International Commission
on Microbiological Specifications
for Foods (ICMSF)

# Microorganisms in Foods 8

## Use of Data for Assessing Process Control and Product Acceptance

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# Contents

# Preface

## ICMSF and the Evolution of Food Safety Management

*Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance* was written by the International Commission on Microbiological Specifications for Foods (ICMSF) with assistance from a limited number of expert consultants. The purpose of this book is to provide guidance on appropriate testing of ingredients, food processing environments, processing lines and finished products to enhance the microbiological safety and quality of the food supply.

ICMSF books represent an evolution in microbiological food safety management principles. In the 1970s and 1980s, food safety control was primarily accomplished through inspection, compliance with hygiene regulations and end product testing. *Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications* (1974, 1986) put forward a sound statistical basis for microbiological testing through the use of sampling plans. Sampling plans remain useful at ports of entry when there is no information on the conditions under which a food has been produced or processed.

At an early stage, the Commission recognized that no single sampling plan could ensure the absence of a pathogen in food. This led the Commission to publish *Microorganisms in Foods 4: Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality* (1988). The value of HACCP for enhancing food safety is recognized globally. *Microorganisms in Foods 4* illustrated the procedures to identify microbiological hazards in food production, to identify the critical points to control the hazards and to establish systems to monitor the effectiveness of control.

Effective implementation of HACCP requires knowledge of hazardous microorganisms and their responses to conditions in foods (e.g., pH, water activity, temperature, preservatives etc.). The Commission's *Microorganisms in Foods 5: Characteristics of Microbial Pathogens* (1996) is a thorough but concise review of the literature on growth, survival and death responses of foodborne pathogens. It is intended as a quick reference to assist in making judgments on the growth, survival or death of pathogens in support of HACCP plans and to improve food safety.

Microbiological food safety management requires an understanding of the microbial ecology of the food being produced. *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (1998, 2005) is intended for those concerned with the applied aspects of food microbiology. It describes the initial microbiota, pathogen prevalence, effects of processing, spoilage patterns, foodborne illness outbreaks and control measures for 17 food commodities. The updated version of *Microorganisms in Foods 6* builds on *Microorganisms in Foods 7* by identifying controls that influence the initial level, increases, and decreases in the microbial population.

*Microorganisms in Foods 7: Microbiological Testing in Food Safety Management* (2002) illustrates how HACCP and Good Hygienic Practices (GHP) provide greater assurance of safety than microbiological testing, but also identifies circumstances in which microbiological testing may play a useful role. It introduces the reader to a structured approach for managing food safety using control measures in three categories: (1) those that influence the initial level of the hazard, (2) those that cause reduction of the hazard and (3) those that prevent increase of the hazard during processing and storage. The concepts of

a Food Safety Objective (FSO) and a Performance Objective (PO) are recommended to industry and control authorities to translate risk into a definable goal for establishment of food safety management systems that incorporate the principles of GHP and HACCP. FSOs and POs provide the scientific basis for industry to design and implement measures to control the hazards of concern in a specific food, for control authorities to develop and implement inspection procedures to assess the adequacy of control measures, and for countries to quantify the equivalence of inspection procedures. In addition, the information on sampling plans presented in *Microorganisms in Foods 2* is updated and expanded.

This new book, *Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance*, consists of two parts. Part I, Principles of Using Data in Microbial Control, builds on the principles of *Microorganisms in Foods 7*. Part II, Application of Principles to Product Categories, provides practical examples for a variety of foods and processing environments. This material updates and replaces similar information presented in *Microorganisms in Foods 2*. Part II also builds on the second edition of *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (2005) by identifying additional tests to evaluate the effectiveness of controls.

*Microorganisms in Foods 5*, *6, 7* and *8* are intended for those involved in microbiological testing or engaged in setting microbiological criteria. These texts are useful for food processors, food microbiologists, food technologists, public health workers and regulatory officials. For students in food science and technology, the ICMSF series offers a wealth of information on food microbiology and food safety management, with many references for further study.

Microbiological testing can be a useful tool in the management of food safety. However, microbiological tests should be selected and applied with knowledge of their limitations, benefits, and the purposes for which they are used. In many instances other means of assessment are faster and more effective than microbiological testing for food safety assurance. The need for microbiological testing varies along the links of the food system, from primary production, to processing, to distribution and sale, to preparation, to point of consumption. Points in the food system should be selected where information about the microbiological status of a food will prove most useful for control purposes.

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# Abbreviations

| | |
|---|---|
| ACC | Aerobic colony count |
| ALOP | Appropriate level of protection |
| ATP | Adenosine tri-phosphate |
| $a_w$ | Water activity |
| °C | Degrees Celsius |
| CCP | Critical control point |
| CDC | Centers for Disease Control and Prevention |
| CFU | Colony forming unit |
| CIP | Clean in place |
| cm | Centimeter |
| D | Decimal reduction units |
| DON | Deoxynivalenol |
| EC | European Commission |
| e.g. | For example |
| EGR | Exponential growth rate |
| EHEC | Enterohemorrhagic *E. coli* |
| i.e. | That is |
| in | Inch(es) |
| EPA | US Environmental Protection Agency |
| EU | European Union |
| FAO | Food and Agriculture Organization |
| FDA | US Food and Drug Administration |
| FSO | Food Safety Objective |
| g | Gram |
| gal | Gallon |
| GAP | Good Agricultural Practices |
| GHP | Good Hygienic Practices |
| GMP | Good Manufacturing Practices |
| h | Hour |
| $H_0$ | Initial microbial contamination level |
| HACCP | Hazard Analysis Critical Control Point |
| ICMSF | International Commission on Microbiological Specification for Foods |
| IFT | Institute of Food Technologists |
| kg | Kilogram |
| kGy | Kilo Gray |
| log | Logarithm in base 10 |
| L | Liter |

| | |
|---|---|
| LAB | Lactic acid bacteria |
| MAP | Modified atmosphere packaging |
| MC | Microbiological Criteria |
| min | Minutes |
| mL | Milliliter |
| MPN | Most probable number |
| NACMCF | National Advisory Committee on Microbiological Criteria for Foods |
| P | Probability or proportion |
| PC | Performance criterion |
| PO | Performance objective |
| ppm | Parts per million |
| RTE | Ready to eat |
| s | Second |
| s.d. | Standard deviation |
| SE | *Salmonella enteritidis* |
| Sect. | Section |
| sqrt | Square root |
| ΣI | Sum of microbial level increase from growth or re-contamination |
| ΣR | Sum of microbial level reductions |
| TTI | Time temperature integrator |
| μg | Microgram |
| UHT | Ultra high temperature |
| UK | United Kingdom |
| US | United States of America |
| USDA-FSIS | US Department of Agriculture – Food Safety Inspection Service |
| WHO | World Health Organization |

# Part I
# Principles of Using Data in Microbial Control

# Chapter 1
# Utility of Microbiological Testing for Safety and Quality

## 1.1 Introduction

This chapter is intended to provide an overview of microbiological testing, as well as an introduction to the related concepts that are discussed in more detail in subsequent chapters or other ICMSF publications. Microbiological testing is applied to food safety and quality management in a number of ways. Governments may use pathogen or indicator testing for lot inspection or verification as a means of lot acceptance, for example at port of entry or for surveillance activity on products in commerce. Industry may also use end product tests for pathogens or indicators for lot acceptance in customer-supplier relationships. Industry also uses microbiological testing to design products and verify the adequacy of performance of process controls for food safety and spoilage control in Hazard Analysis Critical Control Point (HACCP) programs or Good Hygienic Practices/Good Manufacturing Practices (GHP/GMP) programs. These tests may be run on end product, in-process or environmental samples. The target microorganism may be a pathogen, an indicator or a utility microorganism. Investigational sampling is conducted by both government and industry when a microbiological issue is identified to gain information and to identify potential causes of a problem and potential solutions. This testing may examine end product, ingredients, in-process and environmental samples that may be collected at different points in the food system.

Microbiological criteria can be applied at all stages in the food supply chain, from agricultural and aquaculture producers to wild harvesters, through production and retail. The quality and safety of foods at retail may be mandated by governments to protect consumers and meet their expectations, but to achieve this, microbiological limits may need to be applied at earlier points in the supply chain. These criteria are often determined and imposed by businesses rather than governments and may be different than those applicable at the retail level.

When using microbiological tests to evaluate safety or quality of food it is important to select and apply these with knowledge of their limitations, their benefits and the purposes for which they are intended. In many instances, other assessments are faster and more effective than microbiological testing for food safety assurance. It is well recognized that application of prerequisite programs (e.g., Good Agricultural Practices (GAP), GHP, GMP etc.) and a HACCP program is the most effective food safety management strategy (Codex Alimentarius 1997a, ICMSF 1988, 2002a). Control of undesirable microorganisms in foods is best addressed at appropriate steps in the food chain through application of these approaches. However, a variety of different approaches to microbiological testing, which may or may not include pathogen testing, frequently plays an important role in verifying the effectiveness of food safety management programs when used in a thoughtful, well-planned manner.

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Identification of criteria relevant for assurance of microbiological food safety and quality, and their specification within the risk-based food safety management strategies is the main subject of this text. The book aims to provide guidance on appropriate microbiological testing for food safety and quality, including relevant microorganisms, limits and steps in the production and distribution of foods at which testing can be usefully applied. Chapters 2–6 provide more detailed discussions of specific uses of microbiological testing, while Chaps. 8–26 provide guidance of relevant microbiological testing and criteria for specific groups of commodities. Chapter 7 describes the structure of Chaps. 8–26, and explains the approach that led to the suggested microbiological tests and criteria.

This chapter provides a brief introduction to microbiological testing in the management of microbial food safety and quality, as well as providing an introduction to the overall text.

### *1.1.1 Testing as Part of a Food Safety Management Program*

The role of food safety in international trade of foods is governed by the World Trade Organization Sanitary and Phytosanitary (SPS) Agreement (WTO 1994). To determine whether a food should be considered safe the term *appropriate level of protection* (ALOP) has been used, defined as "the level of protection deemed appropriate…to protect human, animal or plant life…" This definition has caused great difficulties for a number of reasons in part because the idea of what is "appropriate" differs from country to country, i.e., "acceptable" risk is culturally defined. Hence, there is increased interest in developing tools to more effectively link the requirements of food safety programs with their expected public health impact.

The risk analysis framework described by ICMSF (2002a) and the Codex Alimentarius Commission (2008b) provides a structured approach to the management of the safety of food and introduced the concept of Food Safety Objective (FSO) as a tool to meet a public health goal such as an ALOP. FSOs and Performance Objectives (PO) can be used to communicate requisite food safety levels, e.g., to industry. FSOs and POs are distinct levels of foodborne hazards that cannot be exceeded at the point of consumption and earlier in the food chain, respectively, and can be met using good practices (GAPs and GHPs) and HACCP programs. While primarily applied for food safety assurance, the principles of these programs can also be applied for proactive assurance of food quality.

The principles of using good practices and HACCP, in order to produce safe foods, do not change with the introduction of these concepts. GHP, GAP and HACCP are the tools for achieving an FSO or PO. Assessing processing and preservation parameters is the preferred option to check that an FSO or a PO is met, but sometimes sampling and testing against microbiological criteria can be used.

Since the FSO is the maximum frequency or concentration of a hazard at the point of consumption, this level is frequently very low. Because of this, obtaining a true measure of this level is impossible in most cases. Compliance with POs set at earlier steps in the food chain can sometimes be checked by microbiological testing. However, in most cases, validation of control measures, verification of the results of Critical Control Point (CCP) monitoring, and auditing GHP and HACCP systems are needed to provide reliable evidence that POs and thus the FSO are met.

To benefit from the flexibility that an outcome based risk management system offers, it is important to be able to demonstrate that the selected control measures actually are capable of achieving the intended level of control on a consistent basis. The successful implementation of HACCP depends on its validation, including the clear identification of hazards, control measures available, critical control points, critical limits and corrective actions. The outcomes of monitoring and verification activities within a HACCP system assist in defining when re-validation may be necessary.

### 1.1.2 Principles of Microbiological Testing and Definitions

The International Commission on Microbiological Specifications for Foods (ICMSF) has written extensively on the principles of controlling microbial hazards in foods (see Introduction). These same principles apply to the control of microorganisms associated with spoilage as well as general indicators of GHP/GMP.

Microbiological tests are frequently performed to reach a decision or make a judgment. If the purpose for collecting a sample cannot be defined, then the analysis should probably not be done. The rationale for testing should be established prior to use and in the context of food safety management falls into four general categories:

1. To determine safety
2. To determine adherence to Good Hygienic Practices (GHPs)
3. To determine the utility of a food or ingredient for a particular purpose
4. To predict product stability

Microbiological testing may also be used to gather background information (e.g., baseline data) that does not involve setting limits. Additionally, microbiological testing may also be done for trace back in the context of an epidemiologic investigation. This has important implications for liability, trade and potential identification of root cause. Because this book focuses on use of data to assess process control and product acceptance, the reader is referred to other references for epidemiological investigation testing (e.g., CLSI 2007) and use of epidemiologic data to measure the impact of food safety control programs ICMSF (2006).

Decision-making based on microbiological data requires that limits be established to differentiate acceptable from unacceptable product or processes. These limits are meaningless without definition of the sampling plan and analytical procedures employed to generate the data, as well as decisions to be made and actions to be implemented as a consequence of the results. Microbiological limits that include methods and sampling plans are defined as *microbiological criteria*. Microbiological criteria should specify the number of sample units to be collected, the analytical method and the number of analytical units that should conform to the limits. Criteria may be established for quality as well as safety concerns (Codex Alimentarius 1997a) and are used in setting standards, guidelines and purchase specifications, which are defined as follows:

*Microbiological standards*: Standards are contained in international, national and regional laws and regulations. Exceeding a standard for a pathogen, such as *Salmonella* or *Listeria*, may lead to a product recall and potentially punitive action.

*Microbiological specification*: Purchase specifications are agreements between the vendor and buyer of a product as a basis for sale. These criteria can be considered mandatory and failure of the vendor to meet specifications can be used as a basis for product rejection.

*Microbiological guidelines*: Guidelines are internal, advisory criteria established by a processor, a trade association or sometimes governments. Failure to meet guidelines serves as an alert to the processor, indicating that remedial action should be taken. A wide variety of criteria fit into this category, such as results on pre-operational swabs from equipment, in-process samples from product or equipment and environmental samples tested for pathogens.

### 1.1.3 Utility Microorganisms, Indicators or Pathogens

Some microbiological tests provide information regarding general contamination, incipient spoilage or reduced shelf life, i.e., the utility of the product. The use of a utility test should be supported by

relevant evidence, e.g., that total aerobic count, rather than enumeration of specific spoilage microorganisms, is a measure of incipient spoilage. Such tests may be useful indicators of product quality. They may involve direct microscopic counts, yeast and mold counts, aerobic plate counts or specialized tests, such as for cold tolerant microorganisms or for species causing a particular type of spoilage (e.g., psychrotrophic pseudomonads in aerobically stored meats, lactobacilli in mayonnaise, or thermophilic spore formers in sugar).

Microorganisms that are not normally harmful but may indicate the presence of pathogenic microorganisms may be used as indirect indicators of a health hazard. For example, for dried egg products Enterobacteriaceae or coliforms can be used as indicators of the potential presence of salmonellae. In dried egg products, any practically applicable sampling plan cannot detect the low level of salmonellae that may be present but that may represent an unacceptable risk to public health. The quantitative information provided by indicator tests can be highly useful for trend analysis and verification of process control. As such, the relative importance of conducting indicator analysis may be higher than that for end product testing in a well designed program that emphasizes useful testing for microbiological safety and quality management. Similarly, indicator microorganisms may be useful in other situations, e.g., when assessing efficiency of cleaning and disinfection or in investigational sampling. Tests for relevant microorganisms can also indicate whether certain foods have been under processed, e.g., high numbers of mesophilic spore forming bacteria in low-acid, shelf-stable canned foods indicate probable under processing when it is certain the container is not leaking.

It is important to recognize that relationships between pathogen and indicators are not universal and are influenced by the product and process and, therefore care must be taken when selecting indicator microorganisms. For instance, coliform counts have been widely used as universal indicators of hygiene, but in many products (e.g., meat or poultry, vegetables, etc.), psychrotrophic Enterobacteriaceae will inevitably be present and the apparently high coliform counts do not necessarily indicate hygienic failure or consumer risk. Similarly, microorganisms naturally present in the product may also interfere with the analysis and interpretation of results, e.g., aeromonads on seafood can mimic coliforms in methods.

### *1.1.4 Risk Based Sampling Using ICMSF Cases*

ICMSF sampling plans are described, and their performance evaluated, in Chap. 7, Applications and Use of Criteria and Other Tests. Sampling plan stringency varies according to the number of samples tested (n), the upper limit on the acceptable concentration (m), the maximum tolerable number of results (c) that exceed m and, for three-class plans, the upper limit of the marginally acceptable level (M). Plans become more stringent as n increases and c, m and M decrease. ICMSF (1974, 1986, 2002a) presented a comprehensive framework for use of acceptance sampling plans based on degree of health risk or concern associated with a food and the change in hazard level, and consequent risk to health, that is expected to occurred between sampling and consumption. The latter is described as *conditions of use.* Five levels of hazard related to the microorganism assessed are differentiated including utility microorganisms, indicator microorganisms and three levels of hazard for pathogens, depending on the severity of the disease they cause. Three conditions of use are differentiated:

1. Those that lead to a reduction in the level of the hazard between the time of production and time of consumption.
2. Those that do not affect the level of the hazard.
3. Those that increase the level of hazard, and thus the risk, between the time of production and time of consumption.

These combinations lead to 15 different cases, each with its own corresponding sampling plan, with higher numbered cases corresponding to more stringent plans. See Sect. 7.4 for additional explanation of cases and how they are used in this book.

Utility tests are not related to health hazard, but to economic and esthetic considerations, therefore the level of concern is categorized as low. Utility tests are included in cases 1–3 and satisfied by relatively lenient sampling plans. Because of the uncertain relationship between indicators and specific pathogens, the level of concern is classified as moderate and it is inappropriate to apply sampling plans with high stringency for indicator microorganisms.

Three-class plans are typically less stringent than two-class plans, and are appropriate where health risk is relatively low (cases 1–9). Two-class plans with $c=0$ are usually used for situations where the health risk is significant and more stringent control is needed (cases 10–15).

## 1.2 GHP and HACCP

As noted above, the production of safe food requires the application of GHP, GAP and similar prerequisite programs, as well as the principles of HACCP, where they can be applied. These approaches enable development and implementation of a total food safety management system that will control most reliably the significant hazards in the food that is being produced. Some hazards are better addressed through GAP or GHP measures (e.g., controlling the initial levels of a hazard through good hygiene) while others are clearly best addressed through HACCP by a defined CCP that has been validated to control the hazard of concern (e.g., reducing the level of a hazard or preventing growth).

It is recognized that in many situations preventative measures such as GHP and HACCP are much more effective food safety management tools than end product testing. Consequently, the use of testing to determine adherence to GHP and validation and verification of HACCP is essential. Chapter 5, Corrective Action to Reestablish Control, discusses the elements of GHP and HACCP, while Chap. 3, Verification of Process Control, discusses methods to evaluate the efficacy and integrity of these essential programs, which differs from the statistical tools and assumptions that help interpret testing results.

### *1.2.1 Validation of Control Measures*

Validation involves obtaining evidence that control measures, if properly implemented, are capable of controlling the identified hazards (Codex 2008a). Validation is essential to demonstrate that GHP and HACCP systems provide the level of safety assurance required and routine sampling plans are not likely to be sufficient for validation studies. Validation focuses on the collection and evaluation of scientific, technical and observational information and generally involves microbiological testing. The scope of validation testing may extend beyond the control measures typically covered by HACCP, to include areas such as primary production and consumer handling, which can also affect the safety of the product at the point of consumption.

Processes can be validated using predictive models, microbiological challenge trials or application of processing criteria (PCs) that have previously been validated or approved to provide adequate levels of treatment and margins of safety, sometimes termed *safe harbors*. Not all of these methods need to be used, and often a combination of approaches is used to establish sufficient evidence to validate a process. Guidelines for validation have been developed by Codex Alimentarius (2008a).

Chapter 2, Validation of Control Measures, provides a detailed discussion of process validation approaches and factors that should be considered. Specific considerations for microbiological studies and approaches, and considerations in planning and undertaking relevant testing and analysis are also considered. Practical advice for microbiological challenge studies to produce reliable results is also presented.

### 1.2.2 Verification of Process Control

Verification of control measures involves "the application of methods, procedures, tests and other evaluations in addition to monitoring, to determine whether a control measure is, or has been, operating as intended" where *monitoring* is defined as "the act of conducting a planned series of observations or measurements of control parameters to assess whether a control measure is under control" (Codex Alimentarius 1997b). Verification can use of a variety of measurements, including:

- Sensory assessments
- Chemical measurements, e.g., acetic acid and preservative levels, water content
- Physical measurements, e.g., pH, $a_w$ and temperature
- Time measurements
- Microbial tests, including tests for toxic metabolites

The development of microbiological criteria relevant to process verification testing, sampling strategy and choice of the sampling plan, and the analysis and interpretation of the data generated for decision-making is discussed in Chap. 3, Verification of Process Control. That chapter addresses consideration of both within-batch and between-batch variability in verification testing. Baseline data on the performance of the food system are used to characterize the quality and safety of product arising from the process when it is functioning as intended. Comparing these baseline data with data from periodic testing can then be used to provide:

1. Assurance that conditions that enable a food process to produce safe products are being maintained.
2. A basis for analyzing performance trends so that corrective actions can be taken before loss of control.
3. Insights into the cause for loss of control (e.g., periodicity of contamination).
4. A warning that conditions have changed sufficiently such that the original HACCP plan may need to be reviewed.

Once established, process control testing typically involves routine testing of a small number of samples. The microbiological limits for a process control testing program ideally include both an action level and an upper limit. The action level allows corrective actions to be taken proactively before the upper limit is reached. To detect such trends towards unacceptable loss of control as soon as possible, and to differentiate them from extreme results that arise simply from normal variation within the acceptable range, comparison of the data over time is needed and is usually done through some form of process control analysis, such as control charting. The specific testing requirements depend on the process control analysis approach employed, and are discussed and exemplified in Chap. 3.

### 1.2.3 Verification of Environmental Control

Assessment and control of microbial loads in food processing environments is important because there is ample evidence that postprocessing contamination can affect product quality and safety. Environmental testing is undertaken to ensure that GHP measures are effective in minimizing product contamination from the processing environment. Microbiological testing is used to:

1. Assess the risk of product contamination.
2. Establish a baseline that characterizes when the processing environment is appropriately controlled.
3. Assess whether control is being maintained.
4. Investigate sources of contamination to be able to implement corrective actions.

Routine environmental sampling is most likely to be applied in food processing plants in which recontamination of product from the environment could occur after a kill step. For ready-to-eat (RTE) products for which there is no effective CCP, monitoring farm environments may also be useful. Environmental sampling is unlikely to be useful at other steps along the food chain. Factors that contribute to and influence postprocessing contamination as well as strategies and actions to control pathogens in food processing environments are described in detail in ICMSF (2002b) and summarized in Chap. 4, Verification of Environmental Control.

### *1.2.4 Corrective Action to Reestablish Control*

Despite the application of food safety management systems, control is sometimes lost with potential implications for product quality and safety. Evidence of loss of control may be obtained from an on-site inspection, monitoring GHPs, monitoring or verifying activities, analysis of samples, consumer complaints or epidemiological information implicating the food operation.

As defined by the Codex Alimentarius Commission (1997b), corrective action is "any action to be taken when the results of monitoring at the CCP indicate a loss of control." Control may not only rely on the HACCP control points, but also on the combined effect of prerequisite programs, other actions and the HACCP plan; thus evaluation of effective control is not always straightforward.

Unlike HACCP systems in which corrective actions in response to loss of control must be documented as part of the HACCP plan, there is less clear description of specific actions to respond to loss of controls relevant to GHP. Chapter 5, Corrective Action to Reestablish Control, describes how visual inspection and microbiological testing are commonly employed to evaluate prerequisite programs, and how they can indicate loss of control and reveal the need for more frequent or more effective cleaning, for more frequent and thorough maintenance of processing equipment, for retraining of staff in hygiene principles and practices or other actions. Specific testing can also be used to identify contamination sources.

For control defined in the HACCP plan, the need for corrective actions for CCPs can be revealed by routine monitoring or from epidemiological or customer complaint data. In these situations, testing can reveal if the document control criteria were incorrect or have become inadequate. The use of appropriate testing according to a relevant sampling plan can help to reveal the microbiological consequences of loss of control and the disposition of the product, e.g., no increased risk, reprocessing required or product must be discarded.

Chapter 5 considers these topics in greater detail, providing practical advice for assessing points/processing requiring control, establishing base-line values so that unacceptable deviation can be recognized, and identifying appropriate use of testing to reestablish control of the operation.

### *1.2.5 Microbiological Testing in Customer-Supplier Relations*

The commercial food chain involves many interacting businesses and supplier-customer relationships, each implying contracts that define expectations of customers and the commitments of suppliers. For perishable and semi-perishable foods or ingredients these may include microbiological aspects of the product, potentially concerning safety, quality and shelf life expectations. For shelf-stable and frozen foods, microbial shelf life is not relevant, but because of persistence of some pathogens, microbiological criteria may be relevant especially if resistant pathogens or microbial toxins could be present through inappropriate handling earlier in the product's life.

Microbiological criteria and testing in customer-supplier relationships can relate to raw materials, ingredients, semi-processed and finished products. They can also consider the potential for microbial

growth in the product. Criteria related to microbial quality and safety can include microbial limits, product formulation specification, packaging, storage and transport conditions, and time/temperature conditions that prevent, or minimize to an acceptable degree, the growth of pathogens or spoilage microorganisms. Evaluation may include microbiological testing, physical-chemical measurements (e.g., pH, $a_w$, residual chlorine assessment etc.) or even visual assessment (e.g., mold affected fruits, grains or nuts in a lot do not exceed some defined, acceptable limit).

Criteria may also relate to processing operations, such as those that might be considered in evaluating a supplier HACCP program. Considerations in defining microbiological or related criteria can include the point in the production chain, the intended further processing or end-use of the product, technological feasibility etc. Microbiological testing considerations specifically relevant to customer-supplier relationships are discussed in further detail in Chap. 6, Microbiological Testing in Customer-Supplier Relations.

### *1.2.6 End Product Testing to Evaluate Integrity*

The relative importance of end product testing must be determined on a product by product basis. For some products, end product testing is the only point where regulatory limits apply. End product testing may be used for lot acceptance when there is insufficient process or testing information available from which to evaluate product safety or utility. Similarly, for products in which no effective CCP is currently available and there is no other means of assessing product integrity, end product testing may offer the only alternative. The suggested criteria for lot acceptance in Part II of this book (Chaps. 8–26) are based on baseline data, experience, industry practice, relative risk when ICMSF cases are considered or existing microbiological criteria that have been developed internationally as a result of the risk analysis process established by the Codex Alimentarius Commission (see Sect. 7.4). Different sampling plans may be appropriate in certain situations. Reducing the number of samples may be entirely appropriate for on-going surveillance activity; whereas increasing the number of samples may be prudent when investigating significant process deviations or outbreaks. For example, in the event of a loss of control, sampling frequency should be increased until confidence is achieved that the process is again under control. Such investigational samples should be analyzed individually rather than as composites, because this will help in identifying the source of the problem.

## 1.3 Limitations in Microbiological Testing of Foods

This book aims to provide practical guidance on relevant microbiological testing of foods to help ensure their safety and quality. Readers should be aware, however, of the limits of confidence one can have in the results of such testing both from a statistical perspective, and also due to the limitations in methods for detection and enumeration of microorganisms in foods.

While methodological considerations are discussed briefly in Sect. 7.5, Limitations of Microbiological Tests, it must be emphasized that estimates for the performance of sampling plans presented in this book (see Table 7.2) do not take into account any errors that might occur from the microbiological methods used to determine either the presence or concentration of microorganisms in foods.

The process of sampling itself can never be completely reliable. The degree to which the microbiological status of the samples taken can be expected to represent the whole lot or batch of food being assessed is discussed in Appendix A, Statistical Aspects of Sampling.

## 1.4 Conclusions

Microbiological testing is applied to food safety and quality management for a number of reasons including development of process controls, monitoring and verification of process control, investigation of the causes of loss of control, and in some situations to directly assess product quality and safety. Assessment of microbiological quality and safety of foods is often laborious and time consuming, and a comprehensive microbiological testing program for many products involves more than routine lot acceptance testing. Currently all microbiological testing methods for end product are destructive. Accordingly, the goal of a comprehensive program is to infer the quality and safety of batches of product using process data augmented by relevant microbiological assessment of samples taken not only from the lot, but also relevant ingredient, in-process, environmental and shelf life. This process has limitations, both due to the confidence that one can have that the samples are representative of the lot, and also because methods of isolation, identification and enumeration of microorganisms from foods are imperfect. These limitations must be understood when designing microbiological testing program for food safety and quality assurance.

The Commission trusts that this book provides practical guidance to those responsible for the assurance of microbial quality and safety of foods to fulfill this important role. Specific recommendations for product categories are provided in subsequent chapters.

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# Chapter 2
# Validation of Control Measures[1]

## 2.1 Introduction

ICMSF previously discussed validation of control measures in the supply chain (Zwietering et al. 2010) and portions of that paper are included in this chapter. The flexibility offered by an outcome based risk management system must be supported by demonstration that the selected control measures actually are capable of achieving the intended level of control on a consistent basis. Validation is defined by the Codex Alimentarius Commission (2008) as:

> "**Validation**: Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome."

The overall effectiveness of the control measures should be validated according to the prevalence of the hazards in the food of concern, taking into consideration the characteristics of the individual hazards(s) of concern, established Food Safety Objectives or Performance Objectives and level of risk to the consumer.

### *2.1.1 Relationship of Validation to Monitoring and Verification*

In addition to the definition of validation cited above, the Codex Alimentarius Commission (2008) adopted the following definitions:

> **"Monitoring**: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control."

> **"Verification**: The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended."

Validation focuses on the collection and evaluation of scientific, technical and observational information and is different from verification and monitoring. Monitoring is the on-going collection of information on a control measure at the time the control measure is applied and verification is used to determine that the control measures have been appropriately implemented. The successful implementation of HACCP requires validation, which includes the clear identification of hazards, control measures available, critical control points, critical limits and corrective actions. The outcomes of

[1] Part of this chapter was published as: Zwietering MH, Stewart CM, Whiting RC, ICMSF (2010) Validation of control measures in a food chain using the FSO concept. Food Control 21:1716–1722.

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monitoring and verification activities associated with a HACCP system assist in determining when re-evaluation may be necessary. To be effective, the scope of validation may go beyond the control measures used in the manufacturing facility and may include control areas such as primary processing and consumer handling.

The production of safe food requires the application of GHP and HACCP principles to develop and implement a total food safety management system that controls the significant hazards in the food being produced. Some risk management principles are best addressed through GHP measures (e.g., controlling the initial levels of a hazard through good hygiene) and others are clearly part of a defined CCP within HACCP (e.g., reducing the level of a hazard, through a decontamination step).

Food manufacturers design processes to meet Performance Objectives (PO) or Performance Criteria (PC), which can be set at specific points throughout the food chain to assure food safety. Regulatory authorities are concerned with whether a group of products or the consequences of a series of processing and handling steps prior to consumption can meet the Food Safety Objective (FSO) and ensure that those foods achieve levels that are consistent with the Appropriate Level of Protection (ALOP) (see Chap. 1, Utility of Microbiological Testing for Food Safety and Quality).

Various control measures include the control of ingredients at the initial stage of food processing or food chain, and intensive protocols to reduce or eliminate the contamination by washing, heating, disinfecting and other measures. Control measures are also designed to prevent an increase of hazards during transportation and storage, by cross-contamination during the processing or cooking, or even by re-contamination after those steps.

Control measures should be validated to determine whether the products meet with objectives; however, different segments of the food industry undertake these activities depending on the situation. Food processors may validate the control measures for the processes they use, and validation should focus on achievement of meeting the given PO or PC. In this case of validation, both within-lot and between-lot variability should be considered. On the other hand, control measures validated under the responsibility of regulatory authorities cover all control actions in the system for multiple products and processes, including consideration of between-lot variability. In this case validation is targeted at assessing the established PCs, POs and FSOs. For example, the effective risk management of a meat production system may include validation of:

- Farm practices aimed at ensuring animal health and minimizing the level of infection in the herd (zoonosis).
- Slaughter practices aimed at minimizing contamination.
- Chilling regimes and temperature control aimed at minimizing the potential for pathogen growth.
- Consumer instructions aimed at ensuring that the product is cooked to the minimum temperature required to inactivate pathogens.

In this chapter, the prevalence and levels of microorganisms from the initial contamination ($H_0$), reduction ($\Sigma R$), growth and re-contamination ($\Sigma I$), and factors that influence these are considered throughout food production until consumption. The influence of these factors on meeting the FSO is represented by the equation $H_0$-$\Sigma R$+$\Sigma I \leq FSO$. Stochastic aspects of the parameters are taken into account as well as deterministic values. Potential key factors, data and data analysis methods are described. However, some of these factors may not be relevant for a particular processing line or processor. Examples of the use of data to validate one or a series of processes, including statistical insights, are provided.

## 2.2 Considerations for Validation

Processes can be validated through the use of a variety of approaches (Codex Alimentarius 2008) including predictive modeling, the literature, microbiological challenge studies and use of safe harbors (i.e., approaches that have been previously approved as delivering a safe product (see Chap. 1)).

Not all these need to be used, but often several approaches are combined to supply sufficient validation evidence. When a safe harbor approach is used, it may not be necessary to conduct validation studies for that process. For example, a safe harbor for milk pasteurization is to deliver a minimum heat process of 72°C for 15s. This process criterion has been validated and therefore can be implemented by manufacturers without re-validation of the process.

Numerous considerations for establishing the efficacy and equivalency of processes are discussed by NACMCF (2006), which proposed the following steps for the development of processes intended to reduce the pathogen(s) of concern:

- Conduct a hazard analysis to identify the microorganism(s) of public health concern for the food.
- Determine the most resistant pathogen of public health concern that is likely to survive the process.
- Assess the level of inactivation needed. Ideally this would involve determining the initial cell numbers and normal variation in concentration that occurs before processing.
- Consider the impact of the food matrix on pathogen survival and possible growth during storage.
- Validate the efficacy of the process.
- Define the critical limits that need to be met during processing so that the food will meet the performance objectives and performance criteria.
- Define the specific equipment and operating parameters for the proposed process.
- Implementation within GHP and/or HACCP.

Regardless of the methods used to determine and validate process criteria, similar microbiological considerations need to be taken into account (NACMCF 2010). These include:

- What is the most resistant microorganism of public health significance for each process? When determining the target microorganism, it is necessary to consider all pathogens that have an epidemiologically relevant association with a product, as the most resistant pathogen may not be present in the highest numbers. Conversely, pathogens controlled by other means may not be of public health significance in a product when growth is required in order to cause illness (i.e., *C. botulinum* controlled by pH).
- Choice of strains used to conduct validation studies
- The phase of growth in which the microorganisms are harvested
- The substrate upon which the culture is grown and the associated environmental conditions (e.g., pH, temperature, atmospheric conditions), including adaptation of culture when appropriate
- The suspending medium
- The food's intrinsic factors, such as pH, $a_w$, and preservative levels
- The sample size, preparation and handling (i.e., compositing, homogenizing, subsamples)
- Packaging conditions (packaging material and atmospheric conditions, including modified atmosphere gas mixtures)
- Cell enumeration methods following the process and selection of appropriate measurement systems
- Processing variability

Three commonly used strategies for process validation include concurrent, retrospective and prospective process validation. *Concurrent process validation* is based on simultaneous collection and evaluation of data from a process concurrently with its application. This is used when there is a change or modification to an established and previously validated process. *Retrospective process validation* is validation of product already in distribution based upon accumulated production, testing and control data. This technique is often used in analyzing process failures that result in product recalls. *Prospective process validation* is a deliberate, forward-looking, planned approach that determines if the process can be relied upon with a high degree of confidence to deliver safe food. Prospective validation is best suited for evaluating novel processes and must consider the equipment, the process and the product (Keener 2006).

A team of experts is required for system validation because of the many skills required such as engineering, microbiology, physical chemistry, etc. Involvement of external experts and regulatory officials in the development of both the master validation plan and the validation protocols is essential to ensure technical adequacy and acceptance by authorities. Process validation requires proper analysis of objective data.

