[27] Annex VI Reg. 1169/2011.

[28] OJ 1997L 181/1.

[29] See Annex VI Dir. 97/23.

Conclusion

Many factors have an impact on the regulatory approach to HPP in the EU, and many aspects, ranging from hygiene to technical standards concerning equipment, have to be taken into account. Because the process was not used to a significant degree before 15 May 1997, it was classified as novel under the scope of the Novel Foods Regulation. Since the inception of the regulation, however, only one application concerning HPP has been submitted and subsequently approved. At the same time, many HPP foods are available on the EU market.

The premarket authorization process under the Novel Foods Regulation starts at the national level, and only when objections from other member states are raised or a product or process requires additional risk assessment, the decision is shifted to the EU level. This situation has resulted in different approaches to HPP by national authorities. Some countries, like the UK, have recognised that HPP as a new process did not fall under the Novel Foods Regulation because no significant changes in composition or structure occur in foods treated with HPP. Other countries, like Germany, have recommended a case-by-case evaluation of possible effects of HPP on different categories of foods.

Although in the implementation of the Novel Foods Regulation it now seems to be widely recognised that HPP foods should not be treated as novel, the provisions of the Regulation have not been changed. Food business operators are primarily responsible for determining whether any significant change is present in HPP food and, consequently, whether premarket authorization is necessary. The term "significant change" remains subject to different interpretations, which further augments uncertainties as to the legal situation of HPP foods on the EU market.

Finally, food business operators are primarily responsible for ensuring that only safe food is placed on the EU market. To this end, they have to maintain a procedure based on HACCP principles and other hygiene control measures to help attain food safety and control over the production process. HPP must be included as a control measure, and critical process factors in HPP must be developed.

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Chapter 31
Consumer Acceptance of High-Pressure Processed Products: American Perspective

Christine M. Bruhn

Abstract This chapter reviews consumer acceptance of high-pressure processed products from an American consumer perspective. Acceptance of products processed by novel processing technologies depends on the consumer's perception of benefits and risks. These include the impact of the technology on taste, the convenience, the nutritional value, the perceived safety of the process or technology, the magnitude of the risk the technology reduces, and the effect of the technology on the environment.

Keywords High-pressure processing • New technology • Consumer acceptance • Consumer attitude • Product characteristics

31.1 Introduction

The array of products in the supermarket today is broader and more diverse than ever. Perishable, lightly processed, and shelf-stable products from around the world vie for consumer attention. The food industry can respond to consumer's increased awareness of the health benefits of good nutrition, a discriminating pallet that seeks appealing flavor, and demand for easy, convenient preparation by utilizing newer technologies with taste closer to freshly prepared. Acceptance of products processed by newer technology depends on the consumer's perception of benefits and risks. These include the impact of the technology on taste, the convenience, the nutritional value, the perceived safety of the process or technology, the magnitude of the risk the technology reduces, and the effect of the technology on the environment. Public attitudes are influenced by perceived credibility of data, rigor of regulatory policy, and impartial action of regulators and demonstrated food safety and environmental responsibility of industry.

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31.2 Consumer Priorities

Consumers expect food processors to deliver products with the characteristics they desire. Most are unaware of the steps in food necessary to produce great-tasting, convenient, healthy products. Food safety and worker safety are basic expectations. Many passively or actively support food production methods that are sustainable with minimal environmental impact. When attention is directed toward a traditional or newer method of processing, some consumers will express concern or reluctance to select the newer product, while others will choose the newer item solely to experience something new. Consumer research can be used to identify the mind-set of target consumers, identify the information the consumer is seeking, and gage response to label or marketing statements.

Consumer beliefs, attitudes, and preferences can be assessed through qualitative and quantitative research methods (Salant and Dillman 1994; Beckley et al. 2012). Qualitative methods such as focus groups or individual in-depth interviews provide insight as to feelings or thoughts on issues (Krueger and Casey 2000). In this approach, a small number of individuals representing the target audience are asked with open-ended thought-provoking questions on key issues with the goal of finding a diversity of opinions that can be organized into specific themes. The number of individuals responding to these themes is then assessed through quantitative research. Quantitative methods include telephone, mail, the Internet, or face-to-face interviews of a large number of consumers (Fink and Kosecoff 1985; Pope 1993). Attitudes can be projected nationwide if the sample is based upon demographic parameters related to the national population. Typically market research organizations use a sample size of 1000 persons.