## 2.3 Validation of Control Measures

Validation generally begins with microbiological studies on a laboratory scale, progresses to a pilot plant scale and ends with full validation on a commercial scale when possible or necessary. Microbiological challenge testing is useful to validate process lethality against a target microorganism(s) to determine the ability of a food to support microbial growth and to determine the potential shelf life of ambient or refrigerated foods. For example inactivation kinetic studies can be conducted over a small range of treatments such as a unique combination of factors and levels (e.g., pH 6.5 and 70ºC). Conversely, studies can also be conducted over a broad range of treatments, and can illustrate where failure occurs and help assess the margin of safety in any process, as well as providing data that can be used in evaluation of deviations. Furthermore this facilitates development of predictive models for future public or private use. Several microbiological predictive models are available, including the USDA Pathogen Modeling Program (USDA 2006) and COMBASE (2010). Challenge studies can also be used to determine processing criteria, although they are of less generic use than models and often are used for particular products or as a way of validating the model predictions. On the other hand models are often generic, and therefore do not contain all factors that are of relevance for a specific food. Therefore models and challenge studies should be combined in an iterative way. This is further discussed by NACMCF (2010). Finally, on a commercial scale, challenge studies can be conducted using nonpathogenic surrogate microorganisms and shelf life studies with uninoculated product can also provide useful information for validating a process.

While microbiological challenge testing can be used to determine the stability of a product with regards to spoilage over the intended shelf life, the remainder of this discussion focuses on microbiological safety of food products. In the following sections, the initial contamination ($H_0$), reduction ($\Sigma R$), growth and re-contamination ($\Sigma I$), and factors influencing these are discussed sequentially, including data needs and experimental considerations.

It is important to note that in this text, diagnostic methods are assumed to be 100% sensitive and 100% specific, which is not the case. These characteristics of methods depend largely on the target microorganism, diagnostic method used and investigated food product. Especially for low level pathogens false negative results might be expected. These aspects need to be clearly considered in validation studies.

### *2.3.1 Initial Level ($H_0$), Standard Deviation and Distribution*

The design of the food process influences the importance of incoming material for product safety. The main source of the pathogen of concern may be from a major or minor ingredient, one incorporated in the initial processing steps or one added later. It is important to understand which ingredient(s) may harbor the pathogen and if there is a seasonal effect on the level of the pathogen. For example, the number of *Escherichia coli* O157:H7-positive lots of ground beef sampled from 2001 to 2009 increased in the June-October period in the USA (USDA-FSIS 2009). The geographical source of the ingredient may also play a role in the likelihood of whether a certain pathogen is present in the raw ingredient. If contamination is not avoidable, the goal is to develop specifications and criteria for the incoming

material that will lead to achievement of the final PO and FSO, in conjunction with the performance criteria for the other steps in the food process. The specifications for accepting the incoming materials include the acceptable proportion above a limit or the mean log level and standard deviation.

Information for validating that incoming materials comply with required specifications can come from:

- Baseline data from government agencies.
- Documentation from suppliers that specifications are met (supplier provides validation and end product testing).
- Baseline data from the processor's experience or
- Test results for incoming lots.

Microbiological testing is one of the tools that can be used to evaluate whether a food safety system is providing the level of control it was designed to deliver. A number of different types of microbiological testing may be employed by industry and government. One of the most commonly used is within lot testing, which compares the level of a microbiological hazard detected in a food against a prespecified limit, i.e., a Microbiological Criterion (MC) (ICMSF 2002). MCs are designed to determine adherence to GHP and HACCP (i.e., verification) when more effective and efficient means are not available. In this context, FSOs and POs are limits to be met, and within-lot testing can provide a statistically-designed means of determining whether these limits are being met (van Schothorst et al. 2009). To assess compliance of a lot to a MC, a sampling plan based on the MC specified and the confidence level desired can be established. To do this, the recommendations for setting MCs as outlined in Appendix A should be followed. The MC should specify the concentration to be met ($m$ in CFU/g), the proportion of defective samples ($c$) allowed above the $m$ value, the number of samples to be tested ($n$) and an evaluation of the implications for a given sampling plan.

A sampling plan appropriate to assess compliance with a specified concentration can be developed using the ICMSF spreadsheet (Legan et al. 2002, http://www.icmsf.org). The calculations underlying the spreadsheet determine the probability that an analytical unit from a lot contains more than any specified number of cells/g. That probability can be estimated from the mean concentration of the cells in the lot, and its standard deviation. It is assumed that the distribution of concentrations of cells in a lot is log-normally distributed. A Performance Objective is determined, e.g., that 99% of units must contain less than a specified concentration of cells, and a corresponding mean log concentration determined from the assumed standard deviation. Then the number of samples required to be taken from the batch, to provide 95% confidence that an unacceptable batch will be rejected by sampling, can be calculated taking into account the size of the analytical unit. In an example on *Listeria monocytogenes* in cooked sausage (ICMSF 2002), the initial number in the raw materials prior to cooking is assured to be no more than $10^3$ CFU/g (i.e., $H_0 = 3$). Often a PO for $H_0$ can also be regarded as the PO for the output of a previous stage of the food chain.

In any sampling process in microbiology, the actual number of organisms recovered in a sample taken from a lot will also be affected by the random distribution of cells within the region that is actually sampled. This randomness is described by the Poisson distribution. The relative effect of this randomness is relatively small when large number of cells are contained, and counted, from the sample (e.g., the standard deviation when the true mean is 100, is ± 10), but it is relatively large when the target concentration is one cell per sample, such as in presence absence testing. Including this consideration in design of a sampling plan is more important when the result of testing is presence or absence, and has also been incorporated into the spreadsheet calculation (van Schothorst et al. 2009). As for the evaluation of sampling plans based on testing against a specific number of cells, for evaluation of sampling plans based on presence/absence testing it is also assumed that the distribution of the concentration of cells in the batch is log-normally distributed, and is characterized by a mean log and standard deviation. The Poisson effect is also included in the calculations for the first alternative, but is relatively minor.

## *2.3.2 Inactivation Studies (ΣR)*

### 2.3.2.1 Modeling Studies

A microbiological predictive model can describe or predict the growth, survival or death of microorganisms in foods. These models typically relate the microbial growth, survival or death responses to the levels of the controlling factors, such as temperature, pH, water activity etc. Models generally should not be used outside the range of the factors used to create them because there is no underlying principle on which to base extrapolation. Thus consideration of the range over which they will be used is required before beginning experimentation (Legan et al. 2002). Where extrapolation is necessary, tests should be conducted to confirm that the extrapolation is valid, e.g., confirm that the established process destroys a specific population of the target microorganism. However, models that can predict the rate of death of pathogens can be used to design safe and effective processes.

Several authors describe experimental design for modeling in food microbiology (Ratkowsky et al. 1983; Davies 1993, Ratkowsky 1993, McMeekin et al. 1993). Guidelines for data collection and storage are also available (Kilsby and Walker 1990, Walker and Jones 1993). A practical guide to modeling, supported by references to primary sources of modeling information is discussed by Legan et al. (2002). The reader should consult these references for details on development of a microbiological predictive model.

### 2.3.2.2 Microbiological Challenge Studies

Detailed information on the design and implementation of microbiological challenge studies has been described (IFT 2001, Scott et al. 2005, NACMCF 2010). Microbiological challenge testing is useful to validate process lethality against a target microorganism(s).

When designing and carrying out a microbiological challenge study, some factors to consider include the selection of appropriate pathogens or surrogates, the level of the challenge inoculum, the inoculum preparation and method of inoculation, the duration of the study, formulation factors and storage conditions, and sample analyses (Vestergaard 2001). Multiple replicates of such studies should be done to reflect variation in the production lots and other factors. The extent of replication and the impact on the results of the study must be considered.

### 2.3.2.3 Challenge Microorganism Selection

The ideal microorganisms for challenge testing are those previously isolated from similar formulations. If possible, pathogens from known foodborne outbreaks should be included. In contrast to kinetic studies, challenge studies frequently use a mixture of five or more strains of the target pathogen because a single strain may not be the most resistant to each of the multiple stress factors involved in the product/process combination. Additionally, strains with the shortest generation time may not have the shortest lag time under the test conditions. Likewise, strains may vary in response to changes in the inactivation treatment (Scott et al. 2005). The strains in the cocktail should be present in approximately equal numbers. It is also important to incubate and prepare the challenge suspension under standardized conditions and format.

When possible, it is desirable to use a pathogen rather than a surrogate microorganism for validation studies. However, surrogates are sometimes used in place of specific pathogens, for example, in challenge studies conducted in a processing facility. The characteristics of the surrogate in relation to those of the pathogen should be determined and the difference accounted for in the interpretation of the challenge studies (Scott et al. 2005). Detailed information on the desirable attributes for surrogates can be found in IFT (2001).

#### 2.3.2.4 Inoculum Level

The inoculum level depends on the purpose of the study; whether the objective is to determine product stability or shelf life, or to validate a step in the process designed to reduce microbial numbers. When validating a process lethality step, it is usually necessary to use a high inoculum level, such as $10^6$–$10^7$ CFU/g of product or higher, to demonstrate the log reduction of the challenge microorganisms. The actual concentration of the inoculum before and after inoculation should be confirmed. Also uninoculated samples should be analyzed to investigate intrinsic product contamination. Total inactivation of the inoculum may not be necessary, especially in situations where the $H_0$ is likely to be low (e.g., when the initial population is $<10^3$ CFU/g a 5D process is required and the inoculum level in the experiment is $10^7$ CFU/g). This may be relevant when validating post lethality treatments, where the process is being designed to inactivate low levels of pathogens resulting from recontamination of product after an initial lethal treatment, such as might occur during slicing or packaging operations.

#### 2.3.2.5 Inoculum Preparation and Inoculation Method

Preparation of the inoculum is an important component of the overall protocol. Typically, the challenge cultures should be grown in media and under conditions optimal for growth of the specific challenge culture. In some studies, specific challenge microorganisms may be pre-adapted to certain conditions.

The method of inoculation is another important consideration. It is essential to avoid changes in the critical parameters of the product formulation undergoing the challenge. For example, the use of a diluent adjusted to the approximate water activity of the product using the humectant present in the food minimizes the potential for erroneous results in intermediate moisture foods. Preliminary analyses should be done to ensure the water activity or moisture level of the formulation is not changed after inoculation. For guidelines for inoculation of low water activity products or for challenge studies with spores refer to IFT (2001).

#### 2.3.2.6 Duration of Challenge Studies for Potential Growth

It is prudent to conduct the challenge study longer than the desired shelf life to determine what would happen if users stored and consumed the product beyond its intended shelf life. Additionally, when validating inactivation processes, it is possible that sublethal injury may occur in some products, leading to a long lag period (Busta 1978). If the product is not tested for at least its entire shelf life, it is possible to miss the recovery and subsequent growth of the challenge microorganism late in shelf life. Some regulatory agencies require data for 1.3 times the shelf life of the product when stored as intended. Shorter times may be considered for refrigerated products that are stored under abuse conditions.

The frequency of testing is governed by the duration of the challenge study. If the shelf life is measured in weeks, the test frequency is typically no less than once per week. It is desirable to have a minimum of 5–7 data points over the shelf life to have a good indication of inoculum behavior. All studies should start with "zero time" testing, i.e., analysis of the product right after inoculation and, for inactivation studies, right after processing. It may also be desirable to test more frequently early in the challenge study and then reduce the frequency of testing to longer intervals.

A sufficient quantity of product should be inoculated so that a minimum of three replicates per sampling time is available throughout the challenge study. In some cases, such as in certain revalidation studies and for uninoculated control samples, fewer replicates may be used.

#### 2.3.2.7 Formulation Factors and Storage Conditions

When evaluating formulation, it is important to understand the range of key factors that control its microbiological stability such as pH, preservative level and water activity. These intrinsic properties should be documented. It is useful to collect data on the inherent manufacturing variability of the critical parameters and ensure that the challenge test conditions encompass this variability by a specified margin (e.g., with 95% confidence). These parameters should be adjusted to the worst case condition expected for the product with respect to microbial growth or inactivation (e.g., highest pH). One approach would be to use the 95% confidence interval for the parameter or the mean plus 2 standard deviations. If there is only one critical parameter, this 95% confidence would mean that one out of 20 times reality could be outside this range. However, if there are many critical parameters, setting all at their 95% confidence level might simulate an unrealistic condition. The level of confidence desired must be considered in evaluating these parameters.

It is important to test each key variable singly or in combination under worst case conditions. For example, if the target pH is $4.5 \pm 0.2$ (95% confidence interval) and the processing capability is within that range, the challenge product should be on the high side of that range (pH 4.7). This should be carefully assessed for different parameters. For example, decreasing the water activity of a product may delay or prevent growth of microorganisms; however, using a different humectant in the system is a change in the critical factor even if the same water activity ($a_w$) is achieved because growth rates may vary with different humectants. Further, decreasing the $a_w$ of a system may reduce the lethality of a process (Mattick et al. 2001). Inclusion of the impact of variability in critical factors helps to ensure that the challenge study covers the process capability range for each critical factor in the formulation.

#### 2.3.2.8 Sample Analysis

Typically, enumeration is conducted at each sampling time. It is desirable to have at least duplicate and preferably triplicate samples for analyses at each time point. The selection of enumeration media and method depends on the microorganisms used in the challenge study. In situations where toxin-producing microorganisms are used, test for appropriate toxins at each sampling time using the most current validated method. Growth may occur without the formation of toxin.

It is prudent to analyze inoculated product and uninoculated control samples at each selected sampling time to determine how the background microbiota behaves during shelf life. It is also important to track pertinent physical and chemical parameters over the shelf life as they may influence the behavior of the microorganism. Understanding how factors such as $a_w$, moisture content, salt level, pH, Modified Atmosphere Packaging (MAP) gas concentrations, preservative levels and other variables may change over product shelf life is important to understanding the microbiological stability of the product. Quality attributes should also be noted.

#### 2.3.2.9 Data Interpretation

Once the challenge study is completed, the data should be analyzed to determine how the microorganisms behaved over time. For toxin-producing pathogens, no toxin should be detected over the designated challenge period. Combining quantitative inoculum data for each time point with data on the background microbiota and the relevant physical and chemical parameters provides a broad representation of the microbiological stability of the formulation under evaluation. A well-designed challenge study can provide critical information on the microbiological safety and stability of a food formulation. Such studies are also invaluable in validating the key lethality or microbiological control points in a process.

### 2.3.3 *Growth Studies ($\Sigma I$)*

An increase in the numbers of pathogen or spoilage microorganism can occur through growth or recontamination. This section addresses growth.

Growth may occur if the food, temperature and packaging atmosphere support growth, and sufficient time is provided under favorable conditions. Growth potential should be assessed for raw ingredients, intermediate points during the manufacturing and after manufacture during distribution, retail, food service and home storage and use. Generally, public health cannot be assured unless the potential for growth is minimized. If the pathogen is not completely inactivated and growth is possible, then an accurate estimation of the amount of growth that may occur is important in validating product safety and stability.

As previously described for validating inactivation, estimates for growth may be obtained from a variety of sources including the literature, models and challenge tests (Scott et al. 2005). Increasing reliance is given to studies with experimental conditions that more closely reflect the actual conditions of the food. Satisfactory validation of a pathogen's growth in a food includes challenge tests with the normal background microbiota. Models and broth studies can provide support for evaluating minor changes in formulation and strain differences and for interpolating to conditions not explicitly tested in the challenge tests. Applications of predictive models in food microbiology include models that predict the growth rate of bacterial pathogens in response to product or environmental factors such as $a_w$, temperature or pH. Growth models can be used to design safe product formulations, to set appropriate storage conditions, to explore the maximum interval between cleaning and sanitizing of process equipment, and can also be used to inform decisions about when a challenge study is needed and to design the test parameters.

Factors that should be considered when evaluating growth include the strain(s) used, surrogates, physiological state of the inoculum, inoculation method, simulation of the experimental or pilot plant conditions to the commercial process, inclusion of all environmental factors in the food (pH, $a_w$, acid anions) and external factors (temperature, packaging), and inclusion of the spoilage microorganisms. Many of these factors were described in the inactivation section; considerations particular to estimating growth are discussed below.

#### 2.3.3.1 Inoculum Level

IFT (2001) provided a list of microorganisms that can be used in microbiological challenge studies and recommendations for selection and assessment of tolerable growth. When the objective is to determine product safety and the extent of growth over its shelf life ($\Sigma I$), an inoculum level of between $10^2$ and $10^3$ CFU/g of product is frequently used. Lower or multiple inoculum levels may be considered if microbial spoilage is a common mode of failure and low numbers are anticipated in the product. See Sects. 2.3.3.3 and 2.3.3.6, for additional considerations on inoculum level.

#### 2.3.3.2 Formulation Factors and Storage Conditions

When similar products are under evaluation, testing formulations that are more favorable to growth can limit the need to conduct challenge studies on formulations less favorable to growth. For example, studying products with a pH near neutrality may represent a worst case when similar products have a lower pH.

Test samples should ideally be stored in the same packaging and under the same conditions (e.g., MAP) used for the commercial marketplace. The storage temperatures used in the challenge study should include the typical temperature range at which the product is to be held and distributed.

Refrigerated products should be challenged under representative abuse temperatures. Some challenge studies may incorporate temperature cycling into the protocol.

#### 2.3.3.3 Lag Phase

A lag phase occurs when cells require time to adjust to a new environment. The lag phase is influenced by the magnitude of the change and the favorability of the new environment. In general, a lengthy lag phase occurs when cells experience a significant shift to a less favorable environment such as to a lower temperature or water activity.

The physiological state of the cell also plays a role in the length of the lag phase. Generally, cells in the exponential growth phase adapt more rapidly than cells in the stationary phase. Cells that are starved in nutrient poor environments such as water, frozen or desiccated on a food contact surface typically have an increased lag time compared to the other cells. Following an inactivation treatment or other severe stress, surviving cells may need time to repair, which can also appear as a lag phase before growth. Significant lag times are most likely when certain ingredients are added (e.g., salt, acidulant) or after a stressful process (heating, thawing, sudden temperature change). A lag phase as result of temperature changes is less likely in a finished product because the mass of the food, retail packaging and box/pallet moderate temperature changes. Validation should recognize that the temperature reduction during a cooling period may extend over one or more days, especially if the food is boxed and palletized. Validation of a process should strive to replicate the initial physiological state and environmental changes in order to accurately determine the length of the lag phase, if any.

The length of the lag phase can be affected by the initial number of cells because a log normal distribution exists for the lag times of individual cells. Validation studies with high cell numbers (>$10^2$ CFU/package or unit) will inevitably have some cells with the shortest lag times and daughter cells will almost entirely originate from these cells. When low levels of contamination occur, it is possible that none of these fastest cells are present in some of the packages and the apparent lag times will become longer and more varied in those packages.

#### 2.3.3.4 Exponential Growth Rate

The exponential growth rate (EGR) increases with storage temperature up to the pathogen's optimum temperature (typically 35–45°C for pathogens). The EGR depends on other intrinsic characteristics of the food such as acidity, water activity and inhibitors in a complex manner that can be estimated by models. However, challenge studies are required to demonstrate that the model's prediction is accurate for a specific food. Once a model is validated, it can be used to estimate the impact of the environmental factor changes (T, pH, $a_w$ etc.) on the EGR.

#### 2.3.3.5 Maximum Growth Level

A pathogen has a maximum level of growth that it achieves in a microbial medium or food. In broth and in pure culture, this level is typically $10^8$–$10^9$ CFU/mL; however, it is sometimes lower in a food. The maximum in a food is affected also by storage temperature. For *L. monocytogenes* in the FDA-FSIS risk assessment the maximum growth levels (CFU/g) selected were $10^5$ for temperatures of <5°C, $10^{6.5}$ for 5–7°C and $10^8$ for temperatures >7°C (FDA-FSIS 2003) based on various literature sources.

#### 2.3.3.6 Competition and the Spoilage Flora

Competition between the pathogen and spoilage microorganism is difficult to predict. For many pathogen-spoilage microorganism pairs, growth of both groups is reasonably independent until the spoilage microorganisms have grown significantly. Spoilage microorganisms may decrease the pH or produce inhibitors such as bacteriocins. Pathogens are typically at low populations and do not interfere with the spoilage microorganisms. Typical microbiota found in commercial settings should be present in challenge studies. Pathogens should be inoculated in the appropriate physiological state, location in the food (e.g., surface, interior or interface of components as appropriate) and concentrations that will likely occur in the commercial setting.

Another important consideration in determining the safety of a food is the storage conditions that lead to spoilage, particularly spoilage before the pathogen reaches the PO. Evaluation of growth during storage requires knowledge of the typical times and temperatures characteristic of that stage. This may be easy for the relatively short growth periods during the commercial phases of the food chain. However, time and temperature are highly variable in the home or food service operation. A temperature of moderate abuse should be selected and the maximum length of the storage period before spoilage at that temperature ascertained for determination of the amount of growth. Foods should be tested for 1.25−1.5 times their intended shelf life unless spoilage occurs first.

#### 2.3.3.7 Effect Variation on Growth

In addition to determining the average increase in cell population during each growth period, it is important to estimate the variation about that estimate (for example the 95% confidence interval). This variation is the consequence of the different characteristics of various strains, fluctuations in the environmental conditions within the food (pH, salt levels) and the ranges in times and temperatures of storage. The challenge test can provide an estimate of the mean log value; varying the parameters within a model can provide additional data to estimate the variation. This variation includes the differences in growth from the factors calculated above but may also be increased by the analyst to account for uncertainties because of a lack of high quality data.

### *2.3.4 Recontamination (ΣI)*

If a food process includes a lethal step that eliminates the pathogen, then any pathogen present at consumption is the result of recontamination. Foods receiving 6−8-log reductions rarely have a contaminated package immediately after that step. For example, if a product initially has a homogeneous contamination of $10^2$ CFU/g in every 100 g package, after a 7 log reduction only one in 1,000 packages will be contaminated and it will have ~1 CFU/package. When determining whether such a food meets an FSO or PO at a further step, calculation begins after the lethal step. The frequency and level of contamination represent the new $H_0$.

Little literature exists on the frequencies and levels of recontamination and few applicable models have been developed to estimate the results of recontamination. Sufficient sampling of the specific process at this step or at a subsequent step with a back calculation is the only way to obtain valid data on recontamination. A food process without a lethal step and with several potential points of additional recontamination is difficult to predict, especially since quantitative information related to recontamination is usually not available. Sufficient sampling of the food after the last point of recontamination is a possible way to validate whether a PO or FSO is being

achieved. Another approach is environmental monitoring and monitoring of food contact surfaces. Other factors to consider are packaging integrity and proper training of employees on handling practices.

## 2.4 Effect of Process Variability on FSO Compliance Validation

One way to demonstrate compliance to an FSO is by using the equation:

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO}$$

By combining information on the initial level ($H_0$), reductions ($\Sigma R$) and increases ($\Sigma I$) of the microbial hazard throughout the production and distribution chain, one can determine if the FSO or PO will be reliably met. The variability of the microbial levels at different steps in the process and food chain will influence the ability to meet the FSO.

The following examples illustrate the impact of including the effect of statistical distributions for $H_0$, $\Sigma R$ and $\Sigma I$ on the hazard level and the percent of nonconformance (% product above the PO or FSO) is calculated. First, a point estimate, without considering variability is used; then the impact of variability in the initial levels, reductions delivered through processing, and increases due to growth during food distribution are included to evaluate the ability to meet the PO or FSO. Fresh cut, washed and packaged lettuce is used as an example, with *L. monocytogenes* as the pathogen of concern. For illustrative purposes, it is assumed that to reach an ALOP, a maximum exposure of *L. monocytogenes* of $10^2$ CFU/g (i.e., an FSO = 2 log CFU/g or $10^2$ CFU/g) for ready-to-eat foods is set.

### *2.4.1 Point Estimate Approach*

Szabo et al. (2003) estimated the initial contamination level of *L. monocytogenes* on precut lettuce, reduction using sanitized washing, and the increases after packaging and during storage and distribution. For a given initial level of *L. monocytogenes* on lettuce and the expected level of growth ($\Sigma I$) during storage and distribution, the necessary reduction level to achieve a given FSO can be determined. From Szabo et al. (2003), the initial population was $H_0 = 0.1$ log CFU/g, the potential increase was $\Sigma I = 2.7$ log CFU/g during storage for 14 days at 8°C, a $\Sigma R \geq 0.8$ log CFU/g was deemed necessary to achieve the FSO of 2 log CFU/g:

$$H_0 - \Sigma R + \Sigma I = 2 \rightarrow 0.1 - 0.8 + 2.7 = 2.$$

In this example, the process can be considered to achieve the FSO exactly. However, this calculation does not consider the impact of process variation.

### *2.4.2 Including Variability in the Process*

#### 2.4.2.1 Variability for One Parameter

The next example illustrates the impact of variability on calculations using data from Szabo et al. (2003). Assume the standard deviation for $\Sigma I$ is 0.59, and assume the log increase of *L. monocytogenes* is normally distributed. For ease of calculation and explanation, $H_0$ and $\Sigma R$ levels do not include variation. Because of the distribution of $\Sigma I$, the producer must target a lower average level of

**Table 2.1** Results of various levels of reduction ($\Sigma R$) on the proportion of defective units (P) with a standard deviation for the increase of 0.59, assuming the log increase is normally distributed

| Reduction ($\Sigma R$) | $H_0-\Sigma R+\Sigma I$ | Probability that FSO = 2 is exceeded P ($H_0-\Sigma R+\Sigma I$)>2 (sd = 0.59) |
|---|---|---|
| 0.8 | 0.1−0.8 + 2.7 = 2 | 0.5 (50%) |
| 1.2 | 0.1−1.2 + 2.7 = 1.6 | 0.25 (25%) |
| 1.77 | 0.1−1.77 + 2.7 = 1.03 | 0.05 (5%) |
| 2.17 | 0.1−2.17 + 2.7 = 0.63 | 0.01 (1%) |
| 2.62 | 0.1−2.62 + 2.7 = 0.18 | 0.001 (0.1%) |

*Note*: The proportion above the FSO determined by the cumulative normal distribution $F(2;\mu,\sigma^2)$ calculated in Excel by 1-NORMDIST(2,x,s,1). For example, for the last line = 1-NORMDIST(2,0.18,0.59,1) = 0.001019

**Table 2.2** Results on the proportion of products that do not meet the FSO (packages of fresh cut lettuce calculated to have greater than 2 log CFU/g *L. monocytogenes* present at the point of consumption), with various mean log and standard deviation values for $H_0$, $\Sigma I$ and $\Sigma R$

| | $H_0$ | $\Sigma R$ | $\Sigma I$ | | Total[a] | |
|---|---|---|---|---|---|---|
| mean log | −2.5 | 1.4 | 2.7 | | −1.2 | $H_0-\Sigma R+\Sigma I$ |
| sd | 0.80 | 0.50 | 0.59 | | 1.11 | $sd = sqrt(sd_1^2+sd_2^2+sd_3^2)$ |
| | | | | $P$(>FSO) | 0.2% | |

[a] The level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

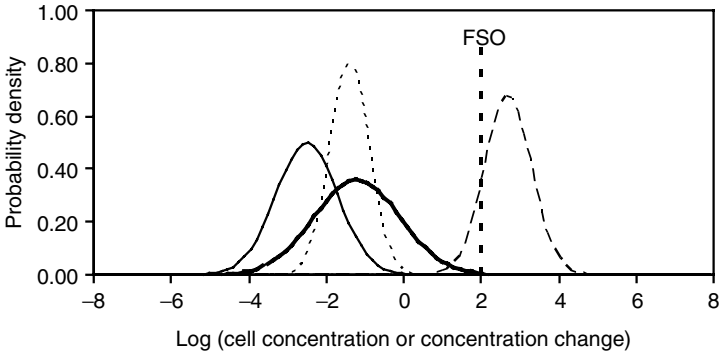


**Fig. 2.1** Probability distribution of initial cell level ($H_0$ ___), reduction in concentration (-$\Sigma R$- – -) and increase in concentration ($\Sigma I$– – –) of *L. monocytogenes* on fresh cut lettuce, and resulting cell concentration distribution (▬) in packages of lettuce at the point of consumption using input values in Table 2.2

*L. monocytogenes* in the finished product to reliably meet the FSO. If the same average level was targeted (i.e., FSO=2 log CFU/g), 50% of the products would be above the FSO to some extent. The processor can consider other sanitizing wash methods to provide a greater reduction step to help to achieve the FSO through process control. The level of reduction needed to achieve different levels of conformity is presented in Table 2.1. For example, if the $\Sigma R$ is 2.62, the proportion product above 2 logs, for a log normal distribution with mean log 0.18 and standard deviation 0.59 is 0.1%.

### 2.4.2.2 Including Variability in the Process for all Process Stages

The example in 2.4.2.1 did not include estimates of variability for $H_0$ or $\Sigma R$, but variation does exist. This section assumes variation for $H_0$, $\Sigma I$ and $\Sigma R$ (values in Table 2.2). The resulting total describes the distribution of levels of *L. monocytogenes* in packages of fresh cut lettuce at the point of consumption, and is equal to the sum of the log means for $H_0$, $\Sigma I$ and $\Sigma R$. The mean is not a correct indicator of the risk without considering the variance. The variance of the total distribution equals the sum of the variances, thus the standard deviation is the square root of the sum of the squares of the standard deviations. The distributions are illustrated in Fig. 2.1. Given this distribution of outcomes, the proportion of packages of lettuce not meeting an FSO = 2 in this example is 0.2%.

### 2.4.2.3 Ineffective Washing Step

Assuming that the lettuce washing step ($\Sigma R$) is not effective in reducing the level of *L. monocytogenes* (Table 2.3, Fig. 2.2), the overall effectiveness of the process can be determined. The mean log level of *L. monocytogenes* in packages of fresh cut lettuce increases from –1.2 to 0.2 and the overall standard deviation of the level decreases from 1.11 to 0.99. The proportion of packages that have *L. monocytogenes* levels above the FSO (2 log CFU/g) at the point of consumption increases to 3.5 % (Table 2.3). Note that the standard deviation does not differ much since the overall standard devia-

**Table 2.3** Impact of a lettuce washing step ($\Sigma R$) that does not reduce *L. monocytogenes* levels on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

| | $H_0$ | $\Sigma R$ | $\Sigma I$ | | Total[a] | |
|---|---|---|---|---|---|---|
| Mean log | –2.5 | 0 | 2.7 | | 0.2 | $H_0 - \Sigma R + \Sigma I$ |
| sd | 0.80 | – | 0.59 | | 0.99 | $sd = sqrt(sd_1^2 + sd_2^2 + sd_3^2)$ |
| | | | | $P(>FSO)$ | 3.5% | |

[a]The level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

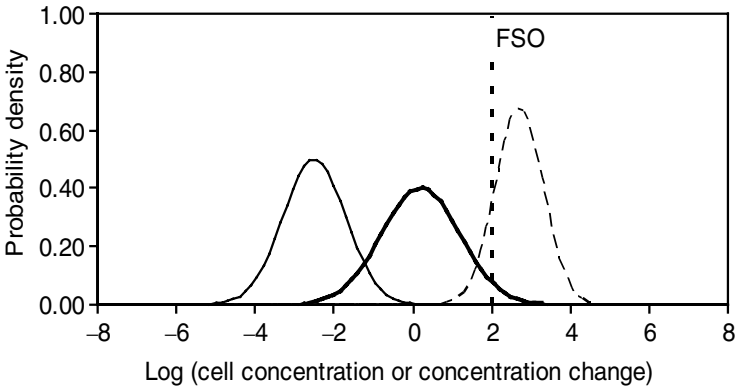


**Fig. 2.2** Probability distribution of the initial cell level ($H_0$ ___), increase in concentration ($\Sigma I$ – – –) and resulting overall final distribution (▬) of the levels of *L. monocytogenes* in packages of lettuce at the point of consumption for a process in which the washing step does not reduce the level of *L. monocytogenes* ($\Sigma R = 0$), following the input values in Table 2.3

**Table 2.4** The impact of shortening the shelf life of the product from 14 to 7 days, thus reducing the level of growth ($\Sigma I$) on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

| | $H_0$ | $\Sigma R$ | $\Sigma I$ | | Total[1] | |
|---|---|---|---|---|---|---|
| mean log | −2.5 | 1.4 | 1.9 | | −2 | $H_0-\Sigma R+\Sigma I$ |
| sd | 0.80 | 0.50 | 0.56 | | 1.10 | $sd=sqrt(sd_1^2+sd_2^2+sd_3^2)$ |
| | | | | P(>FSO) | 0.01% | |

[1] The level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

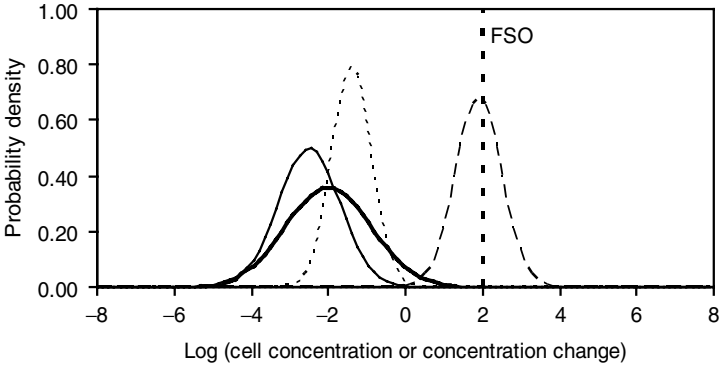


**Fig. 2.3** Probability distribution of the initial level ($H_0$ ___), reduction in concentration (-$\Sigma R$ – -), increase in concentration ($\Sigma I$– – –) and resulting final distribution of *L. monocytogenes* levels in packages of lettuce at the point of consumption (▬) for a product with a shortened shelf life (see Table 2.4)

tion is influenced by the largest contributors, which is $H_0$ in this example. Due to the ineffectiveness of the washing procedure, a higher proportion (3.5%) of packages do not meet the FSO (2 log CFU/g).

#### 2.4.2.4 Effect of Shortening the Shelf Life of the Packaged Lettuce

If the product contains pathogens and supports growth of the pathogen, the length of the shelf life can influence the impact on public health. In this example, the effect of a shorter shelf life on the proportion of packages of lettuce that do not meet the FSO is evaluated by reducing the predicted value for $\Sigma I$. If the product is stored for 7 days at 8°C, rather than 14 days, the increase in *L. monocytogenes* over 7 days is estimated to be 1.9 log CFU/g with a standard deviation of 0.56 (Szabo et al. 2003) (Table 2.4, Fig. 2.3). By decreasing the shelf life, which decreases the extent of growth of *L. monocytogenes*, the proportion of packages of lettuce that do not meet the FSO is decreased to 0.01% compared to 0.2%, over a 10-fold decrease in risk.

#### 2.4.2.5 Meeting the FSO by Changing Levels or Variability

The same proportion of products can meet an FSO, by reducing the variability of one of the inputs. For example, if the variability of the initial levels of *L. monocytogenes* on the raw materials is reduced from 0.8 to 0.4, the level of *L. monocytogenes* reduction required during the lettuce washing step ($\Sigma R$)

**Table 2.5** Effect of reducing variability of $H_0$ and lowering $\Sigma R$ during washing on the proportion of packages of fresh cut lettuce that do not meet the FSO (compare to Table 2.2)

| | $H_0$ | $\Sigma R$ | $\Sigma I$ | | Total[1] | |
|---|---|---|---|---|---|---|
| mean log | −2.5 | 0.7 | 2.7 | | −0.5 | $H_0-\Sigma R+\Sigma I$ |
| sd | 0.40 | 0.50 | 0.59 | | 0.87 | sd= sqrt($sd_1^2+sd_2^2+sd_3^2$) |
| | | | | P(>FSO) | 0.2% | |

[1] The level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

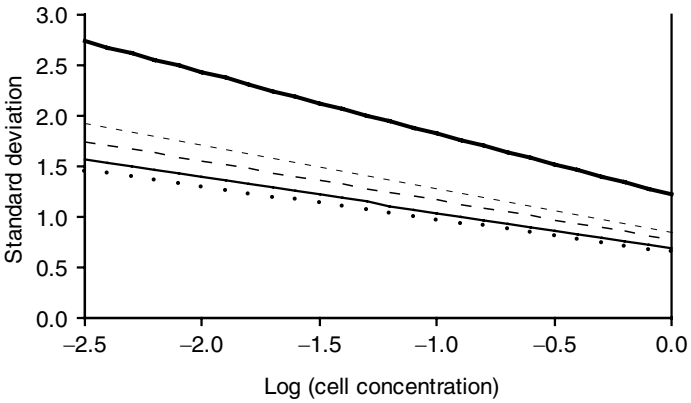


**Fig. 2.4** Various combinations of mean log cell levels and standard deviation of the combined distributions for $H_0$, $\Sigma R$ and $\Sigma I$ resulting in a particular proportion of product that does not meet the FSO=2 log CFU/g. Lines represent percent of products not meeting the FSO. Proportion not satisfying the criterion: 0.1% defective ( ), 0.2% defective (——), 0.5% defective (— —), 1.0% defective (- – -), 2.0% defective (▬▬)

could be decreased from 1.4 to 0.7 with the same proportion of product meeting the FSO (Table 2.5). While the practicality of reducing the standard deviation for a raw agricultural commodity such as lettuce may be difficult to achieve given control measures available at this time, this strategy may be applicable for other product types.

### *2.4.3 Log Mean Value, Standard Deviation and Meeting the FSO*

The proportion of products in which the level of the microorganism of concern is above the FSO or PO is determined by both the mean log levels and the standard deviation of the combined distributions for $H_0$, $\Sigma R$ and $\Sigma I$. Different combinations of the mean and standard deviation resulting in the same overall proportion of products not meeting the FSO can be calculated. The results are shown in Fig. 2.4.

The examples presented in this chapter illustrate the impact of both the mean log level and the variability of $H_0$, $\Sigma R$ and $\Sigma I$ on the proportion of product meeting the FSO. With this deeper level of understanding of the influence of both the levels and variability of the initial microbiological load on the incoming materials, the steps in the process that reduce the level of the microorganism of concern and the increase of the pathogen of concern during storage and distribution, a food manufacturer can determine where they can have the biggest impact on ensuring that the appropriate proportion of product meets the FSO. Control strategies can focus on decreasing variability of the process, decreasing the initial level of the microorganism of concern on the raw materials, or other parameters based on

the levels or variability observed for a particular situation. Calculations used for Fig. 2.4 are presented in Appendix B.

The following assumptions are made with these calculations:

- All variables are assumed log normally distributed, therefore the log of the variables as used in the FSO equation is normally distributed. This also makes their sum in the FSO equation have a normal distribution. If values have other distributions, Monte-Carlo type calculations are needed to determine the statistical distribution of the sum. While a normal distribution for log initial level, log increase and log reduction is often described in the literature, in real life the distribution of pathogens may be highly heterogeneous and not possible to describe by a log normal distribution.
- These examples assume that calculations hold even for very low levels. This may have further implications in some situations. For example, if a 6D inactivation step is applied to containers with a 100-g unit size and an initial concentration of 2 log CFU/g, the calculated level in each unit after inactivation is −4 log CFU/g. If each CFU contains only one microorganism, then this process would actually yield one microorganism in one 100 g unit (i.e., −2 log CFU/g) for every 100 units produced (1% of the units). The other 99% of the units would be free of the microorganism. For some microorganisms, a CFU may contain more than one cell, thus a greater percentage of units could theoretically contain a contaminant. This illustrates the importance of using these calculations as general principles to compare the relative effect of changes to a food safety management strategy rather than as absolute figures.
- If no data on standard deviation are available but minimum and maximum data are known, representing the range where 95% of the data will lie, the standard deviation can be estimated by sd = 0.5 × maximum-minimum)/1.96[2].

## 2.5 Validation of Cleaning and Other GHP Control Measures

Effective application of GHP provides the foundation upon which HACCP systems are developed and implemented. Failure to maintain and implement GHP can invalidate a HACCP system and result in the production of unsafe food.

Effective control of a hazard in a food necessitates consideration of the components of GHP likely to have significant impact in controlling the hazard. For example, incoming material requirements are very important to control the risks of certain hazards in seafood (e.g., paralytic shellfish poisoning, ciguatera toxin, scombroid poisoning). Incoming material requirements are of lesser importance for a food that will be cooked sufficiently to eliminate vegetative pathogens (e.g., salmonellae in raw meat or poultry) that may be present. Thus, the various components of GHP do not carry equal weight in all food operations. It is necessary to consider the hazards that are most likely to occur and then apply those GHP that will be effective for controlling the hazards. This does not mean that the other components of GHP, such as equipment maintenance or calibration, are ignored. Some are very important to ensure a food meets established safety and quality requirements.

In certain situations selected components of GHP may carry particular significance and should be incorporated into the HACCP plan. For example, equipment maintenance and calibration are important for large continuous ovens used in cooking meat products. In this example, the procedure and frequency (e. g., monthly, quarterly) for conducting checks on heat distribution during cooking could

[2] The minimum and maximum 95% limits are minimum = average − 1.96sd; maximum = average + 1.96sd. This results in maximum-minimum = 2 × 1.96sd, so sd = 0.5(maximum-minimum)/1.96.

be incorporated into the HACCP plan as a verification procedure. In addition, it is necessary to verify the accuracy of the thermometers used for monitoring oven temperatures during cooking.

Information on hygienic design of facilities and equipment, cleaning and disinfection, health and hygiene of personnel, and education and training was discussed previously (ICMSF 1988). Preventing contamination or recontamination of the product during processing is a critical component of a control program. Validation means that the facilities and equipment, choice of cleaners and sanitizers, and conduct of the operations are designed to achieve the necessary level of control. Initial considerations in designing the sanitation program include food characteristics, equipment construction and materials, and microorganisms of concern for safety and spoilage. Validation of the program ensures all parts of the system are properly treated to remove food soil and inactivate microorganisms. Residual food soil in wet environments not only provides a source of nutrients for subsequent microbial growth, but also can reduce the effectiveness of sanitation steps. Clean-in-place (CIP) systems require careful verification that all parts are treated and that the system operates as intended.

The effectiveness of many sanitizers is affected by the presence of organic residues from the food and processing environment. Scientific criteria needed to determine a sanitizer's immediate and residual effect include:

- Concentration of the sanitizer and conditions for efficacy (e.g., temperature).
- Immediate and long term antimicrobial effectiveness (stability of the sanitizer).
- Microorganism susceptibility to the sanitizer.
- Characteristics of the surfaces to be sanitized (temperature, organic load).
- Impact of processing steps (thermal treatments, packaging conditions).

As with validation of other components of the food process, validation of the sanitation program is the accumulation of knowledge from laboratory, pilot plant and commercial facility studies. Sufficient information of increasing specificity needs to be acquired to ensure the functioning of the process will be understood. In laboratory studies, pathogens can be inoculated into media or product. Specialized pilot plant studies might use pathogens if exposure to food and humans can be controlled; however, GMP plants must use surrogates. In commercial facilities, data is acquired using surrogates when pathogen presence is a rare event, or from monitoring when naturally-occurring pathogens are present in sufficient frequencies and numbers (e.g., in slaughter operations). Appropriate pathogen strains or surrogates must be used. Chemical agents must be tested according to directions using potable water of appropriate hardness, concentration, pH, temperature and contact time. Variations in the food and process must be considered, the critical factors that determine the margin of safety identified and the minimum lethal treatment specified to be assured that appropriate control will always be achieved. Periodic verification is necessary to ensure that efficacy is not lost over time (e.g., due to development of resistance).

## 2.6 Shelf Life Determination

One approach to management of the safety of the food is to have the food spoil and be rejected by the consumer for poor quality before pathogens that might be present grow to levels that become a public health threat. In the absence of spoilage, other means of limiting shelf life such as use-by labeling or time–temperature indicators could be employed. These issues are discussed below and in more detail in NACMCF (2005).

Distribution and storage conditions may include moderate time and temperature abuse. Process design and validation should include these conditions when validating that the products meet the FSO. Decisions about the temperature abuse can be based in part on retail and home storage temperatures survey databases from e.g., EcoSure (2008) where retail display temperatures varied by product type (5% of home refrigerators exceeded 7.2°C and 0.7% exceeded 10°C). For some products and

regions, a shelf life short enough to cope with the growth at abusive temperatures may result in times that do not permit normal commercial handling or meet consumer's expectations. Specifying the maximum storage temperatures is a public health risk management decision.

Shelf life validation would include determining the distribution of contamination at the end of processing and establishing a PO at that point. The allowable amount of growth that potentially could occur for the food to still meet the FSO can then be determined. With specification of the maximum abuse temperature, laboratory and challenge testing can determine the length of time for repair/lag and growth before exceeding the FSO as explained in previous examples.

For foods that are continually refrigerated from manufacture to consumption, the use-by date can be estimated by the manufacturer. Times for commercial and retail periods and home storage are included in the determination and a calendar date can be applied by the manufacturer. If a food is frozen and then thawed at retail, the growth time is the remaining retail and home storage time. For this product, a label indicating the number of days after purchase is appropriate.

Time temperature integrators (TTI) for retail packages produce a noticeable color change at the end of the allowable storage based on a biological, physical or chemical reaction. The kinetics of the reaction varies among devices and end points may be set for specific time/temperature standards, for quality concerns or theoretically for growth in a specific food-pathogen combination. TTIs are not widely used on consumer packages in 2010 because high cost, complexity of reaction kinetics for different food/microorganism combinations, and lack of consumer awareness and understanding have limited their use. TTIs have a potential benefit of indicating the end of the permissible shelf life because the ongoing reaction rate is continuously affected by the temperature. If the temperature is below the designated optimum, the rate is correspondingly slowed and the time before the indicating color change is lengthened. If the temperature exceeds the designated optimum, the TTI reaction rate appropriately shortens the storage time. Future developments may make it possible to choose a TTI that continuously monitors the temperature during the entire storage period and provides an end point specific to the conditions that a specific individual package experiences.

## 2.7 When to Revalidate

Validation data should be periodically reviewed to determine whether new scientific data or changes in operating conditions would alter the previous validation conclusions. Emergence of a new pathogen requires re-evaluation of the process based on the characteristics of the pathogen. A change in the initial contamination of the ingredients, the formulation of the product, processing parameters or the storage conditions of a food may require the process be revalidated. The impact of specific changes on the concentration, homogeneity or frequency of contamination for the affected step should be estimated. This information may be obtained from the literature, models, and laboratory or pilot plant experiments. The magnitude of the change can be compared to the corresponding mean log and standard deviation of the validated process. If the change is within the values of the original validation, there may be no need for further validation. The final impact of the change at the point of consumption can be estimated and compared to the FSO. For example, a 0.2 log increase in the contamination of an ingredient may increase the contamination by 0.2 log for all subsequent steps to consumption. If this increase does not result in exceeding the FSO, further validation is not needed. However, if the change in the process was an increase in pH that permitted a 1 log increase in pathogen concentration at consumption, this process would likely require revalidation. It would perhaps require redesign of the process to compensate elsewhere for the increased growth and revalidation of the new process.

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# Chapter 3
# Verification of Process Control

## 3.1 Introduction

Many food microbiologists are familiar with sampling plans that use microbiological data to make decisions regarding the quality or safety of a specific lot of food. Ideally, the statistical basis for this type of testing is that analyses are performed on a sufficient number of samples from a single lot such that there is a high degree of confidence that the lot does not have an unacceptable level of microorganisms that affect the quality or suitability of the food.

An important concept in understanding the statistical basis for such *lot-by-lot* or *within-lot* testing is that of defect rates, i.e., the portion of servings or containers that do not satisfy some attribute, such as absence in a defined quantity of product, or below a specified concentration (ICMSF 2002). Such sampling programs become increasingly more resource intensive as the acceptable defect rate becomes smaller. Once a standard method with the appropriate sensitivity has been selected for analyzing samples, achieving the desired test stringency as the defect rate decreases is typically accomplished by analyzing more samples from the lot or by increasing the size of the analytical units examined. When the acceptable defect rate is low (e.g., <5%), the number of samples that need to be analyzed can be a severe practical impediment to using microbiological testing. For example, consider two lots of ready-to-eat food that are required to be free of *Salmonella*, one with 50% of the servings contaminated and a second where 1% of the servings are defective. In the first lot, examining three servings would have a high probability (87.5%) of identifying the lot as contaminated, whereas the probability of identifying the second lot as containing *Salmonella* would only be 63% if 100 servings were examined.

Another important concept associated with within-lot testing is the underlying assumption that there is little or no knowledge about the product and the processes and conditions under which it was manufactured and distributed. In such instances, microbiological testing is used as a control measure to segregate sound and unsound lots. An important consequence of this assumption is that since no prior knowledge of the lot is assumed, the results from testing one lot cannot be considered predictive of the status of other lots.

While within-lot testing plays an important role in food safety particularly for examination of foods at ports of entry for regulatory actions, typically microbiological data collected is not based on traditional within-lot sampling plans and statistics. Instead, sampling is often conducted periodically and on only a portion of the lots. Furthermore, the extent of testing (i.e., number and size of samples analyzed) is typically at a level that it does not provide a high level of confidence that a lot contaminated at a low rate would be detected. This is not to imply that this type of testing does not provide manufacturers or control authorities with important microbiological data; however, too often such testing programs are conducted in a manner that does not provide the best use of the data acquired.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_3,

These testing programs are referred to as *process control* testing or *between-lot testing*, and their usefulness can be enhanced significantly if they are appropriately designed, including appropriate analysis, interpretation and review of the data. When this is done testing programs provide a powerful tool for evaluating and correcting the systems used to control microbiological safety and quality before the system crosses the threshold where the product is no longer suitable for commerce. This chapter provides a brief introduction to the concepts and application of this type of microbiological data acquisition. Detailed requirements for establishing such a testing program are found in other standard references (Does et al. 1996; Roes et al. 1999; ICMSF 2002; Hubbard 2003; NAS US National Academy of Sciences 2003; ECF 2004; NIST/SEMATECH 2006).

Understanding the differences in the goals and assumptions associated with within-lot and between-lot testing is important for successful process control testing. Within-lot testing is used to establish the safety or quality of a specific lot of product, presumably because of a lack of knowledge about the effectiveness of the means for controlling contamination and ensuring safe production, processing and marketing. The purpose of between-lot testing is not to establish the safety of a specific lot; rather safety is assumed to have been achieved by establishing and validating processes and practices that control significant hazards including the variability of ingredients, processes and products. The purpose of between-lot testing is to verify that the process and practices for ensuring safety are still performing as intended. The underlying assumption in this case is that there is detailed knowledge of how the food was manufactured. Thus, process control sampling is most effectively implemented as part of an overall food safety risk management program such as HACCP (ICMSF 1988). To reiterate the different applications of within-lot and between-lot testing – if the testing of *all* lots using within-lot sampling plans was implemented in a HACCP program, that sampling would be both a control measure (that would likely be a critical control point) and part of monitoring activities. Conversely, between-lot testing would be used as part of the verification phase of HACCP. Thus, failure to meet a within-lot sampling plan would indicate a potentially unacceptable lot whereas failure of a between-lot sampling plan would signal a potential loss of control of a HACCP program.

As indicated above, the purpose of process control testing is to determine whether a control system is functioning as designed; i.e., producing servings that have a defect rate below a specified value or within a specified range. An inherent assumption made in conducting between-lot microbiological testing is that actions have been taken to reduce the variability among lots so that the variability between lots is minimized or that the system is consistently operating at a level of control such that the products are substantially better than the specified acceptable level. It is questionable whether a HACCP program could be truly considered under control if there is a large between-lot variation. Thus, between-lot testing is most effective when there is little variation in the mean and standard deviation of the log concentrations of a hazard among lots under normal operation. A small between-lot variance allows a loss of control of the food safety or quality system to be more readily identified with the least amount of microbiological sample analysis.

As a simple example of the difference between within-lot and between-lot sampling, consider a company that has two processing lines, one old and less reliable, and one new and highly reliable, for the same product. The company wants to ensure a defect rate of <1% of that product from either line. For product from the old line, where there is less confidence in the reliability of the process, the company may opt to test each lot. In this case, end product testing is used as a critical control point. Given that the within lot variability of product from the old line is higher, the manufacturer might even choose to use a sampling plan that involves a greater number of samples so as to have more confidence that the results of the sampling plan are representative of the entire lot. Conversely, for the new line, the company could apply the same sampling plan but draw the samples from a greater number of lots; i.e., effectively considering the process as a continuous lot, or a series of large lots, with the lot being defined by a period of time and *lots* overlapping in time. This is the basis of the *moving window* approach, exemplified in Sect. 3.4. In the moving window approach,

an increase in the number of positive results over time indicates a trend toward loss of control. In this case the same sampling plan is used to verify the process.

Appropriate statistical analysis can identify when the incidence of defective units significantly exceeds the tolerable defect rate. If the incidence exceeds that level, the manufacturer should investigate the cause of the elevated defect rate to determine why the process is no longer functioning as intended and should take corrective action. Examination of the system's performance over time also provides useful information and insights into the type of failures that occur (ICMSF 2002). Process control testing is most effective when it can detect an issue at a level or frequency below that which would be considered unacceptable for safety or quality if it were to enter the marketplace. In this way corrective actions can be taken before a critical limit is exceeded.

## 3.2 How to Verify that a Process is Under Control

The actual microbiological methods used to detect, identify and enumerate microorganisms of concern for process control verification are essentially the same as those used for within-lot testing. These methods are available in a variety of standard references (e.g., ISO, AOAC, FDA Bacteriological Analytical Manual, American Public Health Association etc.) and are not discussed further.

Like within-lot testing, microbiological criteria established for a process control testing program can be based on either 2 or 3 class attributes testing plans; i.e., presence/absence or a numerical limit (or limits in the case of three class plans) or variables testing (i.e., full range of quantitative data). Similarly, attribute testing can be based on a 2-class or 3-class sampling plan. Process control sampling plans can be applied to finished products, in-process samples or ingredients. Ideally a decision on the analytical approach used is reached early in the development of the process control sampling program. The approach selected strongly influences the types of data needed during the initial phases of establishing the program. A decision on the approach used should be determined before establishing the microbiological criteria (i.e., decision criteria) for the program.

### *3.2.1 Information Required to Establish a Process Control Testing Program*

As indicated above, use of process control testing is based on detailed knowledge of the product and process. A meaningful process control testing program requires detailed knowledge of the levels or frequency at which the microorganism of concern can be expected in a product when it is produced and handled properly. This includes information on the variation in those levels both between lots and within lots. Thus, the first step in establishing a process control testing program to verify continued successful operation of food safety or quality system is to gather baseline data on the performance of the food safety system when it is functioning as intended. This is commonly referred to as a process capability study. During this period, intensive acquisition of data that characterizes the performance of the system is undertaken, either by generating new data from tests on the system or by collating existing data. The data collected are specific to the system being evaluated. This can be as specific as the performance of a single line within a manufacturing plant or as broad as a commodity class for an industry. However, the latter requires a great deal of forethought and effort to ensure that the acquisition of data is not biased and adequately represents an entire industry. On a national basis, this is typically done through a series of national baseline studies; a major undertaking that is typically done by a national government or industry representative body. The sensitivity of the methods and sampling plans selected should be adequate to provide sufficient data on the true incidence of defects within a lot as well as prevalence (the average rate of defects over time) of the microbiological hazard in the food. Ideally the sensitivity will be set at a level

that is sufficient to detect the pathogen or quality defect at least a portion of the time. Historical within-lot testing results can be highly useful for determining the system's performance and variability.

When conducting a process capability study, care must be taken to ensure that the data collected represent product manufactured when the food safety system is under control. If not, it is likely to increase the variability of the levels (or frequencies) of the microbiological hazard that will form the basis of the reference level against which ongoing performance will be assessed. This could decrease the ability of the process control program to identify when the system is not functioning as intended. The duration of a process capability study will vary with product, pathogen and purpose, but it should be long enough to generate sufficient data to ensure that the variability in the process has been characterized accurately. At a minimum, 30 lots should be examined so that the influence of sampling error is acceptably small and that the performance characterization is reasonably robust. There are instances where the process control study may need to be conducted for longer periods or in phases. For example, if raw ingredient contamination varies substantially over the course of a year, then the process capability study may need to consider seasonality as a factor, thereby extending the duration of the study for a full year. In such instances, it is possible to conduct the process capability study for 30 days, perform initial analyses and set initial control limits; and then review and revise the analysis and control limits, if necessary, as additional data are accumulated. The inclusion of such data in the process control study depends, in part, on a value judgment related to whether the product is deemed under control during those periods when high levels are observed due to season or supplier. If the process is not deemed as being under control, then the data derived from it should not be included in the reference level data set. It also implies that means for preventing the increased defect rates associated with seasonality or supplier will need to be immediately identified since, once implemented, the process control testing program based on the process control study that does not include the period higher defect rate will appropriately identify the process as being out of control during those periods.

As indicated above, process control testing programs are most effective when they detect loss of control *before* a critical limit is exceeded. For that reason, the microbiological limits for process control testing programs employed by companies are frequently established to effectively detect changes before a regulatory limit is exceeded. This allows corrective actions to be taken proactively. However, this proactive approach can be difficult to implement if competent authorities establish limits based on "zero tolerance" instead of specifying a specific microbiological criterion based on risk or on specific testing protocols.

Process control testing can be used for assessing both food safety and food quality, and is not restricted to microbiological testing. Simple, easily performed physical and/or chemical measures of the impact of microbial contamination can offer distinct advantages over more sophisticated testing methods. For example, sterility testing of UHT milk products is amenable to process control testing based on sensory evaluation combined with a pH determination (von Bockelmann 1989).

### *3.2.2 Setting Microbiological Criteria, Limits and Sampling Plans*

The concentration of microorganisms varies in lots of food and is often described by a log normal distribution. Such distributions are open-ended functions and high values can potentially occur even when the system is in control. However, such events should be rare and a high frequency of such occurrences is evidence that the system is no longer under control. A microbiological criterion establishes the decision criterion to assess whether a microbiological testing result could have occurred by chance alone or whether the food safety or quality system has undergone some significant change such that it is no longer functioning as intended.

The microbiological limit associated with a process that is under control effectively establishes that decision criterion, based on the results of the initial process capability study. Assuming that the current level of control within a plant or an industry is deemed acceptable, a limit can be established in combination with an appropriate sampling plan so that the frequency of detecting a positive result or a specific concentration would be unlikely to occur by chance alone. For example, a result that exceeds the 95% probability value would only be expected to occur, on average, about once in 20 samples. If the frequency were higher, it would be indicative that the system is out of control. An increase in the number and size of analytical units examined increases the likelihood of detecting a positive result so that the decision criteria are specific to the microbiological criterion and sampling plan established. Establishing the stringency of a microbiological criterion is a risk management activity. Thus, the specific sampling plan thresholds selected (e.g., 95 or 99% confidence) may take into account a range of scientific and other parameters such as assessed risk, severity of the hazard, technological capability, public health goals, cost of taking action when the process is actually in control, or consumer preferences and expectations. Because this is a risk management issue and not a risk assessment, no specific value of probability of detection serves as a standard criterion. For example, consider two situations that a country or company might assess in establishing a microbiological limit for a food product. First, consider a product where the industry's food safety or quality systems is based on a single, well established technology that is operating with a substantial safety margin to control a relatively mild hazard and has both a low between-lot and within-lot variance. In that instance a microbiological limit based on 99.99% of the baseline distribution (i.e., ≤0.001% of the test values from the program operating as intended would exceed the microbiological limit) might be deemed sufficient to protect public health and the microbiological criterion would be established accordingly. In such a situation, the microbiological limit established would result in the appropriate acceptance of the vast majority of this product. Such a process control standard would have little impact on the industry's current performance. In contrast, consider an industry where there is substantial variability among the technologies, practices and standards of care used by individual companies, leading to substantial between-lot (and in some instances within-lot) variability. In this case, the country or company might establish a microbiological limit at 80% of the current baseline distribution (i.e., 1 in 5 of samples as currently produced would be deemed unacceptable). Over time a process control microbiological limit of such a magnitude would be likely have a large impact on the companies that are poorer performers; i.e., their food systems would be considered as not functioning as intended. Conversely, the limit would have minimal impact on companies that are good performers. The end result would be to decrease both the mean and variance of the log concentration of the hazard in servings of the product entering commerce. A similar outcome would occur over time if the stringency of a within-lot testing program was increased.

## 3.3 Routine Data Collection and Review

Once established, process control testing requires routine testing of only a small number of samples. The number of lots that need to be tested, the frequency of testing and the number of samples from each lot depends on the inherent defect rate when the food safety or quality system is functioning as intended and the degree of confidence that the microbiological limit is not being exceeded by the manufacturer or country. The specific testing requirements of the process control sampling plan depend on the type of process control analysis approach being employed (e.g., CUSUM, Moving Window) (ICMSF 2002). Process control testing programs can also include variations in testing frequency based on process performance; e.g., to increase testing when increased defects are detected or to decrease the frequency of testing when results are consistently acceptable over time. However, rules for variable sampling frequencies should be formulated with a clear understanding of the effect

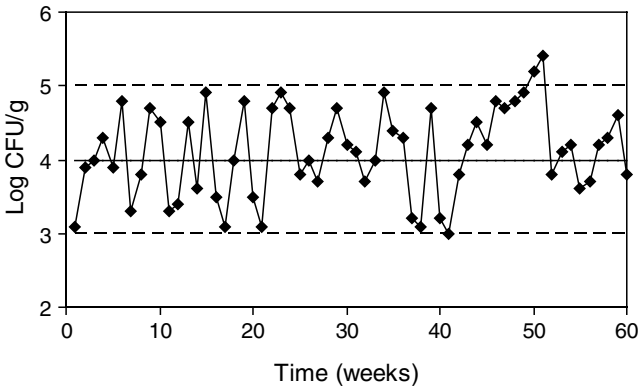


**Fig. 3.1** Hypothetical control chart for a microbial indicator assay conducted weekly. The center *horizontal line* (—) represents the hypothetical microbiological criterion and the two *flanking lines* (– –) represent 95% upper and lower confidence limits

that the alternate sampling frequencies have on the ability of the testing program to detect an emerging loss of process control and to be able to respond in time to prevent unacceptable product from entering commerce.

Implementation of process control testing programs requires effective data management systems and the ongoing evaluation of collected data over time. This is usually done through control charting where the data are arrayed over time (Fig. 3.1). Graphical representation is often a useful tool as an initial evaluation of the data. Comparing these data with the data collected in the routine monitoring of critical control points in HACCP plans and other verification data can be useful for interpreting the results of the process control testing and enhancing the identification of the underlying causes of process deviations For most food microbiology concerns, the lower limit would not typically be considered a decision criterion, with the possible exception of fermented foods or probiotic-containing foods; however, the lower limit may reflect the limit of detection of the test. In the hypothetical example in Fig. 3.1, a loss of control is apparent at weeks 50 and 51 that should have elicited investigation to restore control. Additionally, a general increasing trend began at week 42 and became apparent by week 46–47. This could have stimulated corrective action investigations even before a loss of control occurred.

## 3.4 Competent Authority Process Control Program Examples

The use of process control testing for regulatory verification of food safety programs began in the 1990s as competent authorities began to incorporate HACCP into their regulatory programs. The use of process control analysis techniques provided them with a statistically sound means of establishing microbiological testing as a HACCP verification tool, while minimizing the economic impact of testing on both business operators and the competent authority. While the techniques are increasingly being used by industry and governments, the greatest adoption of this approach has been in North America. Examples of early use of this approach follow.

### *3.4.1 Meat and Poultry*

One of the first uses of process control programs by competent authorities was in the *Pathogen Reduction/Hazard Analysis and Critical Control Point (HACCP) Systems rule* (USDA 1996). This regulation established two microbiological criteria as a means of verifying HACCP plans for meat and poultry products:

1. Testing for *Escherichia coli* as an indicator of fecal contamination and adequate chilling performed by individual business operators.
2. *Salmonella enterica* testing performed by USDA Food Safety and Inspection Service (FSIS).

The microbiological limits established by FSIS were based on extensive review of baseline studies, regulatory testing and industry data for various classes of meat and poultry products (USDA 1995). Built into these standards was a goal of decreasing the incidence of foodborne disease attributable to meat and poultry. The program employed a between-lot moving window approach (i.e., as each new test result is obtained the window moves and the oldest result are discarded), where the results of single samples taken on individual production days are examined over the course of a specified number of days. The frequency of positive samples over that moving time frame is then related to the defect rate that is expected for the specific meat or poultry product. The testing required of manufacturers; i.e., the presence of biotype I *E. coli* as an indicator of fecal contamination, is based on a 3-class attribute sampling plan. The testing by FSIS for *S. enterica* is based on a 2-class plan in conjunction with samples taken periodically by regulatory personnel over a specified number of days. Failure to meet the microbiological limit is considered indicative that the probability that the facility is not achieving the level of control required was >99% (USDA 1996). The *Salmonella* performance standards are not lot acceptance/rejection standards. The detection of *Salmonella* in a specific lot of carcasses or ground product does not, by itself, result in condemnation of the lot. Instead, the standards are intended to ensure that each establishment is consistently achieving an acceptable level of performance with regard to controlling and reducing enteric pathogens on raw meat and poultry products (USDA 1996).

The FSIS regulation and requirements are intended to evolve to address new risks and availability of new data. Development of process control microbiological criteria is being considered by other national governments and intergovernmental organizations. For example, the EU has established process control-based hygiene criteria for controlling *Salmonella* in raw poultry (EFSA 2010), and the Codex Committee on Food Hygiene is considering a process control approach.

### *3.4.2 Juice*

A more limited use of microbiological testing for process control is employed in the US FDA's Hazard *Analysis and Critical Control Point (HACCP); Procedures for the Safe and Sanitary Processing and Importing of Juice; Final Rule* (FDA 2001). In this example the competent authority was concerned about the underlying scientific assumption that enteric pathogens would not become internalized in citrus fruit. The regulation has an exemption for citrus fruit juice producers enabling them to fulfill the required 5-D pathogen reduction by treating the surface of the fruit prior to the juice being expressed. This exemption was based on data that suggest enteric bacteria are limited to the surface of the fruit. This prompted a requirement that manufacturers choosing to use only surface treatments must analyze a 20-mL sample for every 1,000 gallons (~4,000 L) produced per day for generic *E. coli*, using a moving window analysis based on a 7-day window, where two positive samples in a 7-day window are deemed to indicate the process is no longer in control. This requires the manufacturer to investigate the cause of the deviation and divert juice to pasteurization after the juice is expressed. Based on extensive baseline studies of commercial juice operations indicating the range of initial contamination levels, juice that is successfully treated to achieve a 5-D reduction (99.999%) is likely to have <0.5% probability of having two positives in a 7-day window after 20 samples. Conversely, a reduction that yields only 3-D inactivation is calculated to result in a 34% frequency of 2 positive *E. coli* findings within the 7-day window with 20 samples, which would detect the process failure (Garthright et al. 2000; FDA 2001).

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# Chapter 4
# Verification of Environmental Control

## 4.1 Introduction

The microbiological safety of industrially manufactured foods is based on the effective design and implementation of Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP).

Published case studies demonstrate the impact of postprocess contamination (ICMSF 2002). Even when strict control at all CCPs ensures destruction or reduction of pathogens to acceptable levels during processing, foods may become contaminated during subsequent processing and handling. Such contamination typically results from two general circumstances:

1. Addition of contaminated ingredients after the kill step
2. Contamination from the processing environment

The basic elements of GHP are described in the Codex Alimentarius Commissions document "General Principles of Food Hygiene" (Codex Alimentarius 1997). These general principles are supported by numerous product-specific guidelines issued by Codex Alimentarius or organizations. These elements of GHP are defined to minimize or prevent introduction of a pathogen to a product during its manufacture. This is achieved through the implementation of combined measures and multiple protective barriers, which can be described as follows:

1. Prevention of entry of pathogens into areas close to the processing lines.
2. In the event of entry, prevention of establishment in the premises.
3. In the event of establishment, prevention or limitation of microbial multiplication, which would favor persistence and dissemination throughout the plant.
4. In the event of presence, implementation of corrective actions to ensure control of microbial concerns at low levels or eradication where feasible.

## 4.2 Establishing an Environmental Control Program

Elements that contribute to postprocess contamination and measures to control pathogens in food processing environments are extensively discussed and illustrated in ICMSF (2002) and GMA (2009) for *Salmonella* in low moisture food. Testing of in-process and processing environment samples demonstrates that the GHP measures implemented are effective in achieving the desired prevention of contamination. The test results can be used to (1) assess the risk of product contamination, (2) establish

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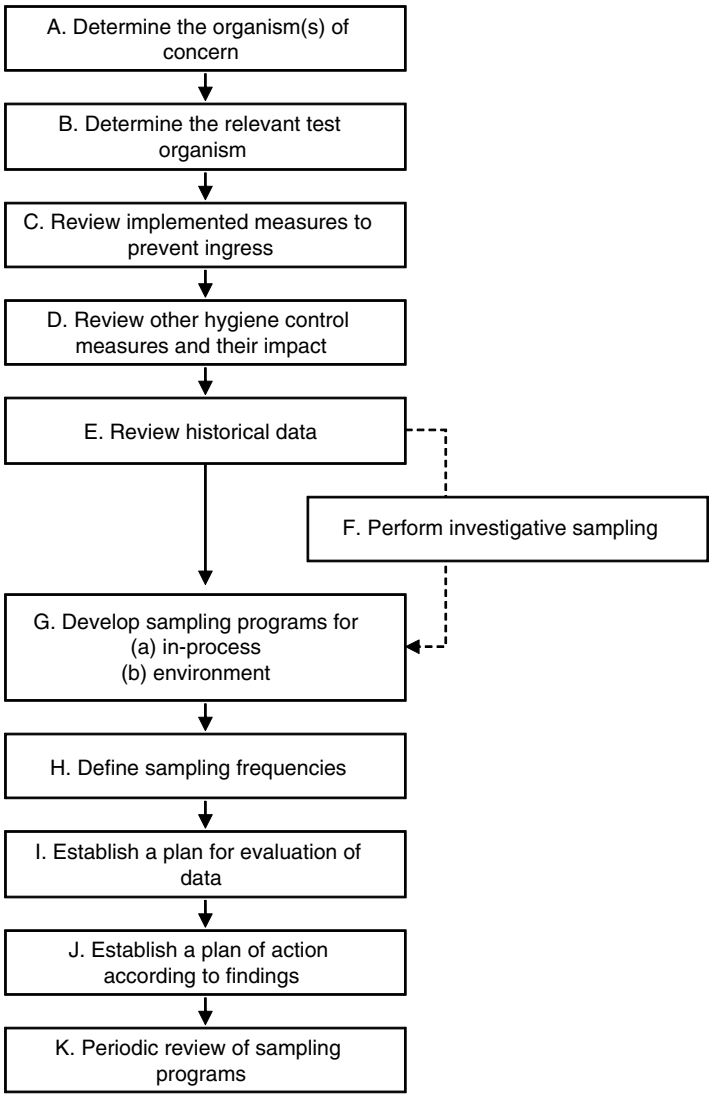


**Fig. 4.1** Proposed approach for establishing an environmental sampling program

a baseline for when the facility is considered under control, (3) assess whether control is maintained over time and (4) investigate sources of contamination in order to apply appropriate corrective actions.

While sampling plans applied to verify environmental control are typically not based on statistical considerations, it is important to consider evaluating results using appropriate statistical tools such as trend analyses. These elements are discussed in detail in ICMSF (2002) and an approach for establishing a testing program is illustrated in Fig. 4.1. This approach can be applied for control of pathogens, hygiene indicators or spoilage organisms.

### *4.2.1 Step A: Determine the Microorganisms of Concern*

Determine the relevant microorganism for the manufacturing process based on a HACCP study, guidance provided in this book or ICMSF (2005). In many cases, a program is established for a single

pathogen; however, it may be done for more than one microorganism if it is deemed necessary for the product under consideration.

### 4.2.2 Step B: Determine the Relevant Test Microorganism

Determine if testing should involve an indicator or the organism of concern. Examples of indicators include Enterobacteriaceae for *Salmonella* or *Cronobacter* spp. and *Listeria* spp. for *L. monocytogenes.* In most of the cases to obtain a full picture of the status, testing for the both the indicator and the pathogen is necessary albeit number of sampling points and frequencies may be different.

### 4.2.3 Step C: Review Measures to Prevent Ingress

Review the existing preventive measures such as zoning within the premises, the layout of different processing lines, interfaces between different parts of the factory, elements such as flow of personnel, equipment and goods (e.g., raw materials, packaging materials, finished products, containers, fork-lift trucks, pallets, waste, rework etc.), as well as the flow of air and water. This is best done using a master plan and having detailed discussions on parameters affecting the preventive measures to avoid the ingress of pathogens in specific areas of the factory, in particular high hygiene areas as described in ICMSF (2002, Chap. 11).

### 4.2.4 Step D: Review Other Hygiene Control Measures and Their Impact

Review other factors that may contribute to the establishment or dissemination of the microbiological concern in the processing areas. This includes reviewing the layout of processing lines, the type of equipment including hygienic design and interfaces with the environment, cleaning procedures used for the environment and equipment (e.g., wet versus dry), cleaning schedules etc. Based on the design of the processing lines, equipment and processing conditions, determine whether the build up of product residues on food contact surfaces may also lead to microbial growth – e.g., at points where condensation is more likely to occur or growth temperatures may be experienced for extended periods of time.