Consumer attitudes don't always predict actual marketplace behavior. Factors other than those addressed in a survey, such as product appearance, price, availability, and quality of substitutes, may alter behavior in either a positive or negative direction. Further, consumer attitudes are affected by information received. Television, newspapers, magazines, the Internet, books, and friends are influential sources of information on food and health (America Dietetic Association 2000; Cogent 2006; International Food Information Council Foundation 2012a, b). Information from these sources in addition to traditional methods of communication like advertising and product labeling affects purchase decisions. Actual purchased behavior is tracked by supermarkets, manufacturers, and other organizations. Limited data is available publicly, such as that reported by the Food Marketing Institute and the Grocers Association of America, with in-depth analysis offered for purchase by various research organizations.

Good flavor, convenience, and health-enhancing properties are key consumer benefits in today's marketplace. An examination of the most successful new products in the supermarket indicates that products with a new flavor, unique flavor combination, or new recipe constitute three of the top five supermarket new product successes (Information Resources Inc. 2004a, b, c, d, 2005a, b, c). In a national telephone survey, over 80 % of consumers indicated that convenience is an important

consideration in purchases (Food Marketing Institute 2005), and foods with added convenience are among top supermarket sellers (Information Resources Inc. 2004a, b, c, d, 2005a, b, c). Dietary fiber, beneficial fatty acids, lycopene, vitamin C, and probiotic cultures are among the top functional food attributes covered by the media (Center for Media and Public Affairs 2004). Products with nutritional appeal are also among the most successful new introductions. The growth in the organic market may reflect interest in "natural" products without pesticides, preservatives, or additives or products believed to be produced in a sustainable or environmentally friendly manner (Williams and Hammitt 2001).

New processing technologies can help realize some of the advantages that consumers seek, but the path from introduction to acceptance is not always clear. Some consumers are skeptical of technology and believe a low-technology approach promotes health and environmental sustainability. The introduction of a food processed by a new technology may create concern among these individuals. The public is generally unaware of methods used or safeguards employed in processed food. Any risks associated with a new technology are seen by the public as imposed by the processor and beyond the control of the consumer. In some consumer's mind an unfamiliar approach presents unknown risks that could be potentially harmful.

31.3 Perceived Risks

Several theories have been developed to explain risk perception. Characteristics of risk, such as severity of consequences, involuntary exposure to risk, harm to the environment, exaggerated reports, and adequate regulations, were found to be important for predicting consumer perception of risk (Yeung and Yee 2005; Yeung and Morris 2006). Risks are enhanced in the public's mind when imposed by others, when not accompanied by clear benefits, or when viewed as unfair (Slovic 1987).

Consumer research can identify the questions consumers have about a new technology. People want to know what risk may be reduced by a new technology and what risk is imposed by the technology. People are primarily interested in how the new process or technology affects them. For example, more people are interested in how eating irradiated food affects human health than how irradiated food tastes (Food Marketing Institute 1998; Information Resources Inc. 2005a, b, c). Taste can be determined by personal experience, but the long-term affect on health requires additional input beyond the individual's capabilities. Similarly, more people are interested in how biotechnology or genetic modification affects food safety rather than how it affects farming practices or how the technology works (Bruhn and Mason 2002).

Massachusetts consumers were asked to describe their level of concern based upon hearing the name of specific food technologies. Respondents indicated concern using a Likert-type scale in which 1 indicated no concern and 4 highest concern. Use of bacteriocins generated the greatest concern, 3.0, followed by irradiation at 2.25, aseptic processing at 2.1, ohmic heating at 2.0, and ultrasound at 1.9

(Cardello 2003). Although aseptic processing is widely used, the term may be unfamiliar. This suggests that the use of less familiar terms may generate concern. Further, some expressed concern about the common process of heat pasteurization (1.25), indicating that the reference line for minimal concern is not 1.0, but somewhere higher.

Most consumers, 74 %, recognize that processing can help foods stay fresh longer, and 72 % agree that processing provides variety year round and 70 % recognize that processing provides additional convenience (International Food Information Council Foundation 2012a, b). Fewer, 59 %, realize that processed food is nutritious, and only 37 % believe that processed foods can provide added health benefits. Further, only 49 % realize that processing improves food safety, and only 48 % believe processed food can be produced in a sustainable way. This suggests that a discussion of high-pressure processing could highlight the advantages this technology offers.

Providing information about the technology reduced concern. Focusing consumer response on the method of processing, such as using the term "minimally processed" and "fewer preservatives" however generated a negative response from some consumers (Cardello et al. 2007). These studies suggest that communicating about the product and providing information about the process are more likely to address consumer information needs and result in product acceptance compared to simply stating "minimally processed."