### 4.2.5 Step E: Review Historical Data

Determine whether historical data on environmental sampling and testing of pathogens or indicator microorganisms exist and if the data still apply to the current environment. For example, if construction events occurred after data were collected, investigative sampling may be appropriate.

### 4.2.6 Step F: Perform Investigative Sampling

If no historical data exist, investigative sampling is recommended to establish a base line that can be used for the development of the sampling program. It may be useful to initially focus this investigative sampling on indicator microorganisms (e.g., aerobic colony counts, Enterobacteriaceae) to evaluate trends that can be used to establish sampling times during production and frequencies for sampling.

### 4.2.7 Step G: Develop Sampling Programs

With historical or investigative sampling data available and considering critical ingredients that may impact the quality and safety of the finished product, an environmental sampling and testing program can be developed. The terminology used to describe in-process and environmental samples may vary depending on the manufacturer. The following definitions have been used in this book.

- *In-process samples*: These samples provide a representative sampling for an entire line and sometimes represent the "worst case." In-process samples include:
  - Intermediate product collected from different process steps that would end up in a container as finished product, such as samples of sauces that would top a pizza or grab samples from a depositor.
  - Samples from equipment or product contact surfaces that could lead to a contamination of product such as process wash water, sifter tailings, fines, line residues or scrapings.
- *Processing environment samples*: The most common method of sampling for the processing environment is with sponges or swabs but it is important to adapt sampling tools to the situation. If air sampling is performed then air collector devices are preferred. These are used to verify that the environment is under control, i.e., free of pathogens or the indicator microorganisms of choice do not exceed target levels. Samples from food contact surfaces taken prior to production and after wet cleaning as part of the preoperational inspection are included in this category.

The sampling sites for both in-process and environmental testing should be based on a thorough knowledge of the premises, processing lines and equipment and the outcome of the HACCP study. Guidance on the relative importance of such sampling programs is provided in individual chapters of this book. Practical details on sampling tools, sampling techniques, routine and investigative samples are provided in ICMSF (2002).

### 4.2.8 Step H: Define Sampling Frequencies

After establishing the sampling plans it is important to determine the sampling frequency. The frequency may vary depending on the type of product manufactured and the duration of production runs. For example, daily sampling may be appropriate for sensitive products such as infant formulae, while weekly or monthly sampling may be appropriate for other product categories. Rotation between different sampling points in the same area may also be appropriate because conditions in manufacturing facilities can change.

It is also important to determine whether the sampling frequencies for indicators and pathogens should differ. Testing for Enterobacteriaceae, for example, provides results within 1–2 days and may therefore be used as a management tool with a higher frequency than *Salmonella* in some facilities.

### 4.2.9 Step I: Establish a Plan for Data Evaluation

To maximize the benefit of an environmental sampling program, it is very important to analyze the data generated in the most effective and proactive way. Different options such as statistical trend analyses, mapping or charting of data and findings etc. exist. The most familiar and convenient method for the establishment should be used. It is important to review the data in a timely manner to allow for corrective action, if necessary.

### 4.2.10 Step J: Establish a Plan of Action to Respond to Findings

When results deviate from standards, guidelines or specifications (e.g., the presence of *Salmonella* in a sample or levels of indicators exceed established internal limits), it is important to take appropriate actions. This is best done according to a preestablished action plan that is "activated" only when a deviation is detected.

Depending on the findings, the action plan may consider the following options: (1) thorough investigational sampling to identify root causes of the deviation and source(s) of the pathogen or indicator, (2) increased sampling frequency over a certain period to demonstrate that control is reestablished, (3) adjustment of the sampling regime for end products; e.g., change from verification to acceptance.

### 4.2.11 Step K: Periodic Review of Sampling Programs

A periodical review (e.g., once per year or when important changes occur) of sampling programs should be performed. This review should consider changes in premises, layout and type of equipment. Historical results should also be considered to optimize sampling plan. For example, sampling points that have not proven to be very useful might be eliminated and new sampling points might be added in areas where more issues have been detected. Changes in sampling frequencies may also be made during such reviews.

Such reviews should be combined with a review of the skills and training level of personnel involved in sampling, as well as a review of the adequacy of sampling tools and techniques.

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# Chapter 5
# Corrective Actions to Reestablish Control

## 5.1 Introduction

The primary goal of a food safety system is to prevent, eliminate or reduce hazards to the extent feasible by existing technology. Food safety systems are based on knowledge of the potential hazards that can occur in food operations, through the process of hazard analysis. Control measures are then selected and applied to ensure the food will comply with requirements established by the manufacturer, customers and control authorities. It is in the interest of manufacturers to produce foods that consumers can rely upon as being safe.

Many countries require food safety systems that incorporate the principles of Good Hygiene Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) programs (Codex Alimentarius 1997a, b). Evidence may reveal that the food operation is not or has not been in control and that corrective action is needed. This evidence may be from an on-site inspection, monitoring GHP, monitoring or verifying a critical control point (CCP), sample analysis, consumer complaints or epidemiologic information implicating the food operation.

In the context of HACCP, corrective action is "any action to be taken when the results of monitoring at the CCP indicate a loss of control" (Codex Alimentarius 1997a). Furthermore, principle 5 of the Codex document on HACCP states:

> Specific corrective actions must be developed for each CCP in the HACCP system in order to deal with deviations when they occur. The actions must ensure that the CCP has been brought under control. Actions taken must also include proper disposition of the affected product. Deviation and product disposition procedures must be documented in the HACCP record keeping.

In this chapter the focus is on microbiological hazards and corrective actions for deficiencies in GHP and from the marketplace are also considered.

## 5.2 Good Hygiene Practices

GHP can be viewed as the basic hygienic conditions and practices that must be maintained to produce safe foods. Effective application of GHP provides the foundation upon which a HACCP plan can be developed and implemented. Collectively, GHP and the HACCP plan constitute the food safety system for a food operation. Failure to maintain and implement effective pathogen controls

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through implementation of GHP can result in production of unsafe food and invalidate the HACCP plan. Spoilage and quality defects may also be more prevalent when GHP is not effectively applied.

The General Principles of Food Hygiene (Codex Alimentarius 1997b) describe the major components of GHP as:

- Design and facilities (location, premises and rooms, equipment facilities)
- Control of operation (control of food hazards, key aspects of food hygiene control, incoming material requirements, packaging, water, management and supervision, documentation and records)
- Maintenance and cleaning (maintenance and cleaning, cleaning programs, pest control systems, waste management, monitoring effectiveness)
- Personal hygiene (health status, illness and injuries, personal cleanliness and behavior, visitors)
- Transportation (general, requirements, use and maintenance)
- Product information and consumer awareness (lot identification, product information, labeling, consumer education, handling/storage instructions)
- Training (awareness and responsibilities, training programs, instruction and supervision, refresher training)

The components of GHP do not carry equal weight for pathogen control. It is necessary to consider the microbial hazards that are most likely to occur in each facility and identify those elements of GHP that contribute most to controlling the pathogens and spoilage microorganisms of concern. Certain elements of GHP may require modification from traditional practice to increase their effectiveness for controlling a specific pathogen. The principles of GHP are intended to provide a certain level of control for a wide variety of microbiological quality and safety concerns. Application of HACCP is targeted towards specific microbial hazards which, if not controlled, can lead to foodborne disease.

The result of verification activities may also indicate a deviation occurred in the implementation or application of GHP requiring the application of corrective actions.

## 5.3 HACCP

HACCP plans are developed following a stepwise process in which:

1. A team of individuals knowledgeable about the food operation is assembled.
2. The food being produced is described.
3. The intended use of the food is described.
4. A flow diagram that describes the steps in the process that are under the manufacturer's control is prepared.
5. An on-site confirmation of the flow diagram is conducted.
6. All potential hazards are listed and a hazard analysis is conducted.
7. CCPs are determined.
8. Critical limits are established for each CCP.
9. A monitoring system is established for each CCP.
10. Corrective actions are established.
11. Verification procedures are established.
12. Documentation and record keeping procedures are established.

The results of monitoring (step 9) may indicate a deviation occurred at a CCP and corrective actions (step 10) are necessary (Codex Alimentarius 1997a).

## 5.4 Assessing Control of GHP and the HACCP Plan

Control means "the state wherein correct procedures are being followed and criteria are being met" and "to take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan" (Codex Alimentarius 1997a). The latter definition incorporates several aspects of the food safety system: establishing critical limits, monitoring to ensure compliance and making adjustments to maintain compliance with the criteria. Chap. 3 addresses verifying compliance with GHP and HACCP plans. This chapter addresses corrective actions to reestablish control. In an ideal food operation:

- Criteria are supported by research and technical literature.
- Criteria are specific, quantifiable and provide a yes/no response.
- The technology for controlling microbial hazards is readily available and at reasonable cost.
- Monitoring is continuous and provides immediate results, while the operation is automatically adjusted to maintain control.
- There is a favorable history of control.
- The potential hazard is prevented or eliminated completely.

Ideal food operations, however, do not exist in the real world. Unfortunately, criteria cannot always be clearly defined and assessments of whether the food operation is in compliance with criteria must be based on the judgment and experience of an observer. In many cases, it may be possible to reduce but not prevent a hazard (e.g., enteric pathogens on raw seafood and agricultural commodities). Control frequently does not rely on a single measure but on a set of measures embedded in GHP and/or HACCP that all need to be functioning as designed during the course of operation. In some cases small changes to the product or processing may impact the effectiveness of control measures. Also, the effectiveness of control measures can range from partial reduction of certain hazards (e.g., salmonellae on raw poultry) to significant reductions of highly resistant hazards (e.g., *Clostridium botulinum* in low acid canned foods). Assessment of whether an operation is under control may vary among individuals with different backgrounds unless there is a common understanding (e.g., guideline, regulation) that clearly defines how to assess control.

### *5.4.1 Assessing Control of GHP*

Many food operations establish written procedures to assess control of the GHP factors listed in Sect. 5.2. The two most common methods to assess control are visual inspection and microbiological sampling. Visual inspection is normally assigned to one or more trained experienced employees in the food operation. Inspections can also be performed by control authorities or third party auditors (ICMSF 2002).

The time of at which inspections are carried out is important and depends on their purpose. Preoperational inspection is performed after the facility and equipment have been cleaned and sanitized to determine whether the equipment and processing environment are acceptable for the subsequent production. Attention may also be given to maintenance activities to be certain personnel follow procedures and do not contaminate the equipment during equipment maintenance, reassembly and start-up. Inspections during production should cover activities that can lead to product contamination, such as employee practices, product flow, build-up of residues, etc. Inspections that address plant construction and layout are less frequent, but are also important.

Results from inspections are recorded and made available for review by those who need the information to respond appropriately. Organizing and evaluating the data for trend analysis can identify situations of improved or reduced control (ICMSF 2002). Timely review is essential so adjustments can be made in a timely manner and a deviation can be avoided.

Visual inspections provide one means of assessing GHP control but in many instances microbiological sampling can provide greater insight and a more accurate assessment of microbial control. For many facilities, it may be relevant to maintain a program of sampling equipment before production commences, as well as collecting samples from the equipment or the food during production. The samples may be tested for indicators (e.g., aerobic colony count, coliforms, Enterobacteriaceae) that reflect the hygienic conditions during processing. Additional tests for pathogens may be performed for certain products. Extensive guidance on microbiological sampling of the processing environment and food has been provided (ICMSF 2002), as well as in this book (see Chap. 4, and product chapters).

For certain food operations the likelihood of resident pathogens and harborage sites must be considered (ICMSF 2002). If this is likely to occur, it may be necessary to establish an environmental sampling program to verify the effectiveness of the GHP procedures (ICMSF 2002). This information could be used to make adjustments in GHP to control one, or more, target pathogens that could become established in the food production environment and lead to contamination of the food.

The basic components of a monitoring program to assess control of persistent pathogens in the processing environment include the following strategies:

1. Preventing the establishment and growth of pathogens in harborage sites that can lead to the contamination of food.
2. Implementing a sampling program that can assess in a timely manner whether the environment where the food is exposed is under control.
3. Detecting the source or route of pathogen transfer that leads to contaminated food.
4. Applying appropriate corrective actions in response to each positive finding of a target pathogen.
5. Verifying, by follow-up sampling, that the source has been detected and corrected.
6. Providing a short-term assessment (e.g., involving the last four to eight samplings) to facilitate the detection of problems and trends.
7. Providing a longer-term assessment (e.g., quarterly, annually) to detect widely scattered incidents of pathogen detection and to measure overall progress toward continuous improvement.

An inherent weakness in industry's ability to detect and respond to pathogens in harborage sites is the difficulty and time needed to collect the samples and perform the analytical tests needed to detect the source(s) of contamination. A common issue is that all the investigational samples may test negative for the target pathogen and a clear direction for appropriate corrective actions is lacking. Furthermore, the pathogen may be detected again at some later date after the routine monitoring program has been resumed.

Microbiological data should be recorded and made available for review by others who need to know the results so they can respond appropriately. In addition, the data should be organized and evaluated for trends toward improved or reduced control (ICMSF 2002). As with visual inspections, this information is essential so appropriate corrective actions can occur in a timely manner.

### *5.4.2 Assessing Control of the HACCP Plan*

HACCP plans are formal, structured documents that are based on the seven principles of HACCP (Codex Alimentarius 1997a). The size and type of food operation will influence the content of the HACCP plan. Food operations that do not have a CCP that can prevent, eliminate or reduce the hazards of concern may not have a HACCP plan. Smaller operations, such as street food vendors, may rely more on regulations or guidelines from health authorities that emphasize GHP.

For larger operations that have HACCP plans, control is assessed through the monitoring and verification activities stated in the HACCP plan. The HACCP plan should include corrective actions for the deviations that are likely to occur (step 10 in Sect. 5.3).

## 5.5 Corrective Actions

### *5.5.1 Corrective Actions for GHP*

Information about how microbial hazards can be introduced is necessary to design a food operation and implement appropriate control procedures. It is not unusual to occasionally detect weaknesses in the design and implementation of GHP, which requires corrective action. Typical corrective actions associated with GHP involve the factors listed in Sect. 5.2. For example, microbiological data might indicate improvements are needed in how processing rooms or equipment are cleaned and sanitized. This could involve training individuals on the correct procedures, changing the method or frequency of cleaning and sanitizing, or performing maintenance and repair on equipment. When food operations increase production or add new products, this may result in an unacceptable increase in risk that the food may become contaminated and may require a change in the plant layout. Another common corrective action for GHP is retraining employees who have not followed established procedures for personal hygiene, food handling or following the traffic pattern that separates raw ingredient processing and areas where ready-to-eat foods are handled.

When equipment is suspected to be a persistent source of contamination, corrective action may include complete dismantling of the equipment to allow more thorough cleaning and sanitizing of the parts before reassembling. For small equipment with many parts, cleaning in a recirculating bath of hot water with detergent (e.g., Clean Out of Place (COP) tank) is effective. COP cleaning requires placement of parts in a way that assures adequate circulation of the cleaning solution for optimum results. These procedures are normally adequate and the preferred corrective action. As equipment is being dismantled, sampling sites suspected of harboring microbial contaminants can provide useful information that can be used to change maintenance and cleaning procedures. For example, the equipment may need to be modified for more effective cleanability. In some situations, lubricants may be a potential harborage site for contamination, and use of food-grade antimicrobial lubricants may be an appropriate corrective action.

Occasionally, even extensive dismantling and cleaning will prove ineffective. For equipment that can be moved, heating with moist heat in a chamber, after sensitive electronics, oil, and grease are removed, can be effective. If this is not possible, the equipment can be covered with a heat-resistant tarpaulin and steam can be introduced from the bottom. When these moist heating techniques are used, an internal temperature of 71°C for 20–30 min is recommended to eliminate vegetative cells. The temperature can be monitored with thermocouples placed within the equipment or thermometers that pierce through the tarpaulin. Of course, equipment such as drying towers for dried milk products and many closed systems must be cleaned and sanitized in-place.

To regain control, it is helpful to determine the source of the contamination so that appropriate corrective actions can be taken. Investigational samples are analyzed individually rather than as composites, samples are collected more frequently (e.g., every four hours) and additional sites are included. A simple map showing the layout of the rooms and the equipment can be beneficial. Positive sites are marked on the map with the dates and times of collection. A very simple schematic drawing or a blueprint of the facility can be used. By organizing the results to show which sites test positive more frequently and where the positive samples first occur, the source of contamination can be more easily located. In an environment that has been in control, this will often identify specific equipment that is a harborage for the contaminant. In general, contamination flows down along or through processing equipment with the flow of product. Fingerprinting isolates can be a very useful tool for identifying the source and pathways of contamination.

Exposed surfaces of equipment may be transfer points but generally are not sources of contaminants due to their ease of cleaning and sanitizing. Of greater concern are enclosed areas (e.g., within a hollow roller for a conveyor) where food deposits and moisture accumulate and cannot be removed

by normal cleaning, scrubbing, and sanitizing. These harborage sites are not necessarily biofilms per se, but rather sites in which a variety of bacteria become established and multiply.

To achieve continuous improvement and long-term control, corrective actions may involve changes in the plant layout, equipment design or maintenance, replacing floors or walls, or changing the procedures for cleaning and sanitizing. In the event construction is required, extra precautions must be taken to control the pathogen and prevent food from becoming contaminated during the construction process.

### *5.5.2 Corrective Actions for HACCP*

Seven possible corrective actions are appropriate to consider when a deviation occurs at a CCP within the HACCP plan:

1. If necessary, stop the operation
2. Place all suspect product on hold
3. Provide a short-term resolution or "fix" so that production can be safely resumed and additional deviations will not occur
4. Verify that the short-term fix has been effective and recurrences do not occur
5. Identify and correct the root cause for failure to prevent future deviations
6. Collect the necessary information to decide what to do with suspect product
7. Record what happened and the actions taken
8. If necessary, review and improve the HACCP Plan

The corrective actions must bring the food operation into compliance with established criteria and ensure safe disposition of the product involved. Corrective actions should be considered in advance for each CCP in the HACCP plan; however, it is unrealistic to anticipate and prepare for all the possible deviations that can occur.

### *5.5.3 Response to Epidemiologic Evidence and Complaints*

When an epidemiologic investigation implicates a specific food as the likely cause of illness or when consumer complaints provide such an implication, the root cause(s) leading to disease may not be immediately apparent. While removal of the implicated food may prevent additional consumer exposure, the corrective actions necessary to prevent future cases of disease may be unclear. Detailed review of the relevant operations before and during the period of likely contamination along with extensive microbiological evaluation of the environment, ingredients and finished foods may reveal information about the root cause(s). Food and environmental isolates should be compared with human clinical isolates to confirm, as clearly as possible, the source(s) of the pathogen and root causes. When the location within the food chain is identified as the likely source, every effort should be made to determine the important factors involved so adjustments can be made to existing control measures (i.e., GHP, HACCP) to prevent additional outbreaks.

It is possible that a thorough evaluation of the food implicated by the epidemiologic investigation will correctly reveal a food system under good control without obvious defects in GHP and HACCP plans or their implementation, despite the presence of pathogens at a frequency and concentration sufficient to cause disease. This scenario is more likely to occur when raw agricultural commodities are involved and existing technology and food safety controls can reduce but not prevent or eliminate the hazard. While it remains appropriate to prevent additional exposures to the implicated food, this situation may require issuance of a consumer advisory for persons at risk. A consumer advisory on

the retail package to properly store, prepare and cook raw meat and poultry products is one such example. Information from public health agencies to physicians and other health care providers who advise high risk patients is another example.

## 5.6 Options for Disposition of Questionable Product

If control is lost and a deviation occurs, several options may be considered for disposition of suspect product:

1. Determine whether the suspect product complies with existing criteria for safety and can be used as intended. To assess the acceptability, a sampling plan can be applied, keeping in mind the limitations of the sampling plan to detect lots with defects that are of very low prevalence (Appendix A and ICMSF 2002). In some situations dividing the lot(s) into smaller portions (e.g., pallet, hourly) may be considered, with sampling and testing of each portion or sub-lot as separate entities. This increases the number of samples across the total production and also provides information about distribution of the defect. Testing sub-lots should be evaluated carefully. See Sect. 5.6.1 for further considerations.
2. The suspect product can be diverted to a safe use. For example, eggs or cooked chicken contaminated with salmonellae could be used as ingredients in the manufacture of a commercial product that will receive a kill step that can ensure the food will be safe.
3. The suspect food could be reprocessed, if reprocessing will destroy the hazard.
4. The suspect food could be destroyed.

Reaching a decision on the appropriate disposition of non-compliant product is influenced by a number of factors. First is the severity or the seriousness of the hazard. For example, does the potential defect consist of spoilage or could it be a severe hazard such as botulinum toxin? Second is the type of microbial hazard. For example, staphylococcal enterotoxin is very heat stable and its presence in a food would render the food unacceptable for human consumption in any manner. Third is the likelihood of the hazard being present in the food. Is it one chance in a million or is it likely to occur every time the deviation occurs? Fourth is how the food will be stored, shipped, and prepared. Fifth is who will prepare the food. Sixth is whether the intended consumers include highly susceptible individuals. Each of these factors and, perhaps, others should be considered before reaching a recommendation on the disposition of the product.

### *5.6.1 Sub-Lot Testing Considerations*

No sampling plan, other than one that tests the entire lot, can prove that the lot is not contaminated. Thus, while the term "zero tolerance" is often used, in actuality sampling to assess compliance can only provide a certain level of confidence that the contamination level is below some mean concentration. That concentration depends on the size and number of analytical units tested, and the variance in the concentration of the pathogen in the lot. From statistical standpoint the size of a lot does not influence the performance of a sampling plan. An example from probability statistics can help explain why this is true. If a die is tossed 100 times and the numbers are recorded and then 3 random numbers from 1 to 6 are determined, there is a certain probability of having a "1" in the set of 3. If the die is tossed 1,000 times, the probability of having a "1" in the set of three random numbers is the same as that for tossing the die 100 times.

If contaminating cells are distributed randomly throughout a lot, creating five sub-lots is equal to taking 5 times the number of samples from the lot, and the average concentration of a microbial population would remain valid for the whole lot and not just the sub-lots. However, in many instances

microorganisms are not randomly distributed. Examples of situations that may alter distribution of contamination during processing include introduction of water from a leaking roof or a drain back-up at one point in time, a change in raw materials, equipment being inserted into the process, mechanical breakdown of equipment, production interruptions for cleaning, a function of production time, and other specific events. In such cases, it is not a good assumption to define this as a uniform lot, and sub-lotting may assist in identifying trends and defective portions of the lot.

The application of testing sub-lots should be evaluated very carefully. Elements to consider are:

- Readily available microbiological data on pathogens as well as indicator organisms from the lot in question
- Data for lots manufactured before and after as well as in-process and/or environmental samples
- Data on processing parameters
- The type of microbiological hazard, its severity and its fate during further handling, i.e., the likelihood that it could increase or decrease prior to consumption, as well as the sensitivity of the consumer, etc.

## 5.7 Repetitive Loss of Control

The HACCP concept has gained wide acceptance because it provides a logical, structured approach to prevent, eliminate or reduce hazards in foods. The system is designed to detect loss of control and, thereby prevent suspect food from reaching consumers. This is an essential component of the food safety system because deviations can and will occur during the normal course of operation. Preventing repetitive deviations for GHPs and CCPs is a desirable goal but may be difficult to achieve in some food operations. Each food operation should strive to prevent repetitive loss of control by implementing a continuous improvement program to achieve greater reliability for controlling GHPs and CCPs within the food safety system.

## References

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Codex Alimentarius (1997b) Recommended international code of practice, general principles of food hygiene (CAC/RCP 1-1969, Rev. 3 1997). Joint FAO/WHO Food Standards Program, FAO, Rome

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# Chapter 6
# Microbiological Testing in Customer–Supplier Relations

## 6.1 Introduction

The complete food chain from farm to fork is characterized by a sequence of supplier–customer interfaces. These interfaces imply the establishment of contracts defining the requirements of the customers with respect to their suppliers. These contracts also reflect the commitment of the supplier to guarantee the delivery of goods in compliance with the agreed-upon requirements.

This sequence of interfaces plays an important role in fulfilling a Food Safety Objective (FSO) at the level of the final consumer as defined by public health authorities. As shown in Fig. 6.1, individual performance objectives (PO) can be established along the whole food chain at these interfaces. These POs should be identical to FSOs if no changes in the level of the pathogen of concern occur in the food chain up to the consumer. Different POs need to be defined to meet the final FSO if either a decrease or an increase in the level of a hazard is anticipated in the food chain (ICMSF 2002). If not done by authorities, customers or manufacturers have to define a PO that is suitable, considering the impact of processing steps and conditions on the relevant pathogen, as well as the impact of distribution and preparation by the consumer. While FSOs and POs are related to a single pathogen, all significant hazards as well as other parameters such as indicators and spoilage microorganisms need to be considered in customer–supplier relationships.

Formal articulation of FSOs by public health authorities is anticipated. Absence in 1, 10, 100 kg have been proposed in the European Union for *Cronobacter* spp. and *Salmonella* in powdered infant formula (EFSA 2004). Thus contracts between suppliers and customers are based on established microbiological criteria, typically applying the worst case scenario established by commercial or administrative people. This chapter discusses the relations between suppliers and customers and the role of microbiological testing in these commercial interactions.

Requirements in contracts established between a supplier and a customer may apply to raw materials or ingredients, semifinished products or finished products. These requirements may include microbiological specifications with relevant parameters such as significant pathogens and indicator microorganism or even spoilage microorganisms. Examples of such requirements can be found in the different chapters in the book. The requirements may also include other elements related to the microbiological conditions or status of the goods in question such as:

- Physico-chemical parameters that may have an impact on growth:
  - Gassing conditions and limits of residual oxygen
  - pH or acidity
  - Temperature maximum during transport and at reception

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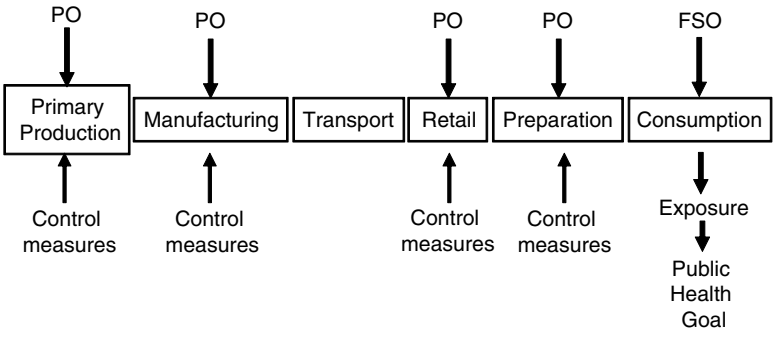


**Fig. 6.1** Application of performance objectives (PO) and Food Safety Objectives (FSO) in the food distribution chain

  - Time lapse for transportation between supplier and customer
  - Requirement for intermediate pasteurization (e.g., liquid whey)
- Parameters related to hygiene:
  - Separation of goods during transport; e.g., according to the risk of contamination, formation and transfer of off-odors etc.
  - Location of containers in a ship to avoid the formation of condensation due to temperature differences
  - Type of packaging material used; e.g., the requirement of strippable bags to avoid contamination during handling and tipping of critical ingredients (e.g., dry mixing)
  - Specific protection of packaging material; e.g., plasticized intermediate cardboard layers between glass jars to avoid the presence of dust in normal cardboard
  - Cleaning procedures for containers and tanks used to transport raw materials or semifinished product

## *6.1.1 Raw Materials and Ingredients Used by Manufacturers*

The choice of parameters included in specifications for raw materials and ingredients depends on several elements such as the point in the food chain, the impact of subsequent processing steps and the regulatory environment.

### 6.1.1.1 Raw Agricultural Commodities

For unprocessed agricultural raw materials, visual qualitative or quantitative parameters play an important role. Examples are:

- Absence or maximum percentage of moldy pieces in a bulk delivery (e.g., cocoa beans, peanuts, grain or maize)
- Absence or maximum percentage level of rotten or unripe fruits or vegetables in a bulk delivery from the field
- Defined characteristics of color or odor (absence of off-odors) for fresh meat or fish

Quantitative microbiological specifications for unprocessed agricultural raw materials that will be further processed may also be included. They may, however, be expressed as percentage of positive findings or as maximum levels of counts; e.g., for *Salmonella* in meat used to manufacture products

such as salami or a maximal level of viable counts in fresh milk beyond which the raw material will be downgraded, respectively. These limits are not necessarily used as acceptance criteria for delivered materials. Rather they may be used to drive improvements by the supplying party through rewarding good quality with a bonus and penalizing poor quality by deduction at payment.

#### 6.1.1.2 Processed Ingredients

For processed ingredients, microbiological specifications are established according to their further use. Skimmed milk powder, for example, is an ingredient that is widely used in the manufacture of many different products such as:

- Dry-mixing operations without any further heat-treatment:
  - Chocolate and confectionery
  - Infant formulae and infant cereals
  - Instant beverages
  - Dehydrated culinary products
- Wet mixing operations with subsequent heat-treatment:
  - Recombined liquid milks (pasteurized or UHT)
  - Fermented dairy products
  - Ice cream
  - Heat-processed refrigerated culinary products
  - Bakery products

The specifications for the skimmed milk powder thus depend very much on use, and they vary from very stringent specifications (e.g., for critical products such as infant formulae) to less stringent ones (e.g., for manufacture of UHT-milk). For example, when used in infant formula, specifications are typically based on standards for finished products established by authorities. Conversely, for use in UHT milk, more lenient specifications may be used for *Salmonella* and Enterobacteriaceae, but limits for process-relevant spore formers are typically included by the customer to minimize the risks of failure of the thermal process (see Chap. 24).

While the adherence to established microbiological requirements can be verified through sampling and testing, limitations of sampling plans need to be considered (see Appendix A). Therefore, it is important for a customer to assess the microbiological hazards and associated risks when using and purchasing a given ingredient. This will allow categorization of the different ingredients according to risk and defining the approach taken in handling ingredients after delivery.

For high risk ingredients used for sensitive products (e.g., skimmed milk for infant formulae) an assessment of the confidence level in the suppliers is also needed. This assessment should be based on audits against key parameters to ensure the manufacture of safe ingredients and may include, but is not limited to, the following:

- Implementation of appropriate preventive prerequisite measures such as GHP
- Implementation of HACCP
- Validation of control measures including critical limits
- Implementation of verification measures such as environmental pathogen monitoring
- Historical data
- Trend analyses techniques
- Release procedures
- Appropriate sampling methods
- Analytical procedures such as the use of validated methods and participation in proficiency tests

### 6.1.2 Interactions with Retailers

Microbiological specifications between manufacturers and retailers and food service are frequently based on national or international criteria established by public health authorities. However, additional or more specific requirements may be established by the retailer. Retailer requirements for raw agricultural commodities, such as fresh fruits or vegetables, or for manufactured products may be similar or identical to those outlined under Sect. 6.1.1. Additional elements may include:

- Elements related to the shelf life of refrigerated products, such as dairy or culinary products, to meet their distribution channel needs
- Elements related to the composition of the products, such as salt or sugar content, or the heat-treatments used to manufacture the product
- Elements related to certification and auditing of the manufacturer

Such requirements may require the manufacturer to conduct challenge and storage tests to demonstrate the stability and safety of the products with the specified recipe modification or the required shelf life. A further requirement may also include monitoring retention samples.

### 6.1.3 Contract Manufacturers

Food manufacturers may subcontract the production of some products for several reasons:

- Small volumes which may benefit from existing production lines dedicated to the same or similar products (cost reasons)
- Proprietary technologies used by contracted manufacturers that are not available at the factory of the contracting party
- Temporary production of new products until it becomes clear that the product will be successful and thus justify the investment for a new processing line
- Insufficient capacity in the manufacturer's own factory thus requiring a contract manufacturer to increase capacity

The main issue related to contracting production is the control over the quality and safety of the product. The required quality can be achieved through the definition of the product characteristics based on the recipe and processing conditions or though use of a contract manufacturer chosen because of the quality attributes of the products they produce. However, ensuring the microbiological safety of the products may not be easy to control. This is particularly true if the standards applied by the manufacturer are different from those of the contracted organization. These differences must be addressed to assure that the level of understanding and implementation of GHP and HACCP are consistent to avoid the potential for increased microbiological risk.

While implementation of the appropriate preventive measures, sampling and testing procedures is usually negotiated as part of the contract, it may not always be possible to impose the requirements of the contracting party. This may be the case if the volumes subcontracted are small in comparison to the total volume produced by the chosen manufacturer. In such cases the contracting party may not be in a position to implement or impose its own quality system and associated standards, and it may be advisable to look for an appropriate alternative. However, different options may be possible and depend on the type of product and its sensitivity in terms of risks for the consumer and risk for the contracting manufacturer. Potential approaches include:

- The contract manufacturer agrees to manufacture and release product according to the specifications and the implemented control measures are approved by the contracting party

- Production lines on which the subcontracted production takes place are under the direct supervision of personnel from the contracting party
- Release is performed by the contracting party's own quality assurance people, who are either located at the contractors site or visit the contracted location during production
- Regular audits conducted by the contracting party (see Sect. 6.2)

## 6.2 Auditing

Auditing suppliers in a supplier–customer relationship plays an important role in assessing whether the agreed-upon requirements will be met consistently and thus the confidence level in a particular supplier. Audits of HACCP and of prerequisite measures such as GHP can be very different in their nature and may range from a simple system audit to a full technical audit. In the first case, audits focus on whether or not a HACCP plan has been established and whether the different steps of a HACCP study have been addressed. In the second case, attention is given not only to the formal aspects, but also to the technical and scientific content, such as the validity of the hazard identification, and the appropriateness of control measures and derived critical limits. This will also include, assessing validation information, the effectiveness of the proposed corrective actions, appropriate verification procedures and improvement of the HACCP plan where necessary. These technical audits require deep knowledge and understanding of the product, possible associated microorganisms, the process and the processing conditions to determine whether the right decisions have been made. These technical audits usually require multidisciplinary teams, including, at a minimum, process or manufacturing specialists and hygienists or industrial microbiologists. This is important because these audits go beyond the sole assessment of the HACCP plan, and also focus on the degree of implementation and effectiveness of GHP, which provide the necessary foundation for a sound HACCP plan. In addition, it is also necessary to audit the procedures designed to verify the effectiveness of the measures. This may include environmental monitoring, verification of end product and review of methods to ascertain if they are appropriate for the particular matrix and for environmental samples. For details on process control, refer to Chap. 3.

Individuals conducting audits need to be qualified and trained to be effective in this role. Two issues are relevant to consider; i.e., training to gain specific competencies and secondly registration of auditors with an appropriate body according to the sector. This is important to avoid an auditor with competence in, for example plastic packaging, from auditing a poultry factory. Ongoing verification of auditor competence also needs to be considered. The auditor training course should be registered with an appropriate training body and if an auditor needs to audit a facility for which they are not competent, then a technical expert should accompany them on the audit. These are all especially applicable where third party audits are used.

## 6.3 Microbiological Data

Usually the only microbiological data provided in supplier–customer relationships are limited to results of the purchased goods and communicated, depending on the agreements or level of confidence in the form of certificates of analysis (CoA) or certificates of conformance or compliance (CoC). The first provide detailed analytical results of the parameters included in the specifications, the latter represent a confirmation or guarantee that based on the implemented control measures and verifications, the products are in compliance with the specification. This provides information on the delivered lots and, since they have been released and shipped, indicates that they comply with the

agreed-upon requirements. However, results of the CoA, will only provide information on the specific lot and not on the overall performance or process capability of the supplier.

A much more useful approach would be for suppliers to share not only results for finished products, but also data on line samples, historical data on lots manufactured on the same processing line or during a time frame around the time delivered lots were manufactured, environmental data or other relevant parameters. These data are more useful for the customer to confirm or modify their confidence level in a particular supplier and could be considered a certificate of conformance and compliance rather than a certificate of analysis.

## References

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# Part II
# Application of Principles to Product Categories

# Chapter 7
# Applications and Use of Criteria and Other Tests

## 7.1 Introduction

As discussed in Chap. 1, it is widely recognized that application of prerequisite programs at preharvest, harvest and postharvest level (e.g., Good Agricultural Practices (GAP), Good Farming Practices (GFP), Good Veterinary Practices (GVP), Good Hygiene Practices (GHP), Good Manufacturing Practices (GMP), etc.) and Hazard Analysis Critical Control Point (HACCP) program is the most effective food safety management strategy. Effective control of undesirable microorganisms in foods is best addressed at appropriate steps in the food chain through targeted and synergistic application of these approaches. Microbiological testing of process hygiene can play an important role in verifying the effectiveness of food safety management programs (prerequisite programs and HACCP) when used in a thoughtful, well-planned manner. In some cases, microbiological testing of the end product may also be used if no prior history of the product is available (e.g., at port of entry). Consistent with previous ICMSF considerations (2002), testing should be required only when the following two conditions exist:

1. The product group has been implicated in foodborne disease or may have an inadequate shelf life or other microbiological issues if effective controls are not applied.
2. The application of testing will reduce the health risk or quality issues associated with a food or will effectively assess adherence to microbiological control measures or process controls.

This chapter provides background on the considerations that the Commission used to propose microbiological criteria for some commodities and not others. It also indicates how the criteria should be interpreted and applied.

The recommendations for end product testing in the following chapters replace those given in *Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications* (ICMSF 1986). Significant advances in the understanding of food production and processing, risk management, and the statistics of sampling have made changes necessary. Additionally, the following chapters provide recommendations not only for end product testing, but also other tests that may provide useful information for microbiological safety and quality management.

Although considerable effort was given to develop appropriate, risk-based criteria, ICMSF recommendations have no official status. Promulgation of official national microbiological standards is the responsibility of governments and articulation of international food safety guidelines is the province of intergovernmental standards setting bodies such as the Codex Alimentarius Commission, which is organized under the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO).

International Commission on Microbiological Specifications for Foods (ICMSF),
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## 7.2 Format of Product Chapters

The product groupings generally follow those used in *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005), which provides details on the microbial ecology of specific commodities and products. The following chapters focus on practical application of testing in the production of microbiologically safe and wholesome foods rather than on the microbial ecology of the products. Each chapter briefly discusses the relevant microbial hazards and spoilage concerns for each sub-commodity and, based on their significance, may recommend tests and criteria for the various stages of production and distribution, as described below.

### *7.2.1 Primary Production*

For some commodities, such as fruits, vegetables, spices, meat, poultry, and fish products, primary production practices can have a major influence on the microbiological quality of the product. Where appropriate and where information is available, recommendations for irrigation or seafood harvest waters, fertilizer, vaccination programs, feeding regime and other on-farm practices may be provided or referenced to national standards.

### *7.2.2 Ingredients*

Many foods are composed of a number of different ingredients. The microbiological quality and safety of some ingredients may be critical to the safety and stability of the final product. Control of a microbiological concern at the ingredient level may be essential for products when there is no subsequent kill step (e.g., cocoa powder in chocolate that has no heat treatment, beef intended for production of unheated fermented salami). For other foods, ingredients may be subjected to a kill step during processing and therefore microbiological criteria are less important (e.g., cocoa powder in ice cream mix that is subsequently pasteurized, beef intended for production of cooked meat products). Anticipated initial levels or criteria for such ingredients discussed in other chapters may be cross-referenced, as appropriate. Testing is generally recommended for an ingredient if the answer to either of the following questions is "Yes" for the commodity under consideration:

1. Is control at the ingredient step necessary for safety or quality?
2. Is testing necessary to verify the acceptability of the ingredient?

### *7.2.3 In-Process*

In this book, the term "in-process" testing is used to describe testing to (1) verify a kill step or (2) monitor whether the product is likely to become contaminated. The concept of HACCP emphasizes the importance of applying validated and verified process controls for the production of safe food. Certain tests may be used to verify that processes are performing as anticipated (e.g., initial in-plant validation to assess the performance of a control measure at certain production step). For example, testing for indicator organisms such as coliforms or Enterobacteriaceae on in-process product emerging from cooking equipment may be useful to verify adequacy of the cooking process.

Sampling intermediate product (e.g., from conveyors, filler heads, holding tanks or vats, etc.) and processing line samples (e.g., process wash water, sifter tailings, fines, line residues, and scrapings) offers an alternative or complimentary approach to the use of swabs or sponge samples to monitor for contamination with microorganisms of concern to public health or spoilage. In-process product

or product residues that accumulate on equipment may represent a worst case when such materials accumulate under conditions that support microbial growth throughout a production period. In-process testing may provide more useful information about potential microbiological concerns than end product testing, particularly when the data are used in a process control system as discussed in Chap. 3 of this book and in *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management* (ICMSF 2002).

In-process testing is generally recommended if the answer to all of the following questions is "Yes" for the commodity under consideration:

1. Does the process need to be controlled to prevent increase, ensure decrease, maintain current level, or prevent spread of a microbial concern?
2. Is testing needed to verify (a) the process is functioning as intended or (b) contamination is not occurring in the process?
3. Are there locations in the process where accumulated product residue may provide a representative or worst case sample that predicts the safety or quality of the final product?

### *7.2.4 Processing Environment*

Maintenance of a hygienic processing environment is essential for the production of safe and wholesome food; however, microbiologically relevant considerations will vary for different food commodities. This section generally addresses the use of swabs or sponges for sampling sites on equipment or in the environment. This type of testing is very useful and effective for verifying that the environment is under appropriate hygienic control for the specific commodity. General guidance on environmental sampling can be found in Chap. 4 of this book and in *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management* (ICMSF 2002). As with in-process sampling, a well designed environmental testing program based on a predetermined clear objective may provide more useful information about potential microbiological concerns than end product testing, particularly when the data are used in a process control system as discussed in Chap. 3 of this book and in *Microorganisms in Foods 7* (ICMSF 2002).

Environmental testing is generally recommended with potential tests to consider, if the answer to the following two questions is "Yes" for the commodity under consideration:

1. Does the environment need to be controlled to prevent contamination of the product with a microbial concern?
2. Will testing be beneficial to verify control of the microbial concern in the environment?

### *7.2.5 Shelf Life*

The shelf life of food commodities is influenced by deleterious changes to product quality that develop over time, many of which are nonmicrobial, such as enzymatic activity, oxidation, structural changes, staleness etc. However, microbial activity can play an important role in the safety or spoilage of some food commodities. Shelf life testing is discussed only if microbial activity is relevant to a particular commodity. For certain products (e.g., bulk shipments) shelf life testing may not be feasible. Shelf life testing is generally recommended if the answer to the following two questions is "Yes" for the commodity under consideration:

1. Is shelf life limited by a microbiological safety or quality concern?
2. Is shelf life testing feasible?

### *7.2.6 End Product*

End product criteria are recommended if they can be used to demonstrate the successful application of food safety controls or to assess the microbiological status of a lot when insufficient information exists to assess its status. For a limited number of foods, available prerequisite programs and HACCP may be inadequate to provide consumer protection. For such foods end product testing may be a necessary step to provide additional protection to consumers.

The determination of the relative importance of end product testing must be made on a product by product basis (see Sect. 7.2.7), and end product testing may be used for lot acceptance when there is insufficient access to other process or testing information. The suggested criteria for lot acceptance are based on baseline data, experience, industry practice, relative risk when ICMSF cases are considered, or existing microbiological criteria that have been developed internationally as a result of the risk analysis process established by The Codex Alimentarius Commission (see Sect. 7.4). Other sampling plans may be appropriate in certain situations. For example, reducing the number of samples may be acceptable for on-going surveillance activity; whereas increasing the number of samples may be prudent when investigating significant process deviations or outbreaks. Testing is generally recommended if the answer to one of the following questions is "Yes" for the commodity under consideration:

1. Is end product testing necessary to verify control of the overall manufacturing process?
2. Is end product testing relied upon for ensuring the safety or quality of the lot?

### *7.2.7 Relative Importance of Tests Recommended*

Tables within each commodity chapter include a rating (i.e., low, medium, high) for the relative importance of the tests recommended. These ratings reflect the level of importance for routine testing during operation under GHP/GMP and HACCP using processes that have been validated to consistently deliver product that is acceptable from the perspective of safety and quality. In assigning ratings, the Commission attempted to identify the types of samples that would provide the most useful information to evaluate the microbiological status of the product being manufactured. It is important to note that the relative importance of a test must be evaluated in the context of the overall microbiological testing program. For example, if ingredient, in-process, and environmental monitoring are routinely conducted in a diligent manner, on a routine basis, in a stable processing environment, with the intent to use the information for trend analysis and process improvement, then the relative importance of finished product testing is likely to be low. However, if upstream testing is done occasionally or in a manner that would not provide confidence that the process is under control, then the relative importance of finished product sampling may increase.

The relative importance and recommended sampling plans may change in nonroutine situations. For example, when validating a new process, qualifying a new ingredient or supplier, performing corrective action for a significant process deviation or investigating a foodborne illness outbreak, more extensive testing is generally warranted. Previous chapters on corrective action, process validation and customer–supplier relationships provide guidance in these areas.

## 7.3 Choice of Microorganisms or Products Thereof

Recommendations for tests are included for microbes or their products (e.g., mycotoxins) that are most important in respect to hazard/risks or noncompliance with GHP/GMP. This choice is based on a hazard analysis and risk categorization (i.e., a qualitative risk assessment) that considers epidemiologic evidence, public health impact, the scientific literature and expert opinion, in-process experimental

validation, and recognizes the limitations of current methodologies. Quality issues are also considered in recommending tests. Detailed discussion of microbial concerns for each commodity is provided in *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005).

## 7.4 Selection of Limits and Sampling Plans

Limits and sampling for in-process and environmental tests are greatly influenced by the site, process, geographic region and other factors, therefore it is not possible to specify limits that are universally applicable in all situations. Typical guidance levels or typical levels encountered may be provided for these tests, but these are not intended to be applied universally. Accordingly, no methods, number of samples, or sample size are specified in most instances. It is important to emphasize that a typical level encountered *does not* indicate a limit. It is expected that levels will periodically exceed a typical level encountered.

For end product testing, the following questions were asked sequentially to help identify the appropriate basis for recommended sampling plans and criteria:

1. Does a risk assessment exist?
2. Has an appropriate level of protection (ALOP) been established that would enable determination of a Food Safety Objective or a Performance Objective?
3. Are sufficient data available to define typical values likely to be encountered that are consistent with safe food, or food of good quality, and do data exist to estimate the variability in values encountered, e.g., within and between batches?

The availability of a risk assessment, dose-response data, consumer exposure data and defined ALOP or FSO/PO, and data on microbial levels typically encountered in a food facilitates development of microbiological criteria that have a link to public health goals. ICMSF (2002) and van Schothorst et al. (2009) reviewed this process in some detail. However, the availability of formal risk assessments for many food types is limited (e.g., qualitative and quantitative). In the absence of a risk assessment, the Commission used the ICMSF cases (ICMSF 2002), generally accepted international regulations (e.g., Codex, national regulations, industry guidelines) or expert opinion to recommend sampling plans and limits.

ICMSF cases, summarized in Table 7.1, consider both the severity of the hazard, the susceptibility of the intended consumer and the potential for the risk to decrease, remain the same, or increase between the time of sampling and when the food is consumed. Sampling plans become increasingly more stringent with increased severity. The following terms are used:

$n$ = the number of sample units to be analyzed
$c$ = the maximum number of sample units allowable with marginal but acceptable results (i.e., between $m$ and $M$)
$m$ = concentration separating good quality or safety from marginally acceptable quality
$M$ = concentration separating marginally acceptable quality from unacceptable quality or safety

Limits ($m$ and $M$) recommended for utility, indicator, and moderate hazards (Cases 1–9) are typically reported on a per gram basis, and quantitative methods are generally used. The $c$ criterion included in Cases 1–9 recognizes that statistical variation may occasionally contribute to results above $m$. Specifying a maximum limit $M$ helps to prevent acceptance of product that may greatly exceed quality or safety indicators without further investigation or action.

For serious and severe hazards (Cases 10–15), when $c = 0$, the maximum acceptable level is $m = M$. For Cases 10–15, test results are greatly influenced by sample size because they are typically reported as being present (positive) or absent (negative) in the sample tested. For this book, the analytical unit for each sample, $n$, for Cases 10–15 is 25 g unless otherwise specified. Thus, for

**Table 7.1** Sampling plan stringency (case) in relation to degree of risk and conditions of use

| Degree of concern relative to utility and health hazard | Examples | Conditions under which food is expected to be handled and consumed after sampling in the usual course of events[a] | | |
|---|---|---|---|---|
| | | Reduce risk | No change in risk | May increase risk |
| *Utility*: General contamination, reduced shelf life, incipient spoilage | Aerobic colony count, yeasts and molds | Case 1<br>$n=5$, $c=3$ | Case 2<br>$n=5$, $c=2$ | Case 3<br>$n=5$, $c=1$ |
| *Indicator*: Low, indirect hazard | Enterobacteriaceae, generic *E. coli* | Case 4<br>$n=5$, $c=3$ | Case 5<br>$n=5$, $c=2$ | Case 6<br>$n=5$, $c=1$ |
| *Moderate hazard*: Not usually life threatening, usually no sequelae, normally of short duration, symptoms self-limiting, can be severe discomfort | *S. aureus*, *B. cereus*, *C. perfringens*, *V. parahaemolyticus* | Case 7<br>$n=5$, $c=2$ | Case 8<br>$n=5$, $c=1$ | Case 9<br>$n=10$, $c=1$ |
| *Serious hazard*: Incapacitating but not usually life threatening, sequelae are rare, moderate duration | *Salmonella*, *L. monocytogenes* | Case 10<br>$n=5$, $c=0$ | Case 11<br>$n=10$, $c=0$ | Case 12<br>$n=20$, $c=0$ |
| *Severe hazard*: For the general population or in foods targeted for susceptible populations, causing life threatening or substantial chronic sequelae or illness of long duration | For the general population, *E. coli* O157:H7, *C. botulinum* neurotoxin; for restricted populations, *Salmonella, Cronobacter* spp.; *L. monocytogenes* | Case 13<br>$n=15$, $c=0$ | Case 14<br>$n=30$, $c=0$ | Case 15<br>$n=60$, $c=0$ |

[a] More stringent sampling plans would generally be used for sensitive foods destined for susceptible populations

Case 10, $n=5$, five individual 25 g samples are analyzed. Statistical considerations underlying the sampling plans recommended in this book are discussed in Appendix A and explained in greater detail with examples by van Schothorst et al. (2009), Whiting et al. (2006) and ICMSF (2002).

## *7.4.1 Comparing ICMSF Cases to Codex Criteria for L. monocytogenes*

The following example evaluates the relative stringency of ICMSF cases, which use a qualitative risk assessment approach for groups of microorganisms, to the Codex Alimentarius Commission criteria for *L. monocytogenes* in ready-to-eat (RTE) foods, which was based on quantitative risk assessments.

### 7.4.1.1 Stringency of Sampling Plans Using ICMSF Cases

The relative stringency of selected ICMSF cases is compared in Table 7.2, using various hypothetical values for $m$ and $M$. The mean concentration that would be correctly rejected with a probability of 95% is provided using the calculations of van Schothorst et al. (2009). A standard deviation of 0.8 and a log

**Table 7.2** Relative performance of ICMSF cases in terms of the mean concentrations (in bold text) that will be rejected with at least 95% probability, assuming hypothetical criteria and a standard deviation of 0.8

| Type and likely change to level of hazard | Reduce | No change | May increase |
|---|---|---|---|
| Indicator, indirect hazard; $m = 1{,}000$/g, $M = 10{,}000$/g | Case 4<br>$n = 5$, $c = 3$<br>**5,100 CFU/g** | Case 5<br>$n = 5$, $c = 2$<br>**3,300 CFU/g** | Case 6<br>$n = 5$, $c = 1$<br>**1,800 CFU/g** |
| Moderate hazard; $m = 100$/g, $M = 10{,}000$/g | Case 7<br>$n = 5$, $c = 2$<br>**2,600 CFU/g** | Case 8<br>$n = 5$, $c = 1$<br>**1,100 CFU/g** | Case 9<br>$n = 10$, $c = 1$<br>**330 CFU/g** |
| Serious hazard; $m = 0$/25 g | Case 10<br>$n = 5$, $c = 0$<br>**1 CFU/55 g** | Case 11<br>$n = 10$, $c = 0$<br>**1 CFU/100 g** | Case 12<br>$n = 20$, $c = 0$<br>**1 CFU/490 g** |
| Severe hazard; $m = 0$/25 g | Case 13<br>$n = 15$, $c = 0$<br>**1 CFU/330 g** | Case 14<br>$n = 30$, $c = 0$<br>**1 CFU/850 g** | Case 15<br>$n = 60$, $c = 0$<br>**1 CFU/2,000 g** |

normal distribution is assumed. As the severity of hazard increases, the stringency of the cases increases and the mean concentration that can be reliably detected decreases (from top to bottom). The mean concentration also decreases as the potential for the hazard increases from left to right.

#### 7.4.1.2 Stringency of Codex *L. monocytogenes* Criteria

The criteria for *L. monocytogenes* in RTE food recommended in this book where developed through the step-wise consensus process within the Codex Alimentarius Committee for Food Hygiene. FAO/WHO (2004) conducted a risk assessment on *L. monocytogenes* in RTE foods to address questions on the risk of serious illness in relation to the level of *L. monocytogenes* in food for different susceptible populations relative to the general population, as well as the risk of serious illness from *L. monocytogenes* in foods that support and do not support its growth at specific storage and shelf life. The risk assessment indicated that the vast majority of listeriosis cases were associated with the consumption of foods that do not meet current standards for *L. monocytogenes* (not detected in 25 g or <100 CFU/g) and that the greatest benefit to public health would be to effect a significant reduction in the number of servings contaminated with high numbers of *L. monocytogenes* (FAO/WHO 2004). Therefore, control measures that reduced the frequency of contamination would be expected to have a proportional reduction in the rates of illness.

The risk assessment used a worst case scenario, where it was assumed that all servings had the maximum level being considered, as well as a more realistic approach that allowed for a distribution of the levels of *L. monocytogenes* to be considered. Both scenarios demonstrated that as the frequency or level of contamination increased the risk and the predicted number of cases also increased. It was assumed that ingestion of a single cell could potentially cause illness. According to the risk assessment and assuming a fixed serving size, if all RTE foods went from having 1 to 1,000 CFU/serving, the risk of listeriosis would increase 1,000-fold (see Table 7.3).

In contrast, the risk associated with introducing 10,000 servings of food that were contaminated with 1,000 *L. monocytogenes* CFU/g into the food supply would, theoretically be compensated by removing a single serving contaminated at a level of $10^7$ CFU/g from the food supply. In interpreting these results and the actual effect of a change in the regulatory limits for *L. monocytogenes* in RTE foods, one also has to take into account the extent to which noncompliance with established limits occurs. Based on data available for the US, where the limit for *L. monocytogenes* in RTE foods was

**Table 7.3** Relative risk of illness and estimated number of cases/year in the United States if all RTE meals were contaminated at that level. Relative risk used the risk from a dose of 1 CFU (FAO/WHO 2004)

| Level (CFU/g) | Dose (CFU) | Relative risk | Estimated number of cases/year |
|---|---|---|---|
| <0.04 | 1 | 1 | 0.54 |
| 0.1 | 3 | 2.5 | 1 |
| 1 | 32 | 25 | 12 |
| 10 | 316 | 250 | 118 |
| 100 | 3,160 | 2,500 | 1,185 |
| 1,000 | 31,600 | 25,000 | 11,850 |

0.04 CFU/g, the estimated number of cases for listeriosis for that population was 2,130, using the baseline level in the US *Listeria* risk assessment (FDA-FSIS 2003). If a level of 0.04 CFU/g was consistently achieved, one could expect <1 case of listeriosis/year. This, in combination with available exposure data, suggested that a portion of RTE food in the US contains a substantially greater number of the pathogen than the limit of 0.04 CFU/g and that the public health impact of *L. monocytogenes* is almost exclusively a function of the foods that greatly exceed that limit. Therefore it could be asked if a less stringent microbiological limit for RTE foods could be beneficial in terms of public health if it simultaneously fostered the adoption of control measures that resulted in a substantial decrease in the number of servings that greatly exceeded the established limit. The results of the risk assessment illustrated that the potential for growth of *L. monocytogenes* strongly influences risk, though the extent to which growth occurs depends on the characteristics of the food and the conditions and duration of refrigerated storage. Using selected RTE foods, their ability to support the growth of *L. monocytogenes* appears to increase the risk of listeriosis 100- to 1,000-fold on a per-serving basis. In order to reflect the difference in relative risk different criteria were developed depending on whether the product will support the growth (Table 7.4).

The criterion for products that do not support the growth of *L. monocytogenes* (i.e., 5 samples with a limit of $10^2$ CFU/g) would reject a lot of food, with a probability of 95%, when the geometric mean concentration was 80 CFU/g, assuming a standard deviation of 0.8 (see Appendix A). This criterion reflects the conclusion from the risk assessment that the vast majority of listeriosis cases result from the consumption of high numbers of *L. monocytogenes* and also the desire to use a level that helps promote compliance within the industry. In contrast, the criterion for products that may support growth is much more stringent. This criterion also uses 5 samples but has a much more stringent limit of absence in 25 g for each analytical unit. This would be able to reject a lot with a geometric mean concentration of 1 CFU in 55 g with 95% confidence (assuming a standard deviation of 0.8). It should be noted that in this example a standard deviation of 0.8 was used to calculate the relative stringency of the ICMSF cases, whereas a standard deviation of 0.25 was used for calculations in the Codex Annex (Codex Alimentarius 2009). The effect of using different standard deviation values from 0.25 to 1.2 on the relative performance of different criteria is given in Appendix A. The risk assessment estimated that products that support growth represent a 100- to 1,000-fold increase in risk per serving. This relative difference in stringency and also comparison to existing ICMSF cases is illustrated in the Fig. 7.1. This criterion provides a higher degree of confidence that *L. monocytogenes* will not be present in foods that represent the greatest risk from illness and is therefore approximately 1,000 times more stringent than the criterion for products that do not support growth.

In this book, the Codex criteria for *L. monocytogenes* are used in place of ICMSF cases.

**Table 7.4** Codex criteria for *L. monocytogenes* in RTE foods (Codex Alimentarius 2009) and relative performance in terms of the log mean concentration (in bold text) that will be rejected with at least 95% probability, assuming a standard deviation of 0.8

| | | | | Sampling plan and limits/g | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Ready-to-eat foods that do not support growth | *L. monocytogenes* | ISO 11290-2 | NA[b] | 5 | 0 | $10^2$ | NA |
| | | **Log mean concentration rejected = 80 CFU/g** | | | | | |
| | | | | Sampling plan and limits/25 g | | | |
| | | | | *n* | *c* | *m* | *M* |
| Ready-to-eat foods support growth | *L. monocytogenes* | ISO 11290-1 | NA | 5[c] | 0 | 0 | NA |
| | | **Log mean concentration rejected = 1 CFU in 55 g** | | | | | |

[a] Alternative methods may be used when validated against ISO methods
[b] NA = not applicable as Codex criterion used in place of ICMSF cases
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

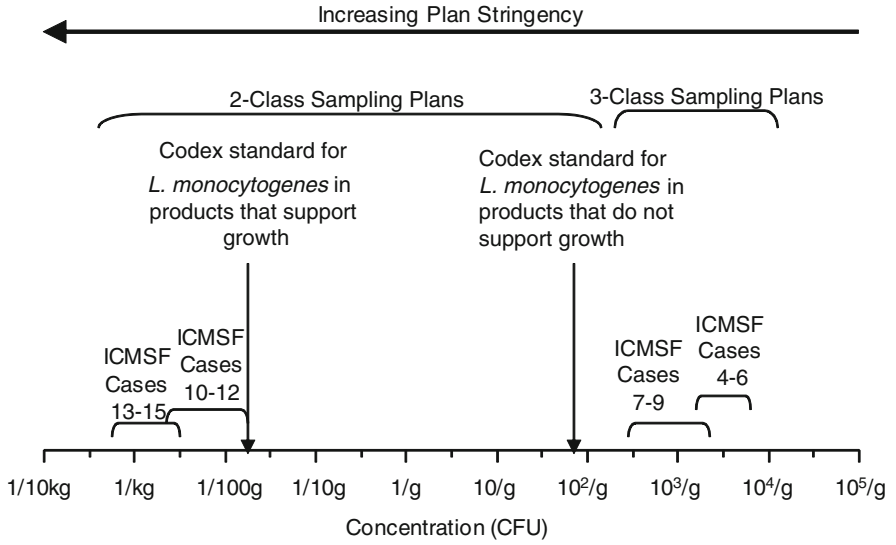


**Fig. 7.1** Geometric mean concentrations of hazard rejected with at least 95% probability for Codex *L. monocytogenes* standards and ICMSF Cases 4–6 ($m = 10^3$/g, $M = 10^4$/g), Cases 7–9 ($m = 10^2$/g, $M = 10^4$/g), and Cases 10–15 ($m = 0$/25 g), assuming a standard deviation of 0.8

## 7.5 Limitations of Microbiological Tests

When used properly and combined with validated process controls, testing can provide actionable information that helps to assure the safety and stability of the products produced. However, testing cannot guarantee the safety of the product. Microbiological testing alone may convey a false sense of security due to the statistical limitations of sampling plans, particularly when the hazard presents an unacceptable risk at low concentrations and has a low and variable prevalence. This is because microorganisms are not homogeneously distributed throughout food and therefore, testing may fail to

detect organisms present in the batch when the sample is taken from an acceptable portion of the batch. Food safety is always a result of several factors and it is ensured primarily through appropriate preventive, *proactive* measures applied along the food chain (e.g., primary production, ingredients, in-process and processing environment) and not through a microbiological testing alone. End product testing alone is *reactive* and deals only with consequences and not with the causes of the problems.

### *7.5.1 Analytical Method*

To be complete, it is important to identify the analytical method that is associated with a microbiological criterion because variation can exist between the results generated by different methods. Considerations in assessing and assuring the performance of microbiological analytical methods are discussed in Appendix A, Sampling Considerations and Statistical Aspects of Sampling Plans. Estimates for the performance of sampling plans presented in this book do not take into account any errors that might occur from the microbiological methods used to determine either the presence or concentration of microorganisms in foods. For consistency, with the Codex Alimentarius Commission, International Standards Organization (ISO) methods are used for most of the criteria identified in this book. Appendix C provides a list of the ISO methods referenced in the product chapters. Other methods may be used if validated against the ISO methods identified.

### *7.5.2 Analytical Units and Compositing*

For serious and severe hazards, enrichment methods are generally recommended to increase the likelihood that contamination can be detected. Enrichment methods rely on growth of the pathogen to a level that can be detected in the enrichment medium and the level of detection can vary depending on the analytical method used. In most instances, this book recommends use of 25 g analytical units for enrichment methods. Each 25 g analytical unit should be selected individually. However, for analysis, multiple units (e.g., 5, 10, 15, 20 etc.) may be composited and run as one test *if the method has been validated* to detect growth of a single cell after the period of enrichment. Jarvis (2007) reviewed the practical considerations to ensure that testing composited samples is as sensitive as testing individual units.

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# Chapter 8
# Meat Products

## 8.1 Introduction

Meat is an important international commodity, consisting of fresh (chilled and frozen) meats and a wide variety of fermented, dry-cured and smoked, as well as cooked products. Shipping whole lamb carcasses and parts occurs. Beef and pork may also be shipped as half-carcasses or converted into primal cuts, retail cuts, boneless meat and trimmings. Raw meat is an important source of human enteric diseases caused by salmonellae, thermophilic *Campylobacter* spp., toxigenic *E. coli* O157:H7 and other enterohemorrhagic *E. coli* (EHEC) strains and *Yersinia enterocolitica*. In general, foodborne disease from these pathogens is due to under cooking or under processing (e.g., improperly fermented meats). The pathogens also may be transferred from the raw meat to ready-to-eat foods. Outgrowth of surviving spores of *Clostridium perfringens* during slow chilling or improper holding of cooked meats is also a problem in foodservice and home settings.

Fresh chilled meat is highly perishable and will spoil under the best of conditions unless frozen. Meat is preserved by adding salt and other ingredients and processing (e.g., fermenting, drying, cooking, canning) in many regions of the world. The conditions of processing and holding can lead to other risks of foodborne illness that are discussed under each product category.

Raw meat is often purchased as an ingredient in chilled or frozen form. While microbiological testing can be performed on the meat, this is an ineffective approach to controlling quality. A preferred approach is for the buyer and supplier to agree on a purchase specification that includes the maximum number of days from slaughter (e.g., 3–10 days), microbial data on process hygiene, and the conditions of chilling, storage and distribution (e.g., ≤5°C). By controlling time and temperature, microbial safety and quality may be better assured for the intended purpose. While there are no standardized procedures for establishing such specifications, they must take into account practical considerations such as the time required to convert carcasses into the desired cuts of chilled meat and shipping, including allowance for nonworking days (e.g., weekends and holidays). The temperature of the meat may vary with the method of chilling (e.g., air chill, $CO_2$ snow) and the size of the portions of meat but, typically internal temperatures of ≤5°C are common when received by customers. An exception may be large beef rounds that are chilled for ≤24 h (at minimum ≤7°C) before shipping.

Another alternative is to purchase frozen raw meat from suppliers that have procedures that control the freezing rate. The method of packing, palletizing and freezing can influence whether microbial growth and spoilage occurs before the meat is frozen in the center of the pack. Manufacturers of certain cooked products prefer mixing chilled and frozen meat to achieve desired temperatures and conditions during processing. Chilled and frozen products may also be mixed during production of products such as salami to keep the fat cold and thus prevent smear when filled into the casing.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_8,

Additional information on the microbiology of meat products is available (ICMSF 2005). The Codex Alimentarius Commission's Code of Hygienic Practice for Meat (Codex Alimentarius 2005) provides guidance for managing microbiological risks associated with meat products.

## 8.2 Primary Production

Conditions for raising livestock differ significantly throughout the world and range from small family-owned farms having one or more animals to large specialized livestock operations. As farm sizes increase and become more specialized, financial investment and concern for animal disease increases. Larger farms must implement more stringent controls to achieve faster growth rates at lower cost with greater yields of higher quality meat and other products. With fewer but larger farms, there is an opportunity to establish national on-farm control programs to improve the conditions necessary to reduce pathogens of concern to human health as well as livestock. For example, regulations that prevent feeding raw, uncooked garbage to pigs successfully reduced the prevalence of *Trichinella spiralis* in pigs and, thereby, reduced the risk of trichinosis among humans in the USA. Likewise, programs adopted in certain countries to improve control of *Salmonella* in livestock at the farm level, for example EU Regulation 2160/2003/EC on control of *Salmonella* or other specified foodborne zoonotic agents.

## 8.3 Raw Meat Products, Excluding Comminuted Meats

This section covers fresh chilled or frozen meat product other than comminuted meats that are typically intended to be cooked.

### *8.3.1 Significant Organisms*

#### 8.3.1.1 Hazards and Controls

Significant hazards for fresh meat are salmonellae and campylobacters. In beef, *E. coli* O157:H7 and other EHEC strains are also a concern, especially in products that may not receive sufficient heat to render the product safe. Fresh pork is a primary source for *T. spiralis* and pathogenic strains of *Y. enterocolitica*. The microbiological content of packaged fresh meat reflects the conditions of the incoming livestock, slaughtering, chilling, cutting, deboning, etc. Control consists of on-farm good animal husbandry practices, contamination prevention during slaughter and microbial contamination reduction by surface treatment of carcasses before chilling. Some surface treatments (e.g., steam, hot water, acid sprays and dips) are not permitted in certain countries.

The Codex Alimentarius Commission's Code of Hygienic Practice for Meat (Codex Alimentarius 2005) provides guidance for managing microbiological risks associated with raw meat.

#### 8.3.1.2 Spoilage and Controls

Four factors influence the microbial spoilage of raw meat at refrigeration temperatures, (1) the numbers and types of psychrotrophic bacteria, (2) the inherent pH of the meat, (3) the storage temperature and (4) the type of packaging, including modified atmosphere or vacuum packaging. These factors should be controlled. Effective implementation of GHP is the primary factor affecting the number and type of psychrotrophic bacteria on raw meat. Equipment should be designed for ease of maintenance and cleanability, and the equipment and processing environment must be cleaned and disinfected at

intervals that can maintain low levels of the psychrotrophic spoilage bacteria. Rooms used for cutting, trimming or deboning chilled carcasses should be maintained at chill temperatures.

The inherent pH of muscle tissue (e.g., pH 5.4–6.5) cannot be altered but should be understood since it is an important factor influencing shelf life of raw, refrigerated meats. Storage temperature, however, can be controlled and storage below 4°C can have a profound beneficial impact on keeping quality. Shelf life is maximized at temperatures approaching the freezing point of meat (about −1.5°C).

The type of packaging can influence the rate of growth and the microorganisms that ultimately cause spoilage. For example, raw meat has a longer shelf life when vacuum packaged or packaged with a gas atmosphere containing carbon dioxide compared with packaging in an oxygen permeable film. Trace amounts of oxygen can influence the rate of spoilage in vacuum packaged meats. Frozen meat typically does not undergo microbial spoilage.

The above information also applies to offal and other by-products (livers, hearts, kidneys, head meat, etc.). Slaughtering operations must provide removal and chilling of these internal organs and meats in a timely manner to prevent incipient spoilage.

## *8.3.2 Microbial Data*

Table 8.1 summarizes useful testing for fresh chilled and frozen meat products, excluding comminuted meats, for microbiological safety and quality.

### 8.3.2.1 Critical Ingredients

Fresh meats available in international commerce, by definition, should not contain added ingredients. Some retail products include added spices or flavorings to marinate the product during refrigerated distribution, storage and display. These ingredients are not likely to influence shelf life unless they introduce psychrotrophic bacteria capable of growing on the product under the conditions of packaging. Certain ingredients, such as vinegar and salt, could reduce the spoilage rate, if present in sufficiently high concentration.

### 8.3.2.2 In-Process

The most common sampling times for control of slaughter process hygiene are before or after the carcasses are chilled. Prechill samples can reflect the level of slaughter process hygiene related to meat safety (e.g., the numbers of *E. coli* or *Enterobacteriaceae* which indicate fecal contamination). Postchill samples reflect all the previous effort to minimize contamination during the slaughtering and chilling. Samples typically consist of swabs, sponges or tissue samples from specified locations on the carcass. Subsequent tissue samples can be collected after the carcasses are cut into portions for further processing or retail packages. Typical levels encountered in operations that apply multiple hurdles during slaughter are an aerobic colony count of $<10^3$ CFU/cm$^2$ carcass surface or $<10^4$ CFU/g of tissue from cut meat when plates are incubated at 35°C. These counts can vary considerably depending on the temperature of incubation and the processing methods used in the region. Because of this, regional or internal company standards will vary and specific recommendations are not possible for this category of products.

### 8.3.2.3 Processing Environment

Swab or sponge samples should be collected before the start of operation to verify the effectiveness of cleaning and disinfecting the meat-contact surfaces and equipment used for cutting, trimming, deboning and other steps in converting carcasses to packaged fresh meat. Analysis for aerobic colony counts is

**Table 8.1** Testing of fresh chilled and frozen meat products, *excluding comminuted meats*, for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Fresh meats generally do not contain added ingredients |
| In-process | Medium | Swab, sponge or tissue samples from carcasses before or after entering the chiller, or tissue samples from cut portions can be useful to assess hygiene process control and conditions that affect microbial levels of subsequent product (ISO 17604). See text for typical levels encountered |
| Processing environment | Medium | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting. See text for typical levels encountered |
| Shelf life | Low | Routine shelf life testing of refrigerated raw meat is not recommended. Shelf life testing may be useful to validate code dates of new retail products or when new packaging systems are implemented |
| End product | Medium | Test for indicators or utility organisms for on-going process control and trend analysis of freshly packaged product using internally developed guidelines (see text). Levels developed for processing do not apply during distribution or at retail (see text) |

| | | | | Sampling plan & limits/g[b,c] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Raw, noncomminuted meat | *E. coli* | ISO 16649-2 | 4 | 5 | 3 | 10 | $10^2$ |

| | | |
|---|---|---|
| Medium | Routine lot acceptance sampling is not recommended for salmonellae on raw meat products. In countries or regions that have established performance criteria for salmonellae, use the required sampling plan and tests. Test in regions where ground beef is a continuing source of *E. coli* O157:H7 illness | |

| | | | | Sampling plan & limits/25g[b,c] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Beef trimmings used in ground beef | *E. coli* O157:H7 | ISO 16654 | 14 | 30[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Swab or sponge samples could also be considered
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

commonly used, but other tests (e.g., ATP-bioluminescence), coliforms, Enterobacteriaceae, occasionally staphylococci may provide useful information. A typical level encountered on thoroughly cleaned, disinfected stainless steel is an aerobic colony count of <500 CFU/cm$^2$. Higher numbers may be encountered on other surfaces (e.g., nonmetal conveyor belts). Regulatory standards may be established in some regions.

#### 8.3.2.4 Shelf Life

Shelf life testing may be performed on refrigerated meats, should the company deem this useful, but testing frozen raw meat is not necessary. Shelf life testing may be useful to validate code dates of new retail products or when new packaging systems are installed. The term "code date" may include "use by," "sell by" and "best-before" dates, depending on the region. Verification of the code date can be based simply on sensory evaluation. Microbiological analysis for specific spoilage microorganisms

may be helpful for certain products. Another method is to conduct in-store surveys to verify sensory acceptability relative to the code dates.