Concern about a technology influences flavor expectations (Lahteenmaki et al. 2002; Cardello 2003). Researchers found that flavor ratings were lower when people were told the product was produced by a new processing method. Flavor ratings increase when people actually see the produce processed by the new technology, when statements about safety are made, and when benefits are described (Schutz et al. 1989; Bruhn 1995; Frever et al. 1997; Cardello 2003). Consistent with these findings, Tuorila and colleagues (1994) found that uncertainty associated with a novel product may introduce a degree of expected disliking, but additional factual information reduces this uncertainty and improves expected liking.

Early users of new technologies have been found to have higher incomes, more prestigious occupations, and more positive self-identities (Rogers and Shoemaker 1971). Women are generally more likely to express concern about a new process or technology than men (Cardello 2003; Food Marketing Institute 2005).

Repeated exposure to neutral or positive information about a technology lowers concern. While examining the effect of an educational intervention on attitudes toward food irradiation, Schutz and colleagues (Schultz 1994) found people expressed less concern about irradiation with repeat testing even though they received no educational intervention. Similarly, Cardello (2003) found post-concern levels for many technologies were reduced by participating in a study in which they sampled products described as processed by a new method.

Generally, negative information is more powerful than positive in influencing public attitudes (McNutt et al. 1986). Market research indicates that positive framing of product attributes, such as meat that is 75 % lean rather than 25 % fat, results in a more positive evaluation than the reverse negative framing (Donovan and Jalleh 1999).

31.4 Product Benefits: A Driving Factor

Research can identify the consumer's view of the most importance product characteristics. Taste is consistently rated as the most important factor that drives consumption and repeat purchase (Information Resources Inc. 2005a, b, c; Cardello et al. 2007; Cardello et al. 2003). The promise of improved flavor was the driving factor for the introduction of the biotechnology modified Flavr Savr (copyright) tomato. Flavor continues to be viewed positively. About two third US consumers surveyed (69 %) indicated they would likely purchase a product modified for improved flavor (International Food Information Council Foundation 2012a, b).

Although people cite nutrition and health as important in product selection (America Dietetic Association 2000; International Food Information Council Foundation 2012a, b), good taste is a more dominant market force. Nutritious products that do not deliver satisfactory flavor do not remain in the market. This is illustrated by the rise and fall in demand for low-carbohydrate foods perceived as helpful in weight management (Information Resources Inc. 2004a, b, c, d). Superior flavor is a driving factor for health professionals as well as the general public. Persons with a background in food and nutrition indicated greatest interest in purchasing products processed by a new technology if the product delivers better flavor (Delgado-Gutlerrez and Bruhn 2008).

The importance of good flavor has increased over the years. In 1994, a national survey found 33 % of consumers indicated they rarely or never gave up good taste for health, while that percentage increased to 43 % in 2004. Similarly, in 2004 those who agreed that they always or usually avoided favorite foods to eat healthier fell to 38 % compared to 42 % in 1994 (Information Resources Inc. 2005a, b, c). In 2012, 87 % acknowledged that taste has the great impact on their food choices, compared to 61 % who reported that healthfulness had a great impact (International Food Information Council Foundation 2012a, b).

Convenience is a driving force in today's market. Convenience, taste, and perceived health advantages of fresh food compared to frozen or shelf-stable food is increasing the demand for refrigerated meal components (Information Resources Inc. 2005a, b, c). Greater convenience is the most important motivation for purchasing minimally processed vegetables (Ragaert et al. 2004).

Demand varies among different demographic groups. The likelihood to buy minimally processed vegetables is higher among better-educated consumers and those with young children. In addition to demographic parameters, higher appeal for specific products can be related to awareness of health benefits. For example, although Belgium consumers rank health and nutritional value relatively low in terms of importance during purchase, consumers with a high awareness of the relationship between food and health attach more importance to these attributes (Ragaert et al. 2004).

Consistent with these trends in the USA, a survey based upon 3000 personal interviews in the UK, Germany, and France found that the perception of personal benefits and environmental friendliness were the most important factors affecting

likelihood to purchase products processed by high pressure (Butz et al. 2003). The importance of improved flavor appears to differ by culture. About half of French consumers indicate they would purchase a product processed by high pressure for better quality, while only 4 % of British consumers indicated that they would purchase for this benefit (Butz et al. 2003). In contrast, almost 40 % of German consumers indicated they would purchase a product processed by high pressure for better health, while this was a driving factor for only 18 % of French consumers (Butz et al. 2003).