#### 8.3.2.5 End Product

Many companies and governments have established criteria for indicators of quality or process hygiene (e.g., aerobic colony count, Enterobacteriaceae, generic *E. coli*). The criteria may be intended for one or more steps in the food chain from slaughter through retail display. Such tests reflect the conditions of slaughter, chilling, and the time and temperature of storage. These values are poor indicators of the prevalence or concentration of enteric pathogens in fresh meats. Also, since psychrotrophic microorganisms increase during storage, distribution and retail display, samples collected at these stages cannot be used to estimate the hygienic conditions during processing and packaging. Samples yielding unacceptable results in distribution and retail display should lead to investigative sampling to determine why they occurred, so that appropriate corrective actions can be implemented. For example, if high levels of *E. coli* are encountered at retail, this may be caused by poor hygienic conditions during manufacture or storage at elevated temperatures (e.g., >7–8°C) that permit growth. Typical levels encountered in operations that apply multiple hurdles during slaughter are an aerobic colony count (incubated at 35°C) of $<10^4$ CFU/g and generic *E. coli* of <10 CFU/g. These counts can vary considerably depending on the temperature of incubation and the processing methods used or allowed in the region. Because of this, regional or internal company standards will vary and specific recommendations are not possible for this category of products.

Indicator tests of frozen products reflect the microbial population at time of freezing and any decrease that may have occurred during distribution and retail display.

There are considerable differences in prevalence rates for salmonellae on fresh meat in different regions and countries. While routine lot acceptance sampling is not recommended for salmonellae on fresh meat products, unique situations can occur where information on the presence/prevalence of salmonellae can provide useful information, such as for outbreak investigations and new supplier qualification.

Of increasing interest is the effort to improve food safety through the application of criteria (e.g., performance objectives) for foodborne pathogens (e.g., salmonellae) at specific steps in the food chain. The growing support for this approach led the Codex Alimentarius Commission to provide guidance to governments for verification of process control of meat hygiene by microbiological testing (Codex Alimentarius 2005). While specific microbiological criteria are not provided, the guidance states that "Establishment of microbiological testing requirements, including performance objectives or performance criteria should be the responsibility of competent authorities, in consultation with relevant interested parties, and may consist of guidelines or regulatory standards." Furthermore, "The competent authority should verify compliance with microbiological testing requirements where they are specified in regulation e.g., microbiological statistical process control requirements, standards for *Salmonella* spp."

Trend analysis is an important component, because the data can be used to measure changes in prevalence rates as industry implements procedures to meet the established requirements. Some countries or regions (e.g., USA, EU) have initiated long-term continuous improvement programs to reduce the prevalence of salmonellae on raw beef and pork products (USDA 1996, 2008; EU 2003, 2005). Ideally, such programs are coupled with guidance that provides science-based, best practices from farm through slaughter and chilling, and relate to a public health goal. It is uncertain whether the approaches (control at the farm, control at the slaughtering plant or a combination of the two) applied by different countries will lead to different degrees of pathogen control and consumer protection. For example, adoption of performance objectives at the plant level for raw meat and poultry has yet to result in reduction of human salmonellosis in the USA that was expected when the pathogen reduction regulation (USDA 1996) was finalized (Cole and Tompkin 2005, CDC 2009).

Lot acceptance sampling of beef trimmings is being used by industry in the USA as a control measure in a comprehensive management system to reduce the risk of *E. coli* O157:H7 in ground beef. For countries or regions where *E. coli* O157:H7 or other EHEC are a pathogen of concern in ground beef, guidance is available for establishing an appropriate sampling plan (ICMSF 2002, Cole and Tompkin 2005, Butler et al. 2006). Epidemiologic data in the USA suggests this practice has contributed to the reduction in disease from *E. coli* O157:H7 in the USA (Cole and Tompkin 2005).

## 8.4 Raw Comminuted Meats

### *8.4.1 Significant Organisms*

#### 8.4.1.1 Hazards and Controls

A wide variety of raw comminuted meat products are produced containing beef, pork, lamb, veal and other meats. The products may contain extenders (e.g., rice, wheat flour, soy protein), spices, herbs and flavoring agents, and are available in many different shapes, sizes and packaging. The hazards of significance in raw comminuted meat products are salmonellae, campylobacters, and when beef and other ruminant species are added, *E. coli* O157:H7 and other EHEC strains. In certain regions, pork products may contain pathogenic strains of *Y. enterocolitica* or *T. spiralis*. Both pathogens can be inactivated by cooking.

#### 8.4.1.2 Spoilage and Controls

See Sect. 8.3.1.2.

### *8.4.2 Microbial Data*

Table 8.2 summarizes useful testing for raw comminuted meats. Refer to the text for important details related to specific recommendations.

#### 8.4.2.1 Critical Ingredients

There are no critical nonmeat ingredients. The primary source of microbial hazards is the raw meat. Since beef trimmings are the primary source of *E. coli* O157:H7, the sampling plan in Table 8.1 is recommended for trimmings to be used for manufacturing ground beef in regions where illness is a concern. Other sampling plans may be proposed. For example, the USDA-FSIS (USDA 2010) refers to "robust" sampling using $n=60$, where each sample is a $1\times3\times0.125$ in. ($2.5\times7.6\times0.32$ cm) surface sample (approximately 340 g). Analysis of trimmings can be used to select suppliers. Working with approved suppliers can lead to improved microbial control of the end products.

#### 8.4.2.2 In-Process

Routine in-process samples are not normally collected. Samples of meat at various stages of processing can be used to establish a baseline and understand changes in the microbial population during processing.

**Table 8.2** Testing of raw comminuted meats for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low to high | Pretesting beef trimmings for *E. coli* O157:H7 may be useful when confidence in supplier control programs is low (see text) |
| In-process | Low | Routine in-process samples are not normally collected. Samples of meat at various stages of processing can be used to establish a baseline and understand changes in the microbial population during processing |
| Processing environment | Low | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting (see text for typical levels encountered) |
| Shelf life | Low | Routine shelf life testing of refrigerated raw meat is not recommended. Shelf life testing may be useful to validate code dates of new retail products or when new packaging systems are installed |
| End product | Medium | Test for indicators or utility organisms for on-going process control and trend analysis of freshly packaged product using internally developed guidelines (see text). Levels developed for processing do not apply during distribution or at retail (see text) |

| | | | | Sampling plan & limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Raw, noncomminuted meat | *E. coli* | ISO 16649-2 | 4 | 5 | 3 | 10 | $10^2$ |

| | | | | Sampling plan & limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Ground beef | *E. coli* O157:H7 | ISO 16654 | 14 | 30[c] | 0 | 0 | – |

Medium: Routine testing is not recommended for salmonellae on raw comminuted meat products (see text). In regions where ground beef is a continuing source of *E. coli* O157:H7 illness, the following criteria are recommended

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to Appendix A for performance of these sampling plans
[c]Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

### 8.4.2.3 Processing Environment

Samples from equipment surfaces before start-up should be used to verify the efficacy of cleaning and disinfecting procedures. Typical aerobic colony counts on thoroughly cleaned, disinfected stainless steel are <500 CFU/cm$^2$. Higher numbers may be encountered on other surfaces (e.g., nonmetal conveyor belts).

### 8.4.2.4 Shelf Life

Shelf life testing of refrigerated raw comminuted meat may be performed if the company finds this useful, but testing of frozen products is not recommended. Shelf life testing may be useful to validate code dates of new retail products or when new packaging systems are installed. Shelf life tests can be performed to periodically verify the code dates applied to retail products.

### 8.4.2.5 End Product

Testing for indicators can be useful for on-going process control and trend analysis of freshly packaged product. Typical levels encountered in operations that apply multiple hurdles during slaughter are an aerobic colony count (incubated at 35°C) of <$10^5$ CFU/g and generic *E. coli* of <$10^2$ CFU/g.

These counts can vary considerably depending on the temperature of incubation and the processing methods used or allowed in the region. Because of this, regional or internal company standards will vary and specific recommendations are not possible for this category of products.

Indicator tests (e.g., aerobic colony count, *E. coli*) of comminuted meats during distribution and retail display cannot be used to assess the hygienic conditions during time of manufacture. If high levels of *E. coli* are encountered at retail, investigational samples are necessary to determine the reason such as poor hygienic conditions during manufacture and/or storage at elevated temperatures (e.g., >7–8°C) that permit growth. Indicator tests of frozen products reflect the microbial population at the time of freezing and any decrease that may have occurred during distribution and retail display.

There are considerable differences in prevalence rates for salmonellae in raw comminuted meats in different regions and countries. A microbiological risk assessment has not been conducted to estimate the risk of salmonellosis as different sampling plans are applied. While routine lot acceptance sampling is not recommended for salmonellae on raw comminuted meats, unique situations (e.g., outbreak investigations, new supplier certification) can occur where data on the prevalence of salmonellae can provide useful information.

The information in Sect. 8.3.2.5 is generally applicable to raw comminuted meats. Due to the public health risk associated with *E. coli* O157:H7 in ground beef, sampling for this pathogen may be appropriate in regions where epidemiological data indicate this can be beneficial. It is important to recognize that the recommended sampling plan cannot ensure that *E. coli* O157:H7 will be absent from the entire lot, particularly with the expected low prevalence. The purpose of the sampling plan is to detect and remove lots of ground beef that have a higher than normal prevalence or concentration of *E. coli* O157:H7 and that will more likely result in illness. Normally, case 13 would apply since ground beef is usually cooked before eating; however, case 14 may be appropriate for regions where *E. coli* O157:H7 or other EHEC are a recognized hazard and undercooking and/or cross-contamination to ready-to-eat foods is likely to occur in homes and food service establishments (ICMSF 2002).

## 8.5 Raw Cured Shelf-Stable Meats

### *8.5.1 Significant Organisms*

#### 8.5.1.1 Hazards and Controls

Two groups of shelf-stable meat products are discussed in this section: (1) traditional raw dry cured hams and (2) dry fermented sausages. The hazards to consider in raw cured shelf-stable meats are salmonellae, EHEC, *Y. enterocolitica*, *Staphylococcus aureus, Clostridium botulinum* and *T. spiralis*. The pathogens of concern depend upon the type of meat (e.g., beef, pork) and the method of manufacture (e.g., dry curing, fermenting, mild heating). While *L. monocytogenes* has been detected in raw cured hams and raw fermented sausages, the product characteristics (e.g., low $a_w$) prevent multiplication. A risk assessment and a risk categorization placed these products in the low category of risk as sources of foodborne listeriosis (FDA-FSIS 2003, FAO/WHO 2004). For dry cured hams, the methods of control are based on traditional practices that have evolved over hundreds of years. Initially, the meat (e.g., pork) is externally coated with salt, which may contain nitrate, nitrite and spices, and held at low temperatures for times sufficient to allow the salt to penetrate throughout the meat. Subsequent drying and aging at higher temperatures for relatively long periods of time (e.g., months) allows additional growth of microorganisms typical for the products (e.g., lactic acid-producing bacteria) and elimination of enteric pathogens.

For dry fermented sausages, use of a commercial starter culture or glucono-delta-lactone (GDL) and processing conditions (e.g., amount of added salt, temperature of fermentation) that favor growth of the

culture, limits growth of *S. aureus* by acidulation process (e.g., pH ≤ 5.3) at a defined period of time and temperature. Another somewhat less reliable method to control *S. aureus* is to hold the sausages at lower temperatures until the moisture content is reduced and, more importantly, enable the indigenous lactic population to multiply. This reduces the likelihood that *S. aureus* will multiply when the temperature is subsequently increased for further processing. Other procedures can be applied.

Survival of *Salmonella*, *E. coli* O157:H7 and *Y. enterocolitica* in improperly manufactured fermented sausage has resulted in illness. These enteric pathogens can be controlled in fermented sausages by applying processes that have been validated to kill the pathogen at levels expected in the raw meat blends and then applying HACCP systems to verify that the required conditions of manufacture are met. Some countries (e.g., Canada, USA) have requirements for validating control of EHEC in fermented meats because the product has been implicated in EHEC infections. These processes may include a mild heating step that may cause the product to lose the raw meat texture traditionally associated with the product. In regions where *T. spiralis* occurs in raw pork, procedures can be applied to inactivate the parasite. One option is to use pork that has been frozen and held for a prescribed time. Another option is to apply processing conditions specified in guidelines or regulations to inactivate the parasite.

#### 8.5.1.2 Spoilage and Controls

By definition these products are shelf-stable and generally do not undergo microbial spoilage during storage and distribution. The method of packaging may be a factor for certain products. Exposure to high humidity can lead to mold spoilage.

### *8.5.2 Microbial Data*

Table 8.3 summarizes useful testing for raw cured shelf-stable meats. Refer to the text for important details related to specific recommendations.

#### 8.5.2.1 Critical Ingredients

The manufacturing processes for meat used in raw, cured, shelf-stable meats should be validated for control of pathogens that occur in the meat. The nonmeat ingredients added to these products are rarely a source of significant pathogens or spoilage organisms. The quantity of some ingredients (e.g., salt,

**Table 8.3** Testing of raw cured shelf-stable meats for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | These products do not contain nonmeat ingredients of significance for microbiological safety or quality |
| In-process | Low | Routine sampling of intermediate products for microbiological testing is not recommended. Critical factors such as time, temperature, rate of pH decline, $a_w$, addition of correct amount of salt and curing agent, must be monitored for safety |
| Processing environment | Low | Routine sampling of equipment and the environment is not recommended |
| Shelf life | Low | These products are inherently shelf-stable |
| End product | Low | Routine sampling of the end products is not recommended |

sodium nitrite) is, however, critical in certain products. Insufficient amounts of salt can lead to pathogen survival and growth. An excessive amount of salt during formulation of sausages to be fermented can slow or prevent the development of the lactic acid bacteria and favor the growth of *S. aureus*.

#### 8.5.2.2 In-Process

For dry cured hams, routine microbial testing at various stages of processing is not performed. Such samples, however, can be helpful in the event a problem occurs and microbiological data are needed. For dry fermented meats, monitoring time, temperature and rate of acid production (decreasing pH) is very important. Routine sampling for pathogens is not recommended since the risk associated with these pathogens is controllable through GHP and the HACCP system. Validated processing conditions should be used for pathogen control.

#### 8.5.2.3 Processing Environment

Sampling the processing environment is generally not recommended for these traditional products. Many of the facilities have a natural flora that has evolved over time and may be beneficial to the process.

#### 8.5.2.4 Shelf Life

These traditional products typically have extended code dates reflecting their stability at ambient temperatures. Shelf life tests are not recommended.

#### 8.5.2.5 End Product

Routine microbiological sampling of these products is not recommended for either safety or quality. Emphasis should be placed on application of GHP, validated processes and monitoring CCPs within the HACCP plan for control of microbiological safety and quality.

## 8.6 Dried Meat Products

### *8.6.1 Significant Organisms*

#### 8.6.1.1 Hazards and Controls

Three general groups of dried meats are produced. The first includes cooked dried meats that are used as ingredients in dried soups and other foods. Cooking and preventing recontamination are important control factors for this class of product.

The second group includes strips of meat or thin sausages that are cooked before drying. These products are sold as snacks or basic ingredients in certain dishes. They may be produced in large quantities in continuous systems or in smaller quantities in batch processing equipment. This product is also produced throughout the world in very small operations, primarily for personal use or local distribution, but this practice can involve fairly wide consumer exposure.

The third group includes a variety of traditional products that are unique to certain regions and have not been cooked (e.g., biltong, charqui).

The microbial hazards to consider in dried meat products are *Salmonella,* EHEC and *S. aureus*. *L. monocytogenes* is not a hazard of concern because the low $a_w$ prevents its multiplication in these products. A risk assessment and a risk categorization have placed these products in the low risk category for foodborne listeriosis (FDA-FSIS 2003, FAO/WHO 2004). Cooking is a CCP for most of these products. Uncontrolled salting and drying conditions can permit growth and enterotoxin production by *S. aureus*. Additional control consists of applying GHP to prevent contamination with enteric pathogens. Extended storage at ambient temperature with high salt (i.e., low $a_w$) can reduce enteric pathogen levels.

#### 8.6.1.2 Spoilage and Controls

Dried meat products are microbiologically stable, although exposure to conditions of high humidity can lead to spoilage by molds.

### *8.6.2 Microbial Data*

Table 8.4 summarizes useful testing for dried meat products. Refer to the text for important details related to specific recommendations.

#### 8.6.2.1 Critical Ingredients

Manufacturing processes for dried meat products should be validated for control of pathogens that occur in the meat. There are no critical nonmeat ingredients.

#### 8.6.2.2 In-Process

Routine in-process samples should not be necessary, but can be helpful in the event of a problem and the source(s) of microbial contamination must be determined.

#### 8.6.2.3 Processing Environment

Routine environmental samples for salmonellae should not be necessary in a controlled operation operating under GHP with adequate separation between raw meat processing areas and where cooked meat products are exposed. Environmental sampling, however, can be helpful in the event a problem does occur and the source(s) of contamination must be determined.

Swab or sponge samples should be collected to verify the effectiveness of cleaning and disinfecting equipment before the start of operation. Analysis for aerobic colony count is typical, but other tests (e.g., ATP-bioluminescence) may provide useful information.

Typical aerobic colony count levels encountered on thoroughly cleaned, disinfected stainless steel are <500 CFU/cm$^2$. Higher numbers may be encountered on other surfaces (e.g., nonmetal conveyor belts).

#### 8.6.2.4 Shelf Life

The final moisture content (i.e., <10%) and low $a_w$ levels make these products microbiologically stable. The strips and thin sausage-shaped products may be higher in moisture for better palatability

**Table 8.4** Testing of dried meat products for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low | These products do not contain nonmeat ingredients of significance for microbiological safety or quality | | | | | | | |
| In-process | Low | Routine in-process samples are not recommended | | | | | | | |
| Processing environment | Medium | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting. (See text for typical levels encountered) | | | | | | | |
| Shelf life | Low | These products are inherently shelf-stable when properly dried and protected from high humidity. The higher $a_w$ of snack products may require verification of stability | | | | | | | |
| End product | Low | Routine sampling is not recommended. If application of GHP and HACCP is in question, sampling for an indicator (e.g., *E. coli*) or *Salmonella* should be considered | | | | | | | |
| | | | | | | Sampling plan & limit/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Low | Dried meat | *E. coli* | ISO 16649-2 | 5 | 5 | 2 | 10 | $10^2$ |
| | | | | | | Sampling plan & limit/25 g[b] | | | |
| | | | | | | *n* | *c* | *m* | *M* |
| | Low | Dried meat | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to Appendix A for performance of these sampling plans
[c]Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

as snacks. If $a_W$ levels are sufficiently high (e.g., >0.70), these products must be packaged in a low oxygen atmosphere to prevent the growth of mold during extended storage or be formulated with a mold inhibitor. Defective packaging seals can contribute to mold spoilage of these products during storage, distribution and retail display.

#### 8.6.2.5 End Product

These products are of low risk to public health and routine sampling is not recommended. If there is reason to question whether GHP and HACCP are being applied in a manner to control enteric pathogens, then sampling for an indicator (e.g., *E. coli*) or salmonellae is recommended. Recommended testing for these products is summarized in Table 8.4.

## 8.7 Cooked Meat Products

### *8.7.1 Significant Organisms*

#### 8.7.1.1 Hazards and Controls

These products are perishable and must be refrigerated or frozen for storage or distribution. Cured and uncured products are included in this section. The microbial hazards to consider in cooked perishable meats include *Salmonella*, EHEC, *L. monocytogenes* and *C. perfringens*. Control of *Salmonella*, EHEC and *L. monocytogenes* requires validated cooking procedures and recontamination prevention;

with cooking managed through the HACCP plan and recontamination managed through effective application of GHP with verification through environmental monitoring (Codex Alimentarius 2009a). Some products are given a final in-package listericidal treatment. Additives may also be used in some countries to inactivate or restrict the growth of *L. monocytogenes*. *Salmonella* and EHEC can survive on cooked refrigerated meat products but cannot multiply if the products are maintained at <7°C.

Control of *C. perfringens* requires chilling cooked meat products at a rate that prevents unacceptable multiplication of surviving spores and storing at <12°C. Historically, a vast majority of *C. perfringens* outbreaks have occurred due to improper chilling or holding in foodservice operations (Brett 1998, Bates and Bodnaruk 2003, Golden et al. 2009). Cured meat products contain sodium nitrite and generally have a higher salt content than uncured products such as roast beef. As a result, cured meat or poultry products rarely are implicated as a source of *C. perfringens* illness.

The microbial hazards on frozen cooked uncured meat products are similar to those for refrigerated products except the vegetative cells of *C. perfringens* are quite sensitive to freezing and decline during frozen storage. Also, *L. monocytogenes* cannot multiply while the product remains frozen.

The Codex Alimentarius Commission's Code of Hygienic Practice for Meat (Codex Alimentarius 2005) provides guidance for managing microbiological risks associated with cooked meat products.

#### 8.7.1.2 Spoilage and Controls

The rate of spoilage is influenced by many factors, such as storage temperature, initial number and type of microorganisms when packaged, type of packaging and chemical composition. Spoilage by psychrotrophic clostridia and lactic acid bacteria has occurred in commercial products having extended refrigerated shelf life (e.g., ≥35 days). Control consists of determining the source of the spoilage bacteria, such as the raw meat or harborage sites in the raw processing environment, and implementing appropriate controls.

### *8.7.2 Microbial Data*

Table 8.5 summarizes useful testing for cooked meat products. Refer to the text for important details related to specific recommendations.

#### 8.7.2.1 Critical Ingredients

The nonmeat ingredients in cooked meat products are rarely a source of significant pathogens or spoilage flora. Some ingredients (e.g., salt, sodium nitrite, sodium lactate, sodium diacetate) can reduce the rate of spoilage and growth of *L. monocytogenes* and clostridia.

#### 8.7.2.2 In-Process

The relative value of testing in-process samples versus processing environment samples for routine assessment of *Listeria* spp. control is debatable. The decision to rely more on in-process over environmental samples may be influenced by regulatory policies and the complexity of the equipment and steps in the process after cooking. Routine in-process sampling is not performed by some manufacturers, while others rely on in-process samples for assessing control. In-process samples can be helpful when investigating a problem and are recommended. Routine sampling for salmonellae, *S. aureus* or *C. perfringens* is not recommended, since the risk associated with these pathogens is controllable through GHP and HACCP.

**Table 8.5** Testing of cooked meat products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | These products do not contain nonmeat ingredients of significance for microbiological safety or quality |
| In-process | High | Monitoring the cooking parameters is essential |
| | Medium | For products that support *L. monocytogenes* growth, postcook samples can assess control of *Listeria* spp. Typical levels encountered postcook:<br>• *Listeria* spp. – absent |
| Processing environment | High | For products that support *L. monocytogenes* growth, during production sample product contact surfaces where cooked products are exposed to potential contamination before packaging. Sponge or swab samples from floors, drains and other nonproduct contact surfaces can provide an early indication of the level of control and a potential risk of contamination for equipment and product. Typical levels encountered:<br>• *Listeria* spp. – absent |
| | Medium | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting. (See text for typical levels encountered) |
| Shelf life | Medium | Shelf life testing may be useful for refrigerated products with extended code dates (see text). Shelf life testing of frozen cooked meats is not necessary |
| End product | Medium | Test for indicators for ongoing process control and trend analysis (see text) |

| | | | | Sampling plan & limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Cooked meat | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $10^4$ | $10^5$ |
| | *E. coli* | ISO 16649-2 | 5 | 5 | 2 | 10 | $10^2$ |
| | *S. aureus* | ISO 6888-1 | 8 | 5 | 1 | $10^2$ | $10^3$ |
| Cooked uncured meat (e.g., roast beef) | *C. perfringens* | ISO 7937 | 8 | 5 | 1 | $10^2$ | $10^3$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Medium | Routine sampling for pathogens is not recommended. If application of GHP or HACCP is in question, the following sampling plans are recommended (see text) | | | | | | |

| | | | | Sampling plan & limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Cooked meat | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |
| Cooked meat: No growth | *L. monocytogenes* | ISO 11290-2 | NA[d] | 5 | 0 | $10^2$ | – |
| Cooked meat: Supports growth | *L. monocytogenes* | ISO 11290-1 | NA | 5[c] | 0 | 0 | – |

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to Appendix A for performance of these sampling plans
[c]Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[d]*NA* not applicable; used Codex criterion

### 8.7.2.3 Processing Environment

The relative importance of verifying control of the processing environment depends on the risk to consumers if the product becomes contaminated between cooking and final packaging. The products of highest concern are those that support the growth of *L. monocytogenes* during normal storage and distribution and do not have a listericidal treatment after final packaging, especially if the intended consumers are highly susceptible to listeriosis. The frequency and extent of sampling also should reflect consumer risk.

Monitoring programs that include sampling equipment and other surfaces that come into contact with exposed cooked products before final packaging are recommended. Sponge samples from large

areas of equipment should be collected during production. Samples can also be collected from nonproduct contact surfaces as an additional measure of control (Codex Alimentarius 2009a). Environmental sampling for products given a validated final in-package listericidal treatment is not recommended. Environmental monitoring for products that do not support growth depends on the products produced in the facility (e.g., some products support growth and others do not), historical trends and regulatory requirements.

The principles for control and monitoring of *Listeria* can also be applied to spoilage microorganisms such as lactic acid bacteria. Swab or sponge samples can be collected before the start of operation to verify the effectiveness of cleaning and disinfecting. Analysis for aerobic colony count is a common analysis, but other tests (e.g., ATP-bioluminescence) may provide useful information. Typical aerobic colony counts on thoroughly cleaned, disinfected stainless steel are <500 CFU/cm$^2$. Higher numbers may be encountered on other surfaces (e.g., nonmetal conveyor belts).

#### 8.7.2.4 Shelf Life

Code dating practices can be validated by holding the product at a controlled temperature and performing sensory evaluation and microbiological analysis at selected intervals, including packages before, on and after the expected expiration date. Subsequent verification can be performed at a frequency that reflects confidence in whether the product will consistently meet the stated expiration date on the package. Shelf life testing of frozen cooked meat products is not necessary.

Validating that growth of *L. monocytogenes* will not occur within the code date applied on the package may also be useful (EU Regulation 2073/2005/EC, Chap. 1, Sects. 1.1, 1.2 and 1.3). This regulation defines the food safety criteria for the validation of RTE products (including meat products) regarding presence or number of *L. monocytogenes* in the end product. The manufacturer should be able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of $10^2$ CFU/g of *L. monocytogenes* throughout the shelf life. Therefore, the operator may establish intermediate limits during the production process that should be low enough to guarantee that the limit of $10^2$ CFU/g is not exceeded at the end of the shelf life and, for RTE products that are able to support the growth of *L. monocytogenes*, that absence of the pathogen in 25 g of sample at the end of the manufacturing process is assured. Guidelines for validation are available (Scott et al. 2005 and Chap. 2).

#### 8.7.2.5 End Product

Recommended end product testing is summarized in Table 8.5. Testing for indicators such as aerobic colony count and *E. coli* is useful to evaluate ongoing process control and trend analysis. Aerobic colony counts typically encountered are <$10^4$ CFU/g and *E. coli* is typically <10 CFU/g. Indicator tests during distribution and retail display cannot be used to assess the conditions during time of manufacture. If high levels of *E. coli* are encountered at retail, investigational samples are necessary to determine the reason such as poor hygienic conditions during manufacture and/or storage at elevated temperatures (e.g., >7–8°C) that permit growth.

The *Salmonella* sampling plan in Table 8.5 assumes that it will not grow under the normal conditions of distribution and storage and that the product will not receive a further cook step (i.e., case 11). Use of case 10 or 12 would be appropriate if the product would be subject to further cooking (e.g., cooked meat used in a frozen entrée that is to be cooked prior to consumption) or if there is considerable potential for produce abuse prior to consumption, respectively. The sampling plans for *L. monocytogenes* are for ready-to-eat foods produced following the general principles of food hygiene for control of *L. monocytogenes* and with an appropriate environmental monitoring program (Codex Alimentarius 2009b).

If the reliable application of GHP and HACCP is in question, sampling for *Salmonella* and/or *L. monocytogenes* may be appropriate. When evidence indicates a potential for contamination with

*L. monocytogenes* (e.g., positive food contact surface results or the effectiveness of corrective actions has yet to be verified) sampling the food should be considered. The stringency of sampling should reflect consumer risk (e.g., whether growth can occur in the food, intended consumers). Guidance on increasing the stringency of sampling by sub-lotting is discussed in Chap. 5.

If the rate of chilling after cooking exceeds the critical limit in the HACCP plan, testing for *C. perfringens* may provide useful information to determine the disposition of the lot. The sample units should be taken from the center of the product or other region that is slowest to chill. Samples should be submitted to the laboratory as refrigerated, not frozen, samples. The decision to test for *C. perfringens* will depend on the available information (e.g., pH, $a_w$, added inhibitors such as sodium nitrite, lactate or diacetate), the extent of the deviation and options that may be available for product disposition. A sampling plan is also provided for products in which temperature abuse is suspected and *S. aureus* is of concern.

If there is a failure to meet the criteria for *L. monocytogenes* or *Salmonella* in Table 8.5, the typical actions to take include (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption, and (3) determine and correct the root cause of the failure.

## 8.8 Fully Retorted Shelf-Stable Uncured Meats

### *8.8.1 Significant Organisms*

The hazards and controls are the same as applied for other low-acid canned foods (see Chap. 24). Spoilage of canned uncured meat products is controllable and should rarely occur. Incipient spoilage may occur if the product is not retorted in a timely manner. This can occur when equipment breaks down and the food is held for an extended period of time before retorting.

### *8.8.2 Microbial Data*

There are no critical nonmeat or meat ingredients for these products. Routine in-process, environmental, and end product testing are not recommended for either safety or quality. Current recommended procedures for commercial processing based on GHP and HACCP yield products that are commercially sterile and stable for the expected conditions of storage and distribution.

## 8.9 Shelf-Stable Cooked Cured Meats

### *8.9.1 Significant Organisms*

#### 8.9.1.1 Hazards and Controls

The hazards of significance in the raw meat ingredients used for these products are salmonellae, *C. botulinum* and, in the case of products containing beef, *E. coli* O157:H7 and other EHEC strains. The heat process used for shelf-stable canned cured meats destroys vegetative microorganisms, some spores and sublethally damages other spores. Safety and stability depends upon the combined effect of thermal destruction or injury of a low indigenous number of spores and inhibition of the survivors by an adequate amount of added salt and sodium nitrite.

For shelf-stable liver, blood and bologna-style sausages, important factors to control are initial spore load, heat treatment, pH, $a_w$, and nitrite. For products like Italian mortadella and German bruhdauerwurst, stability is achieved by heating to >75°C to inactivate vegetative cells, reducing $a_w$ to <0.95 and heating in a sealed container to prevent recontamination.

Brawns are made shelf-stable by adjusting the pH to 5.0 with acetic acid and protecting the product from recontamination after heating. Gelder smoked sausage (a traditional Dutch product) is made shelf-stable by adjusting the pH to 5.4–5.6 with GDL, reducing $a_w$ to 0.97, vacuum-packing, and heating for 1 h to a center temperature of 80°C.

#### 8.9.1.2 Spoilage and Controls

These products are shelf-stable and generally do not undergo microbial spoilage during storage and distribution. Spoilage might occur due to postprocessing contamination through leaks in the container (e.g., in the seams of cans or through the clip-seals of plastic casings) or from growth of *Bacillus* spp. just under the casing. The extent of growth is determined mainly by product composition and the oxygen permeability of the casing or container.

### *8.9.2 Microbial Data*

The ingredients added to these products are rarely a source of significant pathogens or spoilage microorganisms. However, the level of some ingredients, such as salt, sodium nitrite, and acidulants is critical for safety and spoilage control. Insufficient amounts of these ingredients can permit growth of surviving spores, including *C. botulinum*, if present.

Routine in-process and environmental samples are not recommended. Products produced following recommended guidance and programs based on GHP and HACCP should not experience microbial spoilage. Routine sampling of these products is not recommended for either quality or safety.

## 8.10 Snails

### *8.10.1 Significant Organisms*

The hazards to consider include salmonellae, shigellae, EHEC and parasites. The conditions of growing and harvesting influence the potential presence of enteric pathogens. Snails should be cooked to inactivate enteric pathogens and parasites. Freezing is another means to inactivate parasites. Recontamination of the cooked snails should be prevented through GHP. Snails are also sold as a canned shelf-stable food (see Chap. 24). Freezing or canning prevents microbial spoilage. Time and temperature of storage of fresh snails and frozen snails after thawing will influence the rate of spoilage.

### *8.10.2 Microbial Data*

There are no critical ingredients. Routine in-process and environmental samples are not normally collected. Code dating practices for fresh snails can be validated as described for most other raw foods. Enteric pathogens should be assumed to be present and cooking or canning will eliminate these pathogens before they are eaten. Routine sampling of fresh and frozen snails for pathogens is not recommended.

## 8.11 Frog Legs

### *8.11.1 Significant Organisms*

Frog legs are typically distributed as a raw frozen product, which may be thawed during retail display. The hazard of significance is *Salmonella*. *Shigella* may be a concern if frogs are raised in insanitary ponds that may contain human waste. The time between capture and slaughter should be minimized. Care should be exercised in removal of the legs to avoid cutting the intestinal tract. Processing water should be chlorinated and equipment and contact surfaces should be cleaned and disinfected. Guidance for the hygienic processing of frog legs is available from the Codex Alimentarius Commission (Codex Alimentarius 1983). Freezing prevents microbial spoilage. Time and temperature of storage after thawing will influence the rate of spoilage.

### *8.11.2 Microbial Data*

There are no critical ingredients. Routine in-process and environmental samples are not normally collected. See Sect. 8.3.2.3 for guidance assessing cleaning and disinfecting procedures. Microbial spoilage of frozen frog legs should not occur. The Codex Alimentarius Commission guidance for end product specifications is very general: "Frog legs should be free from microorganisms in amounts harmful to man, free from parasites harmful to man and should not contain any substances originating from microorganisms in amount which may represent a hazard to health" (Codex Alimentarius 1983). Salmonellae should be assumed to be present on raw frog legs. Routine sampling of frozen frog legs for salmonellae and other pathogens is not recommended.

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# Chapter 9
# Poultry Products

## 9.1 Introduction

Fresh and frozen raw poultry products are considered important sources of human illness due to salmonellae and thermophilic *Campylobacter* spp. Two scenarios are typically involved – undercooking or cross-contamination from raw poultry to ready-to-eat foods. Raw poultry meat is highly perishable and spoils under the best of conditions unless frozen. As storage temperature increases, raw poultry spoils at a faster rate due to the increased rate of microbial growth and metabolism.

Cooked, perishable poultry products have also been associated with foodborne disease when *L. monocytogenes* has multiplied during distribution and storage. Dried poultry products are rarely involved in foodborne illness, although survival of salmonellae due to undercooking or contamination during drying and packaging has occurred in operations with poor control of GHP.

Many companies and institutions purchase fresh or frozen raw poultry as an ingredient, and the sensory quality of fresh raw poultry for further processing should be controlled. The preferred means of control is for the buyer and supplier to agree on specifications for the maximum number of days from slaughter and the conditions of chilling, storage and distribution (e.g., ≤4°C). By controlling time and temperature, sensory quality can be managed for the intended purpose. Another alternative is to purchase frozen raw poultry from suppliers that have procedures to control the rate at which the poultry meat is frozen. The method of packing, palletizing and freezing can influence whether microbial growth and spoilage occurs before the meat is frozen in the center of the pack. Some manufacturers of cooked products prefer mixing fresh and frozen poultry meat to achieve desired temperatures and conditions during processing. While microbiological testing can be performed on the meat, this is a less desirable approach for controlling sensory characteristics than time-temperature control.

Additional information on the microbiology of poultry products is available (ICMSF 2005). The Codex Alimentarius Commission Code of Hygienic Practice for Meat (Codex Alimentarius 2005) provides guidance for managing microbiological risks associated with poultry products. Risk assessment documents are also available for *Salmonella* (FAO/WHO 2002) and *Campylobacter* spp. (FAO/WHO 2009a) in broiler chickens, and in chicken meats (FAO/WHO 2009b).

## 9.2 Primary Production

The conditions for raising poultry differ significantly around the world and range from small family-owned farms having a few chickens or other fowl to large specialized poultry operations. As farm sizes increase and become more specialized, financial investment and concern for poultry disease increases.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_9,

Modern poultry complexes implement more stringent controls to achieve faster growth rates at lower cost. With fewer but larger farms there is an increasing opportunity to establish national on-farm control programs to reduce pathogens of concern to human health as well as poultry flocks. For example, the Scandinavian countries implemented long-term, on-farm programs to minimize the prevalence of *Salmonella* in poultry operations and on raw poultry meat. These and similar programs in other countries have achieved significant reductions in the prevalence of salmonellae on poultry meat. In Denmark, for example, the prevalence of salmonellae among slaughtered flocks decreased from 62% in 1993 to about 3% in 2000 (DVFA 2004).

A baseline survey on the prevalence of *Salmonella* in turkey flocks was conducted in Europe between October 2006 and September 2007 (EFSA 2008). The prevalence of *Salmonella*-positive breeding flocks and fattening flocks was 13.6 and 30.7%, respectively. The prevalence rates varied within country for which data were available and ranged from 0 to approximately 80%. The data may be used to set targets for future reductions in selected serovars of public health significance (EFSA 2008). Another baseline study evaluated the prevalence of *Campylobacter* and *Salmonella* in broilers in European countries and provides information about the efficacy of the on-farm control strategies applied in some countries (EFSA 2010).

Similar efforts to establish baselines and institute controls may reduce the prevalence of *Campylobacter*. The information collected from many years of research and risk assessments from the farm to the consumer is being used to develop internationally recognized draft guidelines for control of *Campylobacter* and *Salmonella* spp. in chicken meat (CCFH 2010).

## 9.3 Raw Poultry Products

### *9.3.1 Significant Organisms*

#### 9.3.1.1 Hazards and Controls

The hazards of significance are salmonellae and *Campylobacter*. Outbreaks of salmonellosis are usually due to inadequate cooking, recontamination of cooked poultry or cross-contamination to ready-to-eat foods. Risk assessment suggests that a 50% reduction in the prevalence of contaminated chicken would result in a 50% reduction in the expected risk per serving, and a 40% reduction in the concentration of *Salmonella* cells on chicken carcasses exiting the chiller would result in a 65% reduction in risk per serving (FAO/WHO 2002).

*Salmonella* and *Campylobacter* are present on live birds at the farm and upon receipt at the slaughtering plant. The degree of control over factors that contribute to horizontal or vertical transmission of pathogens during egg production, hatching and growing strongly influences the prevalence of these human pathogens on raw poultry carcasses and parts because no control measures can eliminate the pathogens during the slaughtering and chilling process. The types of salmonellae and campylobacter on raw carcasses and parts reflect those present on the live birds before slaughter. This suggests these pathogens are not acquired within the slaughtering facility from harborage sites. During slaughter, salmonellae can be transferred from one flock to following flocks. Thus, if possible, positive flocks should be processed after negative flocks. Rosenquist et al. (2003) reported that this is not necessarily the case for *Campylobacter*.

Considering the perishable nature of raw poultry meat, it is important to exercise control during slaughtering and chilling to minimize contamination with psychrotrophic spoilage bacteria. Typically these efforts also reduce the potential for pathogen contamination.

The hazards of significance on frozen raw poultry products are similar to those for refrigerated products with the possible exception that some *Campylobacter* may be inactivated by freezing.

Although some decline in *Campylobacter* (Sandberg et al. 2005, Georgsson et al. 2006) and vegetative cells of *Clostridium perfringens* can occur during frozen storage, freezing cannot be relied upon to ensure microbial safety. Salmonellae, for example, can survive for a year or more.

The Codex Alimentarius Commission Code of Hygienic Practice for Meat (Codex Alimentarius 2005) provides guidance for managing microbiological risks associated with raw poultry.

#### 9.3.1.2 Spoilage and Controls

Four factors influence the rate of growth and type of spoilage of raw poultry at refrigeration temperatures – (1) numbers and types of psychrotrophic bacteria, (2) inherent pH of poultry tissue, (3) storage temperature and (4) type of packaging such as modified atmosphere or vacuum packaging. Effective implementation of GHP is the primary factor affecting the number and type of psychrotrophic bacteria on raw poultry meat. In particular, it is necessary to design equipment for ease of maintenance and cleanability. The equipment and processing environment must be cleaned and disinfected at intervals that can maintain low levels of spoilage bacteria.

The inherent pH of poultry tissue cannot be altered but should be understood since it is an important factor influencing shelf life of raw poultry products. The higher pH of dark meat (e.g., thighs and legs) results in more rapid spoilage than white meat products (e.g., breasts). Storage temperature, however, is controllable. Reductions in storage temperature below 4°C can have a profound beneficial impact on keeping quality. As temperatures approach the freezing point of poultry meat, shelf life can be maximized.

The type of packaging can also influence the rate of growth and the microbiota that ultimately cause spoilage. For example, raw poultry has a longer shelf life when vacuum packaged or packaged with a gas atmosphere containing carbon dioxide compared with packaging in an oxygen permeable film.

Frozen poultry typically does not undergo microbial spoilage.

### *9.3.2 Microbial Data*

Table 9.1 summarizes useful testing for raw poultry products. Refer to the text for important details related to specific recommendations.

#### 9.3.2.1 Critical Ingredients

Raw poultry meats available in international commerce generally do not contain added ingredients. Some retail products are produced with added spices or flavorings to marinate the product during refrigerated distribution, storage and display. These ingredients are not likely to influence shelf life unless they introduce psychrotrophic bacteria capable of growing on the product and under the conditions of packaging. Certain ingredients (e.g., vinegar and salt) could reduce the rate of spoilage, if present in sufficiently high concentration.

#### 9.3.2.2 In-Process

The most common sampling location for process control is after chilling. Sampling immediately after defeathering may also be used to determine the extent of microbial reduction by the interventions during further processing. Postchill samples reflect all previous efforts to minimize contamination.

**Table 9.1** Testing of raw poultry products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Time and temperature should be controlled for raw poultry ingredients. Routine testing of the nonmeat ingredients, if any, is not recommended |
| In-process | Medium | Test whole carcass rinse or tissue samples (e.g., neck skin) to establish a baseline at various stages of processing and to evaluate where changes in the microbial populations occur during processing. Typical levels for psychrotrophs, *E. coli* and *Salmonella* depend on sampling site, sampling method and processing conditions within each facility |
| Processing environment | Medium | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting procedures. See text for typical levels encountered |
| Shelf life | Low | Routine shelf life testing is not normally performed on refrigerated products, testing of frozen products is not recommended. Shelf life testing may be useful to validate code dates of new retail products or when new packaging systems are installed |
| End product | Medium | Test for indicator microorganisms for on-going process control and trend analysis of freshly packed product using internally developed guidelines. Levels developed for processing do not apply during distribution or at retail (see text). Typical levels encountered at processing:<br>• Aerobic colony count – $<10^5$ CFU/g<br>• *E. coli* – $<10^2$ CFU/g<br>Routine lot acceptance sampling is not recommended for salmonellae or *Campylobacter* on raw poultry. Outbreak investigations or new supplier certification may benefit from determining the prevalence of salmonellae or *Campylobacter* in some situations (see text)<br>In countries or regions that have established performance criteria, the required sampling plan and tests should be applied |

In-process sampling is not recommended unless the postchill data indicate investigational samples at earlier steps in the process would help to identify sites contributing to contamination. In-process samples should be the same as those used for postchill sampling. Aerobic colony count, *E. coli* and/or *Salmonella* could be used for investigational purposes. The selection depends on the nature of the problem (e.g., premature spoilage, unacceptable levels of *Salmonella*). Two common sampling procedures include removing a portion of neck skin and the whole bird rinse (Cox et al. 2010). Testing for psychrotrophs could provide useful data when investigating premature spoilage problems. Testing for *E. coli* or *Salmonella* could provide data to better understand the occurrence of unacceptable levels of *Salmonella*. Typical levels of psychrotrophs, *E. coli* and *Salmonella* encountered depend on the method of sampling, sampling location, processing conditions and other factors. Development of internal standards based on trend analysis and methods is appropriate.

#### 9.3.2.3 Processing Environment

Swab or sponge samples collected before the start of operation can help verify the effectiveness of cleaning and disinfection of the equipment used for slaughtering, chilling and other steps in converting carcasses to packaged fresh poultry meat. Analysis for aerobic colony count is commonly used, but other tests (e.g., ATP-bioluminescence, coliforms, Enterobacteriaceae) may provide useful information in some instances. A typical level encountered on thoroughly cleaned, disinfected stainless steel is an aerobic colony count of <500 CFU/cm$^2$. Higher numbers may be encountered on other surfaces (e.g., nonmetal conveyor belts).

#### 9.3.2.4 Shelf Life

Shelf life testing of refrigerated raw poultry products may be performed should the company deem this useful, but testing of frozen raw poultry is not recommended. Shelf life testing can be useful to validate code dates of new retail products or when new packaging systems are installed. Verification can be based simply on sensory evaluation. Microbiological analysis for specific spoilage microorganisms may be helpful for certain products. In-store surveys to verify sensory acceptability relative to the code dates may also be considered periodically.

Shelf life testing is not necessary for raw poultry meat to be used as ingredients for manufacturing further processed products.

#### 9.3.2.5 End Product

Many companies and governments have established criteria for indicators of quality or process hygiene (e.g., aerobic colony count, Enterobacteriaceae, *E. coli*). The data are most useful when incorporated into a process control program and used for trend analysis. Typical levels encountered are an aerobic colony count of $<10^5$ CFU/g and *E. coli* $<10^2$ CFU/g. However, numbers that exceed these may not indicate loss of control at the slaughter plant. Several factors including flock health will result in a wide variation in the quantity and type of bacteria present on chicken skin when birds are presented for slaughter.

Criteria established by control authorities in the producing or importing country should be considered. The criteria may be based on samples collected from specific steps in the food chain from slaughter through retail display or at point of entry. The test results reflect the conditions of primary production, slaughter, chilling, and time and temperature of storage. These values are poor indicators of the prevalence or concentration of enteric pathogens in fresh poultry meat.

Samples collected during storage, distribution and retail display do not provide a reliable estimate of the hygienic conditions during processing and packaging because psychrotrophic microorganisms may increase. Samples yielding unacceptable results at these stages should lead to investigative sampling to determine why they occurred so that appropriate corrective actions can be implemented. Potential causes of high levels may include poor hygienic conditions during manufacture or storage at elevated temperatures (e.g., >7–8°C) that permit growth during distribution, storage or display. Indicator tests on frozen products reflect the microbial population at time of freezing and any decrease that may have occurred during distribution and retail display.

The prevalence rates of salmonellae on fresh poultry meat vary considerably in different regions and countries. While routine lot acceptance sampling is not recommended for salmonellae on fresh poultry products, unique situations (e.g., outbreak investigations, new supplier certification) can occur where information on the prevalence of salmonellae can provide useful information.

Application of criteria (e.g., performance objectives) for foodborne pathogens (e.g., salmonellae, *Campylobacter*) at specific steps in the food chain is of increasing interest to improve food safety. This led the Codex Alimentarius Commission to provide guidance to governments for verification of process control of meat hygiene using microbiological testing (Codex Alimentarius 2005). While specific microbiological criteria were not provided, the guidance states that "Establishment of microbiological testing requirements, including performance objectives or performance criteria should be the responsibility of competent authorities, in consultation with relevant interested parties, and may consist of guidelines or regulatory standards." Furthermore, "The competent authority should verify compliance with microbiological testing requirements where they are specified in regulation e.g., microbiological statistical process control requirements, standards for *Salmonella* spp."

Trend analysis is an important component because the data can be used to measure change in prevalence rates as industry implements procedures to meet the established requirements. Some countries or regions (e.g., USA, EU) have initiated long-term continuous improvement programs to

reduce the prevalence of salmonellae or *Campylobacter* on raw poultry (USDA 1996, 2008, EU 2003, 2005, NZFSA 2008). Ideally, such programs are coupled with guidance that provides science-based, best practices from farm through slaughter and chilling and relate to a public health goal. It is uncertain whether the approaches (control at the farm, control at the slaughtering plant or a combination of the two) applied by different countries will lead to different degrees of pathogen control and consumer protection. For example, adoption of performance objectives at the plant level for raw meat and poultry has yet to result in reduction of human salmonellosis in the USA that was expected when the pathogen reduction regulation (USDA 1996) was finalized (Cole and Tompkin 2005, CDC 2009). The portion of human salmonellosis originally attributed to poultry may be lower than previously thought or interventions at other steps in the farm to fork continuum may need to be addressed.

The value of microbiological testing of intermediate products, the processing environment or end products will depend on the refrigerated or frozen product being produced, its intended use and the expected benefit of the data. Table 9.1 summarizes the relative importance of testing for raw poultry products.

## 9.4 Cooked Poultry Products

This section addresses fully cooked poultry products. Some partially cooked (e.g., par-fried) and ready-to-heat products may be treated as raw products.

### *9.4.1 Significant Organisms*

#### 9.4.1.1 Hazards and Controls

These products are perishable and must be refrigerated or frozen. The microbial hazards to consider in cooked perishable poultry products include *Salmonella*, *L. monocytogenes* and *C. perfringens*. Control of *Salmonella* and *L. monocytogenes* involves use of validated cooking procedures and prevention of recontamination. Cooking is managed through the HACCP plan. Recontamination is managed through effective application of GHP designed for *Listeria* control and verification through environmental monitoring (Codex Alimentarius 2009). Some products are given a final in-package listericidal treatment. Additives may be used in some countries to inactivate or restrict the growth of *L. monocytogenes*.

*Salmonella* introduced through recontamination after cooking can survive on cooked refrigerated poultry products but cannot multiply if the products are maintained below 7°C.

Control of *C. perfringens* requires chilling cooked poultry products at a rate that prevents unacceptable multiplication of surviving spores and storing at <12°C. Historically, more than 90% of *C. perfringens* outbreaks have occurred due to improper chilling or holding in foodservice operations (Brett 1998, Murrell 1989). It also has been suggested that improper retail and consumer refrigeration accounts for the majority of *C. perfringens* illness in the USA (Golden et al. 2009). Cured poultry products contain sodium nitrite and generally have a higher salt content than uncured products such as turkey or chicken breast. Cured poultry products have rarely been implicated as a source of *C. perfringens* illness.

The microbial hazards on frozen, cooked, uncured poultry products are similar to refrigerated products except the vegetative cells of *C. perfringens* are quite sensitive to freezing and decline during frozen storage. Also, *L. monocytogenes* cannot multiply while the product remains frozen.

The Codex Alimentarius Commission Code of Hygienic Practice for Meat (Codex Alimentarius 2005) provides guidance for managing microbiological risks associated with cooked poultry products.

#### 9.4.1.2 Spoilage and Controls

The rate of spoilage is influenced by many factors (e.g., temperature, initial number and type of microorganisms, type of packaging, chemical composition). Spoilage by psychrotrophic clostridia and lactic acid bacteria has occurred in commercial products having extended refrigerated shelf life (e.g., ≥35 days). Control requires determining the source of the clostridia (e.g., the raw poultry meat or harborage sites in the raw processing environment) and implementing appropriate controls.

### *9.4.2 Microbial Data*

Table 9.2 summarizes useful testing for cooked poultry products. Refer to the text for important details related to specific recommendations.

**Table 9.2** Testing of cooked poultry products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | These products do not contain nonpoultry ingredients of significance for microbiological safety or quality |
| In-process | High | Monitoring the cooking parameters is essential |
| | Medium | For products that support *L. monocytogenes* growth, postcook samples can assess control of *Listeria* spp. Typical levels encountered postcook:<br>• *Listeria* spp. – absent |
| Processing environment | High | For products that support *L. monocytogenes* growth, during production, sample product contact surfaces where cooked products are exposed to potential contamination before packaging. Sponge or swab samples from floors, drains and other nonproduct contact surfaces can provide an early indication of the level of control and the potential risk of equipment and product contamination. Expected levels encountered:<br>• *Listeria* spp. – absent |
| | Medium | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting procedures. See text for typical levels encountered |
| Shelf life | Medium | Shelf life testing may be useful for refrigerated products with extended shelf life. Shelf life testing of frozen cooked poultry is not necessary |
| End product | Medium | Test for indicators for on going process control and trend analysis in manufacturing. Typical levels encountered:<br>• Aerobic colony count – $<10^4$ CFU/g from product surface<br>• *E. coli* – absent<br>Routine sampling for pathogens is not recommended. Follow the sampling plans below when conditions occur as described in Sect. 9.3.2.5 |

| Product | Microorganism | Analytical method[a] | Case | Sampling plan & limits/g[b] *n* | *c* | *m* | *M* |
|---|---|---|---|---|---|---|---|
| Cooked poultry | *S. aureus* | ISO 6888-1 | 8 | 5 | 1 | $10^2$ | $10^3$ |
| Cooked poultry: No growth | *L. monocytogenes* | ISO 11290-2 | NA[c] | 5 | 0 | $10^2$ | – |
| Cooked uncured poultry | *C. perfringens* | ISO 7937 | 8 | 5 | 1 | $10^2$ | $10^3$ |
| | | | | Sampling plan & limits/25 g[b] | | | |
| Cooked poultry | *Salmonella* | ISO6579 | 11 | 10[d] | 0 | 0 | – |
| Cooked poultry: Supports growth | *L. monocytogenes* | ISO 11290-1 | NA[c] | 5[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] *NA* not applicable due to use of Codex criteria
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 9.4.2.1 Critical Ingredients

The nonpoultry ingredients in cooked poultry products are rarely a source of significant pathogens or spoilage flora. Some ingredients (e.g., salt, sodium nitrite, sodium lactate, sodium diacetate) can reduce the rate of spoilage and growth of *L. monocytogenes* and clostridia.

#### 9.4.2.2 In-Process

The relative value of in-process samples versus processing environment samples for routine assessment for the control of *Listeria* spp is a debatable issue. The decision to rely more on in-process over environmental samples can be influenced by regulatory policies, the complexity of the equipment and steps in the process after cooking. Routine in-process sampling is not performed by some manufacturers while others rely on in-process samples for assessing control. Experience indicates that in-process samples can be helpful when investigating a problem and are recommended. Routine sampling for salmonellae, *Staphylococcus aureus* or *C. perfringens* is not recommended, since the risk associated with these pathogens is controllable through GHP and HACCP.

#### 9.4.2.3 Processing Environment

The relative importance of verifying control of the processing environment depends on the risk to consumers if the product becomes contaminated between cooking and final packaging. This section focuses on control of *L. monocytogenes* because it is a significant concern for products that support its growth and have a long refrigerated shelf life. In an environment demonstrated to control *L. monocytogenes* to a manageable level, *Salmonella* is likely to be controlled.

Of highest concern are products that do not have validated growth inhibitors (e.g., lactate, diacetate), that support growth during the normal time and temperatures for storage and distribution, that do not receive a listericidal treatment after final packaging and are intended for consumers who are highly susceptible to listeriosis. The frequency and extent of sampling also should reflect consumer risk.

Monitoring programs that include sampling of equipment and other surfaces that come into contact with exposed cooked products before final packaging can be very helpful and are recommended. Sponge samples from large areas of equipment should be collected during production. Samples can also be collected from nonproduct contact surfaces as an additional measure of control (Codex Alimentarius 2009). The benefit of environmental sampling for products given a validated final in-package listericidal treatment is questionable.

The principles for control and monitoring of *Listeria* can also be applied to control spoilage microorganisms (e.g., lactic acid bacteria) of cooked poultry products. Swab or sponge samples should be collected before the start of operation to verify the effectiveness of cleaning and disinfecting. Analysis for aerobic colony count is a common analysis, but other tests (e.g., ATP-bioluminescence) may provide useful information. Typically, aerobic colony counts on thoroughly cleaned, disinfected stainless steel are <500 CFU/cm$^2$. Higher numbers may be encountered on cleaned, disinfected nonmetal surfaces such as conveyor belts.

#### 9.4.2.4 Shelf Life

Code dating practices can be validated by holding the product at a controlled temperature and performing sensory evaluation, microbiological analysis or both at selected intervals, including packages before, on and after the expected expiration date. Subsequent verification can be performed at a frequency that reflects confidence in whether the product will consistently meet the stated expiration date on the package. Shelf life testing of frozen cooked poultry products is not necessary.

Validating that growth of *L. monocytogenes* will not occur within the code date applied on the package may be of interest in some regions. Considerations for validation are available (Scott et al. 2005).

#### 9.4.2.5 End Product

Test for indicators (e.g., aerobic colony count, *E. coli*) for on going process control and trend analysis. Typical aerobic colony counts are $<10^4$ CFU/g from product surfaces and *E. coli* is usually not detected in cooked product.

Apply validated processes, managed through HACCP plans, to destroy salmonellae and *L. monocytogenes*, and apply effective GHP to prevent recontamination from the processing environment. If the reliable application of GHP and HACCP is in question (e.g., indicator tests are higher than anticipated), sampling for *Salmonella* and *L. monocytogenes* may be appropriate. When evidence indicates a potential for contamination with *L. monocytogenes* (e.g., positive food contact surface results or the effectiveness of corrective actions has yet to be verified) sampling the food should be considered. Some fully cooked products are ingredients in further processed products that may receive another kill step, while the final use of others may be difficult to determine. The stringency of sampling should reflect consumer risk (e.g., whether growth can occur in the food, intended consumers etc.) as well as uncertainty about final use of the product. Guidance on increasing the stringency of sampling by sub-lotting is discussed in Chap. 5.

The *Salmonella* sampling plan in Table 9.2 is for foods in which *Salmonella* will not grow under the normal conditions of distribution and storage (i.e., case 11). The sampling plans for *L. monocytogenes* are for ready-to-eat foods produced following the general principles of food hygiene for control of *L. monocytogenes* and with an appropriate environmental monitoring program (Codex Alimentarius 2009). As an example of the performance of this sampling plan, assuming a log normal distribution, the sampling plan for products that do not support the growth of *L. monocytogenes* would provide 95% confidence that a lot of food containing a geometric mean concentration of 93 CFU/g and an analytical standard deviation of 0.25 log CFU/g would be detected and rejected based on any of the five samples exceeding $10^2$ CFU/g. Such a lot may have 55% of the samples below $10^2$ CFU/g and up to 45% of the samples above $10^2$ CFU/g, whereas only 0.002% of all the samples from this lot could be above $10^3$ CFU/g.

The typical actions to take when the criteria are not met would be to (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption and (3) determine and correct the root cause of the failure.

In the event a chilling deviation occurs after cooking (i.e., the rate of chilling exceeds the critical limit in the HACCP plan), the product can be tested for *C. perfringens* to provide additional information when considering disposition of the lot. The sample units should be taken from the center of the product or other region that is slowest to chill. Samples should be submitted to the laboratory as refrigerated samples (i.e., not frozen). The decision to test for *C. perfringens* depends on the available information (e.g., pH; $a_{W;}$ added inhibitors such as sodium nitrite, lactate or diacetate), the extent of the deviation and options, and predictive models to estimate growth that may be available for product disposition. A sampling plan is also provided for products when temperature abuse is suspected and *S. aureus* is of concern.

## 9.5 Fully Retorted Shelf-Stable Poultry Products

The hazards and controls for fully retorted shelf-stable poultry products are the same as those for other low acid canned foods (see Chap. 24). Spoilage of low acid canned foods, including canned poultry products is controllable and should rarely occur. The potential exists for incipient spoilage if the

product is not retorted in a timely manner. This can occur for several reasons such as when equipment breaks down and the food is held for an extended period of time before retorting.

Current recommended procedures for commercial processing are based on GHP and HACCP yield products that are commercially sterile and stable for the expected conditions of storage and distribution. Routine microbiological testing of these products is not recommended for either safety or quality. See Chap. 24 for additional information.

## 9.6 Dried Poultry Products

### *9.6.1 Significant Organisms*

#### 9.6.1.1 Hazards and Controls

Dried poultry products are cooked and processed to provide shelf stability. They are generally available in two basic groups. One consists of diced, powder, bouillon and paste products that are used in soup mixes and flavorings. The other consists of poultry meat formulated with salt, flavorings and spices and then formed into flat strips or thin sausages that are cooked and dried. The significant microbial hazard to consider is *Salmonella*. *L. monocytogenes* is not a hazard of concern because the low $a_W$ prevents multiplication in these products. A risk assessment and a risk categorization have placed these products in the low category of risk as sources of foodborne listeriosis (FDA-FSIS 2003, FAO/WHO 2004). Cooking is a critical control point in the manufacture of these products.

#### 9.6.1.2 Spoilage and Controls

Dried poultry products are microbiologically stable until they are rehydrated or exposed to conditions of high humidity.

### *9.6.2 Microbial Data*

Table 9.3 summarizes useful testing for dried poultry products. Refer to the text for important details related to specific recommendations.

#### 9.6.2.1 Critical Ingredients

There are no critical nonpoultry ingredients.

#### 9.6.2.2 In-Process

Routine in-process samples should not be necessary but can be helpful in the event of a problem and the source(s) of microbial contamination must be determined.

#### 9.6.2.3 Processing Environment

Routine environmental samples for salmonellae should not be necessary in a controlled operation operating under GHP with adequate separation between raw poultry processing areas and where cooked poultry products are exposed. Environmental sampling, however, can be helpful in the event a problem does occur and the source(s) of contamination must be determined.

**Table 9.3** Testing of dried poultry products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | These products do not contain nonpoultry meat ingredients of significance for microbiological safety or quality |
| In-process | High | Monitor cooking and formulation parameters such as pH, $a_w$, and preservatives. The manufacturing processes should be validated for control of salmonellae that are present in poultry meat |
| | Low | Routine microbiological testing in-process samples are not recommended |
| Processing environment | Medium | Sample equipment surfaces before start-up to verify efficacy of cleaning and disinfecting procedures. See text for typical levels encountered |
| Shelf life | Low | These products are inherently shelf-stable when properly dried and protected from high humidity. The higher $a_w$ of snack products may require verification of stability (see text) |
| End product | Low | Routine sampling is not necessary. If application of GHP and HACCP is in question, sampling for *Salmonella* may be considered |

| | | | | Sampling plan & limit/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Dried poultry | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

Swab or sponge samples should be collected to verify the effectiveness of cleaning and disinfecting equipment before the start of operation. Analysis for aerobic colony count is a typical analysis but other tests (e.g., ATP-bioluminescence) may provide useful information. Typical aerobic colony counts on thoroughly cleaned, disinfected stainless steel are <500 CFU/cm$^2$. Higher numbers may be encountered on other surfaces (e.g., nonmetal conveyor belts).

#### 9.6.2.4 Shelf Life

The final moisture content (i.e., <10%) and low $a_w$ make these products microbiologically stable. The strips and thin sausage-shaped products may be higher in moisture for better palatability as snacks. If $a_w$ levels are sufficiently high (e.g., >0.70), these products must be packaged in a low oxygen atmosphere to prevent the growth of mold during extended storage or be formulated with a mold inhibitor. Defective packaging seals can contribute to mold spoilage of these products during storage, distribution and retail display.

#### 9.6.2.5 End Product

These products are of low risk to public health and routine sampling is not recommended. If there is reason to question whether GHP and HACCP are being applied in a manner to control enteric pathogens, then sampling for an indicator (e.g., *E. coli*) or salmonellae is recommended.

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# Chapter 10
# Fish and Seafood Products

## 10.1 Introduction

Finfish and shellfish are an important source of animal protein in most parts of the world. In 2006, the total world production was approximately 144 million metric tons, of which more than 52 million metric tons were produced by China. Wild fish catches contributed approximately 92 million metric tons. Aquaculture production has increased steadily since 1990, and yielded 52 million metric tons in 2006 (FAO 2009). In 2005 almost 40% of fish and shellfish used for human consumption were reared in aquaculture. Most of the production (110 million metric tons) is used for human consumption and a large fraction is used for fish meal and fish oil. Seafood products are traded around the world, and South East Asia and China are major exporters of farmed crustaceans (FAO 2009).

Seafood products can be the vehicle of foodborne diseases caused by parasites, toxins, viruses or pathogenic bacteria. They can also carry heavy metals, pesticides or antibiotic residues. Seafood products were the cause of approximately 20% of foodborne disease outbreaks with known causes in the US from 1997 to 2006, but it should be noted that relatively few cases are associated with each outbreak. The major causes are histamine poisoning and ciguatera toxin (CSPI 2007). Histamine is heat stable and if produced in the raw material, it will not be eliminated by hot-smoking or canning.

Fish and shellfish are cold-blooded animals caught or harvested from a multitude of environmental conditions, ranging from warm tropical freshwater lakes to cold arctic marine waters. The microbiota of fish reflects the aquatic environment in which the fish are caught (ICMSF 2005). Several potential foodborne hazards reside naturally in the marine or freshwater environment and control of these hazards must be considered during handling and processing. Examples include parasites, aquatic toxins such as ciguatera and shell fish toxins, and *Vibrio* species such as *V. parahaemolyticus* and *Vibrio vulnificus*. Vibrios receive a lot of attention as etiological agents of seafood borne disease and several risk assessments are available (FAO/WHO 2005a, b, 2011, FDA 2005). Only some strains of *V. parahaemolyticus* are capable of causing gastroenteritis and these are often but not always positive for a thermostable direct hemolysin (*tdh*) or a *tdh*-related hemolysin. Most environmental strains are *tdh* negative. The percentage of the *tdh* positive *V. parahaemolyticus* population in coastal waters varies from 0.1 to 4% (FAO/WHO 2011). Additionally, the percentage of pathogenic *V. parahaemolyticus* in seafood is typically low, but occasionally the percentage may be higher (e.g., 1–4% in oysters) depending on geographic area (FAO/WHO 2011). Methods to quantify pathogenic *V. parahaemolyticus* are being developed and future microbiological criteria should be based on levels of the pathogenic strains. Currently there is no experience with sampling for *V. parahaemolyticus* in the processing environment and it is suggested to investigate if *Vibrio* spp. may be useful indicators in facilities processing fish for raw consumption.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_10,

This category includes a multitude of fin fish (e.g., tilapia, cod, tuna), crustaceans (e.g., shrimp, lobster) and mollusca (e.g., squid, octopus, bivalves such as mussels, clams or oysters). The range of products produced is very large and includes foods prepared by a broad spectrum of traditional and modern food technology methods such as freezing, cooling, salting, drying, smoking and acidification, and products are packaged under different atmospheres. Despite the heterogeneity in raw material and processing techniques, seafood products can be grouped by commodities with similar microbial ecology (ICMSF 2005).

Most fish and seafood products, if not frozen, are very perishable and may spoil rapidly due to bacterial growth. One of the most important control parameters is temperature, and fresh fish should, preferably, be stored in melting ice to retard spoilage. Packaging, salting and acidifying or heat treatments are common processes in extending shelf life of seafood products.

The reader is referred to *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005) for more information on the microbial ecology and control of fish and seafood product quality and safety. Also, the Codex Alimentarius Commission has published a Code of Practices for Fish and Fish Products (Codex Alimentarius 2008) and there is a range of codes and standards for a several seafood sub-commodities.

## 10.2 Raw Finfish of Marine and Freshwater Origin

This product category includes whole, head-on and filleted finfish. The fish may be caught or farmed and originate from marine or fresh water. These products should preferably be stored between 0 and 2°C. The products may be distributed and sold on ice but may also be packed under vacuum or in modified atmosphere and distributed at temperatures just above freezing. Low temperature and perhaps atmosphere are the only preserving parameters. The water activity is high and pH is typically between 6.0 and 6.8. Most finfish are processed before consumption by cooking, but very fresh fish may be consumed raw (e.g., sushi or sashimi).

### *10.2.1 Significant Organisms*

#### 10.2.1.1 Hazards and Controls

Foodborne disease associated with finfish is typically caused by aquatic biotoxins (ciguatera) or histamine. Histamine is the dominant biogenic amine and its production is associated with temperature abuse. Most cases of histamine poisoning (also called scombroid poisoning) involves levels >500–1,000 ppm (Lehane and Olley 2000). If the fish is consumed raw, parasites, some *Vibrio* species and enteric pathogens from fecally contaminated waters may be a concern. Hazards associates with marine and fresh water fish and shellfish have increased due to climate change and related temperature changes and excessive fishing. Under these conditions, certain oceanic cyanobacteria (also known as blue-green algae) may form toxins. *Clostridium botulinum* type E is an indigenous aquatic microorganism, thus it may need to be considered for vacuum and modified atmosphere packed products because it is capable of growth at 3–4°C under anaerobic conditions (ICMSF 1996). Fish or crustaceans that are produced on "integrated farms" may feed on chicken, pig or other manures, thus microorganisms such as *Salmonella* may be present on the raw fish. Finally, procedures to control antibiotic residues should be in place when dealing with farmed species.

Algal toxins are controlled by surveying harvesting waters for algal blooms. Ciguatera is an issue in warm tropical reef waters and avoiding fish from such areas during periods of harmful algal blooms is the most efficient way to prevent foodborne disease. Some parasites are controlled by removal during visual inspection of finfish, and all parasites are destroyed by appropriate freezing or

cooking. The presence of low levels of *C. botulinum* is not a risk, but the potential growth and toxin formation under anaerobic conditions must be controlled by keeping the fish below 3°C at all times. *Vibrio* species are of concern in warmer waters only if the fish is eaten raw. Contamination with enteric pathogens is controlled by avoiding contaminated waters and by observing good hygienic practices during processing. Farmed fish species treated with antibiotics should be held for temperature dependent specified periods to clear them of residues before harvesting.

The Codex Alimentarius Commission Code of Practice for Fish and Fishery Products (Codex Alimentarius 2008) provides advice on appropriate technology practices and HACCP systems to manage risks from fish and seafood products.

#### 10.2.1.2 Spoilage and Controls

Fresh fish are very perishable and spoil due to bacterial growth. At ambient temperature, mesophilic Gram-negative bacteria are the main cause of spoilage which occurs within ½–2 days. At chilled temperature, spoilage is mostly caused by Gram-negative psychrotrophic bacteria. Vacuum packing may delay spoilage in some warm water fish species but it is not as efficient in preserving fish as it is for meat products. Controlling growth of fish spoilage bacteria is based on low temperature, sometimes combined with packaging in controlled atmosphere (vacuum or $CO_2$). In $CO_2$-packed, chilled products, either photobacteria or Gram-positive bacteria are the main spoilage microorganisms. The most common and meaningful test for spoilage is a sensory evaluation of the product. If the specific spoilage microorganism of the product is known (e.g., *Shewanella* species of iced gadoid species), then a count of these may be used to estimate the remaining shelf life of the product; however, the number will not describe the sensory quality.

### *10.2.2 Microbial Data*

Table 10.1 summarizes testing that may be useful for fresh, raw fish. Refer to the text for important details related to specific recommendations.

#### 10.2.2.1 Aquatic Environment

The water from which fish and shellfish are harvested or reared has an impact on safety. Toxins from cyanobacteria in fresh water aquaculture are an increasing concern. Algal toxins are typically produced by dinoflagellates and algal blooms are the cause of ciguatera toxin and other toxins. Surveying catching waters for algae or avoiding fish from tropical reef areas during periods of algal blooms can control this hazard. End product testing is not an efficient way to control risk, although high performance liquid chromatography (HPLC) analyses are available for some toxins. If no prior knowledge of the product is available, sampling and analyzing for toxins by HPLC can provide information about the product.

#### 10.2.2.2 Raw Materials

Several of the hazards listed for fresh fish originate in the aquatic environment, thus must be assumed to be present on the raw material, albeit at low levels. Nematodes are likely to be present in many fish caught in the wild and visual inspection is often carried out; e.g., on cod fillets after filleting. This hazard is controlled by further processing (e.g., cooking, acidification or freezing). Trematodes are common especially in farmed fish in the Asian countries and should be controlled by processing procedures and improved sanitation (e.g., breaking the fecal-oral route of contamination). Several bacterial pathogens (*C. botulinum*, histamine forming bacteria and *Vibrio* species) are common in the

**Table 10.1** Testing fresh fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Live fish | Medium | Survey waters for algal blooms in at risk areas and halt catching during bloom periods |
| Critical ingredients | Low | Raw fish do not contain added ingredients |
| In-process | Medium | Wild caught fish are likely to harbor parasites, and some (nematodes) may be removed during visual inspection |
| | High | To kill parasites, some countries require freezing (24 h at −20°C) for fish to be consumed raw, therefore monitor time and temperature |
| Processing environment | Medium | Samples from equipment surfaces before start-up can be used to verify efficacy of cleaning and disinfecting procedures. Monitoring swab samples over time may be used for trend analysis |
| | | Monitoring for indicators of enteric pathogens e.g., *Salmonella* or levels of *Vibrio* spp. may be done if product is intended for raw consumption and epidemiological data indicate reason for concern |
| Shelf life | Low | Shelf life testing using sensory assessments may be useful to validate code dates of new retail products or packaging systems |
| | | Tests for specific spoilage bacteria (if known) may provide a guide to expected shelf life under known storage conditions. Counts of specific spoilage bacteria above $10^7$ CFU/g indicate on-set of spoilage |
| End product | Medium | Routine testing for pathogens is not recommended. Test for indicators for verification of control. Visual inspection for parasites is recommended if product is intended for raw consumption |

aquatic environment. Testing for any of these organisms on the raw fish will not ensure safety, thus control should be ensured by harvesting, processing and storage parameters. Ingredients such as fish meal used in dry aquaculture feed are typically tested for presence of salmonellae, but a link between their presence in the feed and human disease has not been observed. The aerobic colony count of raw, newly caught fish varies between $10^4$ and $10^7$ CFU/cm$^2$, while properly skinned fillets can have much lower counts. For fish of the *Clupeidae*, *Scombridae*, *Scombresocidae*, *Pomatomidae* and *Coryphaenedae* families, that will be raw ingredients in the manufacture of other fish products, the Codex Alimentarius Commission standards referring to quality recommend that these should not contain more than 10 mg of histamine per 100 g fish (100 ppm), (e.g., Codex Alimentarius 2004).