Although consumer research provides valuable insight, an inquiry about a technology's safety may itself create uncertainty about the processing method. When asked about new products, consumers indicate that the potential risk associated with a product is the most important determinant in product use (Cardello et al. 2007). Hicks and coworkers found that 15 % of volunteers from a national sample admitted that they were fearful of new food processing technologies (Hicks et al. 2009). Consumers express concern about potentially harmful by-products and unknown health risks. Presence in the supermarket provides reassurance that potential risks are under control. Consumers expect them to have been evaluated by regulatory agencies, the supermarket, or others. Consumers then respond to product appearance, taste, and convenience. Products processed by high pressure and labeled "fresher under pressure" generated repeat purchase because they met consumer expectation of quality and convenience.

Providing information on the benefits to consumers may receive increased acceptance. When consumers in Hicks and colleagues' sample (2009) are given a brief explanation of high-pressure processing and its benefits, 40 % indicated that they would be willing to pay more for processed products, while 45 % were not sure. The majority of those willing to pay indicated they would pay $0.25 or $0.50 more per item.

31.5 Communicate with the Public

Continuous consumer communication plays a pivotal role in acceptance. Communication is more than advertising. Effective communication is a two-way process which involves listening, identifying, and responding to consumer questions.

Information should be presented in a variety of sources, with preferred sources varying by age and gender. Consumers find television, newspaper, magazines, and supermarket brochures convenient. More men and younger consumers prefer web-based sources than women or older persons (Li-Cohen and Bruhn 2002; Food Marketing Institute 2008; Hicks et al. 2009).

When deciding about controversial or complex issues, consumers will likely be influenced by opinion leaders, trusted people, or organizations that are knowledgeable about technology (Rogers 1995). Endorsement by respected experts increases the acceptance of food processed by new methods.

Issues other than those that can be scientifically measured determine if a technology is accepted (Belton 2001). When message components were segregated, trust in the spokesperson was significantly more important in explaining attitudes than accuracy of information (Bord and O'Conner 1989). For example, Sapp et al. (1994) found that word of mouth and trust in government and industry were more important than demographic factors in predicting consumer acceptance of irradiation.

Trust is greatest for groups perceived as knowledgeable, unbiased, and acting with the public's best interest in mind (Frewer et al. 1996). While no one organization is trusted by everyone, patterns of trust have emerged. In the USA, health organizations such as the American Medical Association and other health professionals are viewed as trustworthy by the largest percentage of consumers (America Dietetic Association 2000; Pew Initiative on Biotechnology 2004; International Food Information Council Foundation 2012a, b). University scientists and government agencies such as the US Food and Drug Administration are also viewed as trustworthy by a majority of consumers. Industry groups, advocacy groups, and the media are seen as trustworthy by the smallest percentage of consumers. Those who prefer low-technology approaches to food processing appear to have lower levels of trust in government sources. For example, compared with those who select conventional products, those who buy organic foods have a greater feeling of distrust toward regulatory agencies (Williams and Hammitt 2001).

To increase trust and the likelihood that communications are understood, educational programs should be built around what the public wants to know, as determined by consumer research. New technology should be described using lay terminology. The new method may be compared to a similar or more familiar technology. Communication should acknowledge that risks can never be completely eliminated. Consumer benefits should be emphasized. Transparency is the operative word, sharing what is known and not known both in regard to risk and benefits. Consumers today are interested in the impact of a technology on worker safety and environmental stewardship, as well as personal welfare.

Developing descriptions of products processed by new technologies should be done systematically. Statements describing new technologies should be evaluated by focus groups prior to adoption to test message clarity. Messages should anticipate and respond to concerns special interest groups may express. When these approaches are used, public acceptance generally increases (Schutz et al. 1989; Bruhn 1995; Resurreccion et al. 1995; Bruhn and Mason 2002; Fox 2002; Johnson et al. 2004; Zienkewicz and Penner 2004; Delgado-Gutlerrez and Bruhn 2008). A guide on developing and testing communication messages is available from the US Food and Drug Administration (Fischhoff et al. 2011).

31.6 Summary

Products will have the greatest likelihood of success when developers address consumer needs, respond to consumer concerns, and offer tangible benefits. Researchers have demonstrated that statements about the benefits associated with a particular

food or food processing technique will reduce concerns and increase likelihood of consumption. Factual information from a trusted source, clear statements about safety and benefits, and exposure to a product that delivers quality and convenience will increase the likeliness of consumer acceptance. Building consumer knowledge of how and why food is processed will benefit the public by increasing use and acceptance of newer technologies.

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