#### 10.2.2.3 Processing Environment

Raw fish undergo little processing except for bleeding, gutting and filleting. The processing environment can be a source of spoilage bacteria and human pathogens but routine cleaning and sanitizing procedures can control this. Monitoring the aerobic colony count on surfaces may be used to assessing the cleanliness of the processing environment. In particular cases, such as where the fish is used to produce cold-smoked fish, monitoring the environment for *Listeria monocytogenes* may be required (see Sect. 10.9), as the raw fish entering the smoke house can be a source of the microorganism.

#### 10.2.2.4 Shelf Life

Fish are cold-blooded animals and the natural microbiota is often adapted to low temperatures. Fish do not accumulate glycogen, thus pH does not decrease post mortem as in warm-blooded animals. Storing fish in melting ice (0°C) is recommended to delay spoilage. Shelf life of fresh fish stored under controlled conditions (typically in ice) range from 7 to >30 days depending on the fish species.

Spoilage bacteria cause off-odors and off-flavors of fish. The specific bacteria differ between fish species, e.g., Gram-negative psychrotrophic bacteria (shewanellae) for many iced fish from marine temperate waters and pseudomonads for many iced fresh water species. Spoilage is typically detected when specific spoilage bacteria are $>10^7$ CFU/g.

When the specific spoilage bacteria have been identified for a fish species, levels of these can be used to predict remaining shelf life. Counts of spoilage bacteria or total aerobic colony counts generally will not indicate sensory quality. Differentials counts at 25 and 35°C may be a useful predictor of shelf life quality. Counts of spoilage bacteria may also have a predictive value in determining the potential remaining shelf life under defined conditions. However, sensory assessment is required to determine code dates and shelf life for products; e.g., with change in packaging atmosphere.

#### 10.2.2.5 End Product

Routine microbiological testing of these products is not recommended for either quality or safety. However, inspection for parasites and, for scombroid species, assessment of histamine is important to ensure safety. Some countries require that all wild caught fish intended for raw consumption should be frozen for at least 24 h at −20°C to kill parasites.

For histamine, various Codex Alimentarius Commission standards for finished seafood products have histamine limits of <20 mg/100 g of fish (200 ppm) (e.g., Codex Alimentarius 2004). This applies only to species of *Clupeidae*, *Scombridae*, *Scombresocidae*, *Pomatomidae* and *Coryphaenedae* families. Approaches for testing for histamine vary between regions. In the US, sensory analysis (detecting odors of decomposition in 18–24 subsamples for processed products) is recommended and if positives are found, at least six subsamples should be analyzed including the subsamples demonstrating decomposition odors. A sampling plan where $n=6$, $c=1$, $m=50$ ppm and $M=500$ ppm is applied. In Europe (EC 2005), for products from fish species associated with high amounts of histidine, a sampling plan where $m=100$ ppm, $M=200$ ppm, $n=9$, $c=2$ is recommended. In Australia and New Zealand, the code states that the level of histamine in fish or fish products must not exceed 200 mg/kg (200 ppm) (FSANZ 2000). Malle et al. (1996) and Duflos et al. (1999) describe the analytical method for measurement of histamine.

If the product is intended for raw consumption, several bacterial and viral pathogens from the human-animal reservoir may present a risk. These may be present on the fish due to cross-contamination and observing good hygienic practices will control these hazards. If no prior knowledge of the product is available, testing for *Salmonella* and *V. parahaemolyticus* may be relevant on a limited basis if the product is intended for raw consumption. It should also be noted that raw fish is typically consumed very fresh and results of bacteriological analyses may not be available before product is consumed. Thus understanding the source and handling conditions is more important than testing to assure safety of raw fish.

## 10.3 Frozen Raw Seafood

This product category is derived from fish (whole or filleted) described in Sect. 10.2, from crustaceans described below or from mollusca (e.g., squid or octopus). The products are typically stored at −18 to −20°C and no microbiological growth occurs under these conditions. Frozen fish or crustacean may be further processed, cooked and consumed, or consumed raw as sushi or sashimi after thawing.

## *10.3.1 Significant Organisms*

### 10.3.1.1 Hazards and Controls

Freezing raw fresh seafood does not change increase the risk profile, and it eliminates the parasites that present a risk in raw and lightly preserved products. Cooking eliminates pathogens of concern. The presence of aquatic toxins and histamine (in scombroid species) is similar to the outline for raw fish, and cooking will not destroy these hazards. Avoiding fish from tropical reefs or areas with algal blooms will control the risk of aquatic toxins. Formation of histamine may be controlled by maintaining low temperature during all steps of storage, handling and processing. Freezing halts the histamine formation process.

### 10.3.1.2 Spoilage and Controls

Microbiological spoilage is not an issue in frozen seafood. Any spoilage of the raw fish before freezing may be determined by sensory assessment. Sensory quality changes during frozen storage, with more rapid change at higher or fluctuating freezing temperatures. The total colony count may indicate the level of hygiene during processing or length of storage before freezing.

## *10.3.2 Microbial Data*

Table 10.2 summarizes useful testing for frozen raw fish. Refer to the text for important details related to specific recommendations.

### 10.3.2.1 Critical Ingredients

Crustaceans may be glazed during freezing to avoid evaporation of water during frozen storage. Water used for this process should be of drinking water quality.

### 10.3.2.2 In-Process

The product passes through a very limited number of processing steps and sampling of these is not useful.

**Table 10.2** Testing frozen raw fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Raw fish | Medium | Parameters as indicated in Table 10.1 should be under control; e.g., algal toxins. Freezing will eliminate parasites |
| Critical ingredients | High | If product is glazed, ensure water is potable |
| In-process | Low | Routine samples are not collected of raw fish during processing to frozen fish |
| Processing environment | Low | Samples from equipment surfaces before start-up can be used to verify efficacy of cleaning and disinfecting procedures |
| Shelf life | Low | Sensory quality of frozen fish typically deteriorates due to biochemical, autolytic changes |
| End product | Medium | Routine microbiological testing is not recommended. Histamine testing of species known to accumulate this biogenic amine may be relevant |

#### 10.3.2.3 Processing Environment

Swabs for aerobic colony counts can be used to determine if the normal cleaning and disinfection procedures are working.

#### 10.3.2.4 Shelf Life

Shelf life of frozen seafood products is not limited by microbiological effects but typically by oxidative changes during frozen storage. High or fluctuating freezing temperatures may accelerate quality deterioration. Monitoring time and temperature during processing will avoid deterioration of sensory quality.

#### 10.3.2.5 End Product

No routine microbiological testing of end product is recommended. If the thawed products are to be consumed raw, the points made in Table 10.1 should be considered, otherwise Table 10.2 is recommended. For histamine, see Sect. 10.2.2.5 for current testing recommendations.

## 10.4 Raw Crustaceans

Crustaceans are animals carrying the skeleton on the outside and include crabs, prawns and shrimp. The two latter are very important in international trade and constitute a major export from South East Asian countries. Crustaceans may be distributed and sold raw (frozen) or cooked (see specific section below).

### *10.4.1 Significant Organisms*

#### 10.4.1.1 Hazards and Controls

Crustaceans are typically processed by cooking (see Sect. 10.5) but may be consumed raw. Presence of human pathogens in the waters may cause disease. Enteric pathogens, including viruses, can be controlled by avoiding catch from fecally contaminated waters but *Vibrio* spp. are indigenous to the aquatic environment.

#### 10.4.1.2 Spoilage and Controls

Fresh crustaceans are perishable products and several spoilage reactions cause sensory spoilage. Proteolytic enzymes in the crustacean digestive gland become active at harvest, and autolysis begins very rapidly resulting in a quick loss of sensory quality. Autolytic reactions produce ammonia, and oxidation may cause black spot (melanosis) development. Bacterial growth may produce spoilage off odors and flavors. Storage at low temperature (melting ice) is the most efficient way of delaying spoilage. Sensory assessment is used to determine quality of the product.

### *10.4.2 Microbial Data*

Table 10.3 summarizes useful testing for raw crustaceans. Refer to the text for important details related to specific recommendations.

**Table 10.3** Testing fresh crustaceans for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | If dipped in metabisulfite to prevent melanosis, measurement of residual sulfite may be required. If sanitizers are used in rinsing waters, monitoring residues may be required |
| In-process | Low | Routine samples are not collected during raw crustacean processing |
| Processing environment | Medium | Swab samples from equipment surfaces before start-up can be used to verify efficacy of cleaning and disinfecting procedures. Monitoring for indicators of enteric pathogens (e.g., *Salmonella* or *Vibrio* species) may be done if product is intended for raw consumption and epidemiological data indicate reason for concern |
| Shelf life | Low | Shelf life of raw, non-frozen crustaceans is short. pH increases during iced storage and may, depending on species, be monitored to indicate spoilage |
| End product | Medium | Routine microbiological testing is not recommended. Test for specific pathogens only when information indicates potential for contamination or when production conditions and history are not known |

#### 10.4.2.1 Critical Ingredients

Normally, raw crustaceans do not contain any added ingredients. To avoid formation of black spots, crustaceans may be dipped in metabisulfite, which may be hazardous to sensitive individuals. This may require that residual sulfur dioxide levels are monitored. In some countries, chlorine or other sanitizers may be added to the rinsing water and in such cases, monitoring residues may be needed.

#### 10.4.2.2 In-Process

Monitor time and temperature during processing to control of spoilage reactions.

#### 10.4.2.3 Processing Environment

Raw crustaceans undergo limited processing. Swabs for aerobic colony counts can be used to determine if the cleaning and disinfection procedures are working.

#### 10.4.2.4 Shelf Life

Crustaceans are highly perishable products and should be stored in melting ice or frozen. Determination of eating quality is done by sensory assessment.

#### 10.4.2.5 End Product

Routine microbiological testing of raw crustaceans is not recommended if the product is intended for cooking. However, if intended for raw consumption, sampling and testing for specific pathogens (salmonellae and *V. parahaemolyticus*) may be useful if no prior knowledge of the product is available. As for raw fish intended for raw consumption, crustaceans for raw consumption are rapidly consumed and end product testing is unlikely to be done before consumption.

## 10.5 Cooked Crustaceans

### *10.5.1 Significant Organisms*

#### 10.5.1.1 Hazards and Controls

Cooking processes used for crustaceans inactivate almost all of the microorganisms present. Both mechanical and manual handling after the cooking (e.g., peeling) may result in contamination from the raw product or human origin including enteric pathogenic bacteria, viruses and *Staphylococcus aureus*. As most competing microorganisms have been eliminated, *S. aureus* may grow and produce enterotoxin if the product is temperature abused. Cooked crab-meat may be manufactured as a refrigerated perishable product and psychrotrophic *C. botulinum* may be a safety issue. In the USA, pasteurized crab meat is given a Type E botulinum cook (e.g., at least 10 min at 90°C). Cooked crab-meat is also manufactured as a shelf-stable product (see Sect. 10.14). If the product is manufactured as refrigerated product, *L. monocytogenes* may become an issue.

#### 10.5.1.2 Spoilage and Controls

Cooked crustaceans will spoil due to bacterial growth; however, no specific microorganisms have been identified as spoilage organisms. Sensory assessment is recommended to determine degree of possible spoilage. If stored frozen, spoilage is not an issue of concern.

### *10.5.2 Microbial Data*

Table 10.4 summarizes useful testing for cooked crustaceans. Refer to the text for important details related to specific recommendations.

#### 10.5.2.1 Critical Ingredients

Crustaceans are typically brined at some point during processing and may be glazed before freezing. The bacteriological quality of brine and glazing water should be checked.

#### 10.5.2.2 In-Process

Time and temperature measurements during cooking procedure are used to control the cooking process. Microbiological sampling of the product during processing is not typically useful.

#### 10.5.2.3 Processing Environment

Cross contamination may occur from the processing environment and the level of bacteria on the final product reflects levels on the incoming raw material (Høegh 1989). There is evidence that crustaceans, such as raw farmed shrimp, may be contaminated with salmonellae. Also, handling, especially manual handling, may cause contamination with human pathogenic microorganisms. Areas in which cooked crustaceans are handled should be treated as high-risk zones. The peeling machines used to remove shells of shrimps may be difficult to clean and disinfect, and special care should be paid to this particular equipment. Swabs of surfaces can be used to determine efficacy of cleaning and disinfection procedures. If the product is intended for distribution under refrigerated conditions,

**Table 10.4** Testing cooked crustacean for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Raw animal | Low | Since the product is cooked during processing, microbiological testing of the raw material is not useful unless confidence in the supplier is low | | | | | | | |
| Critical ingredients | Low | These products may be brined during processing and water of drinking water quality should be used | | | | | | | |
| In-process | Low | Testing of the product during processing is not recommended | | | | | | | |
| Processing environment | Medium | The processing areas following cooking must be treated as high risk zones. Cleaning and disinfection procedures should be checked | | | | | | | |
| | High | Test for *Salmonella* (or indicators of enteric pathogens) in post cook areas during normal operation to verify control of the process. If product is refrigerated and not pasteurized in the container, test post cook areas during normal operation for *L. monocytogenes*. Typical guidance levels:<br>• *Salmonella* – absent<br>• *Listeria* spp. – absent | | | | | | | |
| Shelf life | Low | Microbial shelf life testing is not relevant for frozen, cooked crustaceans<br>For refrigerated, pasteurized crab meat, shelf life testing may be considered when changes are made to the process | | | | | | | |
| End product | | Routine sampling for pathogens is not necessary. Test for specific pathogens only when information indicates potential for contamination or when production conditions and history are not known (see text) | | | | | | | |
| | | | | | | Sampling plan & limits/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Low | Peeled cooked crustaceans | *S. aureus* | ISO 6888-1 | 8 | 5 | 1 | $10^2$ | $10^3$ |
| | | | | | | Sampling plan & limits/25 g[b] | | | |
| | Low | | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |
| | Low | | *L. monocytogenes* | ISO 11290-1 | NA[d] | 5[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[d] *NA* not applicable; used Codex criterion for RTE foods supporting *L. monocytogenes* growth

surveillance of *L. monocytogenes* should be considered in the post cook environment. Environmental monitoring for salmonellae in the post cook environment is also prudent.

#### 10.5.2.4 Shelf Life

Spoilage of cooked crustaceans proceeds quite rapidly; however, there are no solid data on bacterial growth rates and spoilage microorganisms. Counts above $10^6$ CFU/g indicate bacterial growth after cooking but these levels may not necessarily result in obvious signs of spoilage.

#### 10.5.2.5 End Product

Routine sampling for pathogens is not necessary. Test for specific pathogens only when information indicates potential for contamination or when production conditions and history are not known. This is especially true for peeled products where manual handling is likely. If the product is entering refrigerated storage and distribution system, sampling and testing for *L. monocytogenes* may be relevant. Sampling plans for ready-to-eat products that allow growth are given in Table 10.4.

## 10.6 Raw Mollusca

This product category includes filter feeding aquatic animals such as oysters, mussels, clams, cockles and scallops. Also gastropods, echinoderms and tunicates belong to this group. This section primarily deals with oysters that are often distributed alive and eaten raw. The oyster may also be shucked (removed from the shell) and distributed. While the immune defense of the live oyster protects it from deterioration, the shucked oyster spoils rapidly. Also, some products such as New Zealand green mussel are frozen raw (in half shell) and distributed.

### *10.6.1 Significant Organisms*

#### 10.6.1.1 Hazards and Controls

Live bivalve mollusca are relatively often the cause of foodborne disease. The agents causing disease are shellfish toxins, virus, enteric bacterial pathogens and *Vibrio* species. *V. vulnificus* may be a critical issue in some areas. Testing of the live animals is, in general, not an efficient way of controlling these agents of disease. Harvest waters may be monitored for algal blooms. The European Union classifies growing waters according to the content of enteric pathogens of the live animal (EC 2004a, b) and has limits for allowable levels of marine biotoxins (EC 2004a). Depuration is the process in which the live animals are placed in clean water and slowly rid themselves of pathogens. However, some pathogens, for instance virus, may stay in the animals even during depuration. Cooking will kill pathogenic *Vibrio* spp. but may not kill hepatitis A or norovirus. Heating to 90°C for 1.5 min appears to be effective (D'Souza et al. 2007). *Vibrio parahaemolyticus* is increasingly associated with foodborne disease from live bivalves and two major risk assessments have been conducted (FDA 2005, FAO/WHO 2011). *V. parahaemolyticus* and *V. vulnificus* may grow in the live animal and at temperatures >26°C. These bacteria may reach $10^5$–$10^6$ CFU/g, hence chilling is an important control.

The association between live mollusca and foodborne disease has long been recognized and in 1925 a US conference formed the basic principles of the National Shellfish Sanitation Program. This gives a set of general guidelines and notes the importance of clean waters. This program provides guidance on the level of *E. coli* acceptable in shellfish growing waters (Clem 1994). Currently, the USA classifies shellfish growing waters according to content of a coliform level; however, it is generally recognized that high levels of the traditional fecal indicators do not necessarily correlate with the presence of pathogenic vibrios or enteric viruses in raw molluscan shellfish.

Due to the epidemiological link between disease and consumption of raw molluscan shellfish, several agencies have microbiological criteria for these products. Also, in the USA, restaurants must post a note saying that consumption of raw molluscan shellfish may be dangerous. This posting is primarily due to the risk from *V. vulnificus* which appears particularly prevalent in parts of the USA.

The prevalence of norovirus as an emerging pathogen has been reported in many countries, in association with raw and shucked oysters. If it is suspected, the presence of norovirus should be tested specifically.

#### 10.6.1.2 Spoilage and Controls

Bivalve mollusca to be consumed raw are typically stored alive. Spoilage does therefore not occur as the immune system of the animal prevents degradation from occurring. Shucked mollusca should be stored at low temperatures; in ice, as spoilage will proceed rapidly.

## *10.6.2 Microbial Data*

Table 10.5 summarizes useful testing for raw bivalves. Refer to the text for important details related to specific recommendations.

### 10.6.2.1 Harvesting Waters

The USA classifies shellfish growing waters based on coliform levels (NSSP 2007). EU classifies harvesting areas into three categories (A, B or C) based on the level of coliforms, *E. coli* and *Salmonella* in the live animals. Neither may be a good reflection of the level of enteric virus present in the animals. Accumulation of shellfish toxins is a cause of disease and several countries have implemented surveillance programs of harvesting waters. Typically, these are based on environmental observations as well as sampling and analysis for either toxins (e.g., paralytic shellfish poisoning) or toxicity of the animals.

### 10.6.2.2 In-Process

There is limited processing of live bivalve mollusca. In the live state they may be depurated and further processing may include shucking. Water quality must be controlled and some measure of depuration efficiency can be obtained by monitoring fecal indicators.

**Table 10.5** Testing live (raw) bivalves for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Aquatic environment | High | Monitor shellfish growing waters for appropriate indicators of water quality (see text) |
| Critical ingredients | Low | Water and ice used to process or hold (depurate) raw bivalves must come from an uncontaminated source. Test when water quality is in question |
| In-process | Low | Live bivalves pass through only a limited processing |
| Processing environment | Low | Live bivalves pass through only a limited processing. The hygiene status may be monitored by swabs for total count |
| Shelf life | Low | The live animals will themselves prevent spoilage. Shucked bivalves spoil rapidly |
| End product | Low to high | If the product is from known approved waters, end product testing is not useful. Where the status of the growing waters is not known, or where contamination is suspected, testing may be useful (also see text) |

| | | | | Sampling plan & limit/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Live bivalve mollusca | *E. coli* | ISO 7251 | 6 | 5 | 1 | 2.3 | 7 |
| | *V. parahaemolyticus*[c] | ISO/TS 21872-1 | 9 | 10 | 1 | $10^2$ | $10^4$ |
| | | | | Sampling plan & limit/25 g[b] | | | |
| | | | | *n* | *c* | *m* | *M* |
| | *Salmonella* | ISO 6579 | 11 | 10[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Only from waters suspected to harbor *Vibrio* spp. In some areas, lower levels of *M* (e.g., $10^3$) may be more relevant to ensure safety
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 10.6.2.3 Processing Environment

The processing environment is not likely to contribute to safety risks from this product.

#### 10.6.2.4 Shelf Life

Live mollusca do not spoil easily. Dead animals will spoil rapidly and spoilage is easily detected by sensory assessment.

#### 10.6.2.5 End Product

While end product testing will not control disease from this product, it may allow the most contaminated lots to be detected. The EU standard for live bivalves suggests testing of five samples for *Salmonella* and for *E. coli* $n=1$, $c=0$, $M=230$ MPN/100 g in flesh and intra-valvular liquid, with the sample comprised of a minimum of ten animals (EC 2005). The ICMSF case sampling plans suggest case 10 or 11, which suggests a higher number of samples. Setting a limit for these organisms can be useful for areas where *Vibrio* species are at high levels in shellfish growing and harvesting waters.

The Codex Alimentarius Committee on Fish and Fishery Products discussed (Codex Alimentarius 2008) microbiological *Vibrio* standards for live and raw mollusca. An FAO/WHO (2010) risk assessment on *V. parahaemolyticus* in oysters indicates that the establishment of a limit can be an effective means to reduce risk to human health, provided there is compliance with that limit. However, the risk reduction to health comes at a price in terms of the amount of product that would potentially be rejected. The risk assessment considered a balance between these two factors, estimating that a maximum level of $10^3$ CFU/g would lead to a reduction in illness of more than 2/3; however, it would also cause rejection of up to 20% of products. A maximum level of $10^4$ CFU/g would reduce illness between 20 and 90% and lead to rejection of 1–2% of products on the market.

Testing for enteric virus, or viruses indicating this group, may be possible in the future and may be a more relevant parameter for testing.

## 10.7 Cooked, Shucked Mollusca

The meat of bivalves may be removed from the shell using physical force (e.g., forcing the shells apart with a knife) or by subjecting the animals to mild heat before shucking to relax the adductor muscle. The raw meat may be distributed as raw product, in which case the hazards and criteria used for raw live bivalves apply. As opposed to the live animal, the raw meat will spoil rapidly. The shucked meat is often heated either as pasteurized or commercially sterile product.

### *10.7.1 Significant Organisms*

#### 10.7.1.1 Hazards and Controls

Live bivalve mollusca are relatively often the cause of foodborne disease. The agents causing disease are shellfish toxins, virus, enteric bacterial pathogens and *Vibrio* species. The issues outlined in the former section apply also to the raw meat. The heated (pasteurized) meat is similar to cooked crustaceans in terms of hazards to be controlled. The EU microbiological criteria for cooked shucked meat of bivalves are the same as for cooked crustaceans (EC 2005).

#### 10.7.1.2 Spoilage and Controls

Raw bivalve mollusk meat spoils very rapidly. Due to the high glycogen content, a fermentative type of spoilage usually takes place. The spoilage may be monitored by sensory assessment and pH measurements. The products are mostly distributed as frozen products and spoilage is prevented by the low temperature.

## *10.7.2 Microbial Data*

Table 10.6 summarizes useful testing for cooked, shucked mollusca. Refer to the text for important details related to specific recommendations.

#### 10.7.2.1 Harvesting Waters

The issues outlined in the former section apply here.

#### 10.7.2.2 In-Process

Cooking of shucked bivalves is a critical control point because it is a kill step for vegetative pathogenic bacteria. The pasteurization may take place in packed product (pouched) in which case postpasteurization contamination is not a problem.

**Table 10.6** Testing shucked, cooked bivalves for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Aquatic environment | High | Monitor shellfish growing waters for appropriate indicators of water quality (see text) |
| Critical ingredients | Low | Shucked, cooked bivalves do not normally contain any ingredients |
| In-process | High | Water quality and heating steps should be controlled |
| Processing environment | Low to high | If heated in pouch, the processing environment is of low importance<br>If handled after heating, sampling equivalent to other pasteurized products must be in place (see text)<br>Cleaning and disinfection procedures may be monitored |
| Shelf life | Low | If not further preserved (frozen, in-pack pasteurized), the product will spoil rapidly |
| End product | Medium | Routine sampling for pathogens is not recommended. If application of GHP or HACCP is in question, the following sampling plans are recommended (see text) |

| | | | | Sampling plan & limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Shucked, cooked bivalves not processed in pack | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 10.7.2.3 Processing Environment

Shucked, cooked bivalves may be heated in pouch, in which case the processing environment is of low importance. However, if any handling takes place after heating, this becomes a high-risk zone and environmental sampling equivalent to other pasteurized products must be in place. This may include surveying for specific pathogens or indicators thereof. Cleaning and disinfection procedures may be monitored by environmental sampling. The processing environment should be monitored for hygienic status as described for cooked crustaceans.

#### 10.7.2.4 Shelf Life

Shucked, cooked mollusca spoil easily and should be kept at refrigerated temperature.

#### 10.7.2.5 End Product

The heat treatment eliminates Gram-negative pathogens acquired from the growing waters, while inactivation of viral pathogens requires further study. The product is prone to contamination from the processing environment if not processed in-pack. Lots where contamination is suspected can be tested for *Salmonella* and *S. aureus* following the same criteria as for cooked crustaceans.

## 10.8 Surimi and Minced Fish Products

Surimi and other minced fish products consist of washed fish proteins; typically from white-fleshed fish species. Often these are intermediate products intended for further processing into products such as crab sticks or kamaboko.

### *10.8.1 Significant Organisms*

#### 10.8.1.1 Hazards and Controls

There are no special hazards related to these products and many products are heated before consumption. Minced fish products are typically distributed as frozen, cooked products and may be eaten without further processing. These products are equivalent to those described in Sect. 10.13. Pathogenic microorganisms from the human-animal reservoir that may be transferred during cross-contamination may constitute a risk. Observing good hygienic practices during processing will control these organisms. If the products are sold packed and refrigerated, the pathogenic bacteria of interest in other ready-to-eat fish products should be considered. *C. botulinum* can grow and produce toxin in vacuum-packaged surimi and only low temperature storage and short storage lives can effectively control this risk. In the USA, surimi may be given a Type E botulinum cook (e.g., at least 10 min at 90°C). *L. monocytogenes* has been detected in surimi products and is capable of growing. Cooking in package will control this hazard. Sampling plans and standards as developed for lightly preserved fish products apply.

#### 10.8.1.2 Spoilage and Controls

When stored frozen, there are no spoilage issues. If stored refrigerated, spoilage is of bacterial origin (e.g., *Bacillus*) and is easily detected by sensory assessment. Low temperature storage is the most efficient control of spoilage.

## *10.8.2 Microbial Data*

Table 10.7 summarizes useful testing for surimi and cooked minced fish. Refer to the text for important details related to specific recommendations.

### 10.8.2.1 Critical Ingredients

There are no critical ingredients in this product. Cryoprotectants, salt, soy protein and starch may be added but do not influence microbiological safety or spoilage.

### 10.8.2.2 In-Process

In-process samples are not necessary.

### 10.8.2.3 Processing Environment

Swabs for aerobic colony counts can be used to determine if the normal cleaning and disinfection procedures are working. If the product is distributed refrigerated and a risk of *L. monocytogenes* has been identified, then the processing environment should be sampled for *L. monocytogenes*.

**Table 10.7** Testing surimi and cooked minced fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Surimi does not contain critical ingredients |
| In-process | Low | Routine samples are not collected of surimi during processing |
| Processing environment | Low | Samples from equipment surfaces before start-up can be used to verify efficacy of cleaning and disinfecting procedures |
| | High | If products are distributed refrigerated and not pasteurized in-bag, environmental monitoring of *L. monocytogenes* is needed |
| Shelf life | Low | No standard procedures exist |
| End product | Low | Microbiological testing is not recommended for frozen product. If products are distributed and stored refrigerated, sampling and testing for *L. monocytogenes* may be relevant unless pasteurized in-bag |

| | | | | Sampling plan & limits/g[c] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case[b] | *n* | *c* | *m* | *M* |
| Surimi and minced fish No growth | *L. monocytogenes* | ISO 11290-2 | NA[b] | 5 | 0 | $10^2$ | – |
| | | | | Sampling plan & limits/25 g[c] | | | |
| | | | | *n* | *c* | *m* | *M* |
| Growth supported | *L. monocytogenes* | ISO 11290-1 | NA[b] | 5[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] *NA* not applicable due to use of Codex criteria
[c] Refer to Appendix A for performance of these sampling plans
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 10.8.2.4 Shelf Life

Microbiological spoilage should not be an issue for products produced under normal GHP and HACCP programs.

#### 10.8.2.5 End Product

Sampling and testing of frozen products is not recommended for either safety or spoilage. If products are distributed and stored packed and refrigerated, sampling and testing for *L. monocytogenes* may be relevant, if not cooked in the final package.

## 10.9 Lightly Preserved Fish Products

Lightly preserved fish products are typically ready-to-eat products preserved by low levels of NaCl (3–6% in water phase), low levels of acid or food preservatives. Some are based on raw fish (cold-smoked or brined fish) others on cooked product (brined crustaceans). Typically, they are vacuum-packed and marketed as refrigerated products, although some distribution is done with frozen products. Refrigerated shelf life is typically 3–4 weeks for vacuum-packed cold-smoked fish and can be longer for brined crustaceans.

### *10.9.1 Significant Organisms*

#### 10.9.1.1 Hazards and Controls

Products that use raw fish for processing carry some of the same hazards as raw fish, such as the presence of aquatic toxins, parasites and histamine. The preservation parameters are not always sufficient to control growth of two important human pathogens, psychrotrophic *C. botulinum* and *L. monocytogenes.* Combining NaCl, low temperature and shelf life limitation is used to control these hazards. Some products are handled manually during processing and as they are ready-to-eat foods, human enteric pathogens may be transferred to the product if appropriate good hygienic practice measures are not in place.

#### 10.9.1.2 Spoilage and Controls

Several lightly preserved fish products spoil due to microbial growth and metabolism. However, several groups of bacteria may contribute to the spoilage and microbiological testing cannot be used to determine degree of spoilage or expected shelf life. Sensory assessment is used to determine eating quality of the products.

### *10.9.2 Microbial Data*

Table 10.8 summarizes useful testing for lightly preserved fish. Refer to the text for important details related to specific recommendations.

**Table 10.8** Testing lightly preserved fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Consider parasites and histamine according to description in Table 10.1 if confidence in the supplier is low (see text) |
| | Low | If brine injection is used, the brine should be prepared freshly for each batch or checked for presence of *L. monocytogenes*, which should be absent |
| In-process | Low | In-process samples are not routinely collected |
| Processing environment | High | Swab product contact surfaces and close surfaces, and test for aerobic colony count and *L. monocytogenes*. Typical levels encountered after cleaning and disinfection:<br>• Aerobic colony counts – <10–$10^2$ CFU/$cm^2$<br>• *L. monocytogenes* – absent |
| Shelf life | Medium | Shelf life testing through sensory assessment may be useful for products with longer shelf life. The potential for growth of *L. monocytogenes* during shelf life should be determined |
| End product | Medium | Routine sampling for pathogens is not necessary. If application of GHP and HACCP is in question, sampling for *L. monocytogenes* may be considered in lot acceptance |

| Product | Hazard | Analytical method[a] | Case | Sampling plan & limits/g[b] *n* | *c* | *m* | *M* |
|---|---|---|---|---|---|---|---|
| Lightly preserved fish No growth | *L. monocytogenes* | ISO 11290-2 | NA[c] | 5 | 0 | $10^2$ | – |
| | | | | Sampling plan & limits/25 g[b] n | c | m | M |
| Growth supported | *L. monocytogenes* | ISO 11290-1 | NA[c] | 5[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] *NA* not applicable due to use of Codex criteria
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 10.9.2.1 Critical Ingredients

The hazards outlined for raw fish are carried over to this product, unless cooked raw material is used (see Table 10.1). If a supplier program is not in place, testing for histamine in scombroid species may be useful. Fish from waters with algal blooms should not be used. Wild caught fish are likely to harbor parasites and some countries require that they be frozen for 24 h at −20°C to kill parasites.

Fish intended for cold-smoking is brined before smoking. Brining may be done by dry salting, by bath-brining or by injection brining. The brine can be a reservoir of *L. monocytogenes* and should not be reused. The brine should be analyzed for presence of *L. monocytogenes* if *L. monocytogenes* contamination of the final product is detected. If the brine is not prepared fresh for each batch during processing, presence of *L. monocytogenes* should be monitored. The NaCl is not per se a source of contamination, but NaCl-levels in the final product should be measured as this is an essential parameter in controlling *C. botulinum.*

#### 10.9.2.2 In-Process

Microbiological testing of product during normal processing is not recommended. In case of investigational sampling, the fish may be sampled during processing to determine the site of

contamination. Although done at a relatively low temperature (e.g., 22–26°C), the cold-smoking process results in reduction of bacterial counts. This can be verified testing swabs of the fish before and after this processing step. Approximately 1 log reduction is to be expected.

#### 10.9.2.3 Processing Environment

The processing environment is the most common immediate source of contamination with *L. monocytogenes* and sampling surfaces and the processing environment may be helpful to control this microorganism. The frequency and extent of sampling will depend on the potential for growth relative to the shelf life date. If products are stabilized to prevent *Listeria* growth, less frequent sampling is required. The frequency of occurrence of *Listeria* spp. may correlate with finding *L. monocytogenes* in some plants. However, this is not universal and some plants may be completely dominated by non-*monocytogenes* listeriae. The general status of cleaning and disinfection can be monitored by swab sampling and determining the aerobic colony count. In general, product contact surfaces should contain less than 10 CFU/cm$^2$ after cleaning and sanitizing based on swab-samples with the occasional sample reaching 100 CFU/cm$^2$. If agar-contact sampling is used, the number is lower. Codex Alimentarius (2009) provides general guidance on control of *L. monocytogenes* in processing environments.

#### 10.9.2.4 Shelf Life

The shelf life of these products may be determined by safety considerations, such as ensuring that *C. botulinum* or *L. monocytogenes* do not grow to hazardous levels. Procedures for validating that these microorganisms are controlled may involve a combination of measuring growth in naturally contaminated products or in inoculated products as well as using predictive models. In terms of eating quality, the shelf life of these product types may vary dramatically between processors. Sensory assessment is used for this purpose and may be used when validating code dates.

#### 10.9.2.5 End Product

Application of GHP and HACCP should ensure prevention of cross-contamination. If the conditions of manufacture are not known or if the reliable application of GHP and HACCP is in question, sampling for *L. monocytogenes* may be appropriate. Depending on the potential for growth during storage, either the microorganism should be absent in 25 g or its presence in low levels is tolerable.

Sampling for *C. botulinum* is not recommended as control of this microorganism is ensured by elevated salt levels and low temperature. Scombroid species (e.g., tuna, mahi-mahi) may contain histamine and products can be tested if no prior knowledge is available. See Sect. 10.2.2.5 for current recommendations.

## 10.10 Semi-Preserved Fish Products

These products are typically based on raw fish or roe being preserved by salt, acid and food preservatives. The level of preservatives is typically higher (more salt, more acid) than in the lightly preserved products described above. Examples are marinated herring, roll-mops, anchovies or caviar. As compared to the lightly preserved fish products, the products are more preserved and have a longer shelf life. Shelf life is often several months.

## *10.10.1 Significant Organisms*

### 10.10.1.1 Hazards and Controls

Few pathogens are relevant to semi-preserved fish, but parasites may be considered due to the use of raw fish. Products are typically packed under oxygen-limited conditions and growth of *C. botulinum* can be a risk if not controlled by the combination of high NaCl, acid and low temperature. The products do not support growth of *L. monocytogenes*. Preformed histamine should be considered.

### 10.10.1.2 Spoilage and Controls

Few spoilage microorganisms can grow in semi-preserved fish products but yeasts can cause spoilage especially in products with low acidification (pH>4.5).

## *10.10.2 Microbial Data*

### 10.10.2.1 Critical Ingredients

The products contain no ingredients that affect microbiological safety and spoilage

### 10.10.2.2 In-Process

In-process sampling is not useful for these products.

### 10.10.2.3 Processing Environment

Sampling of the processing environment is generally not recommended for the semi-preserved fish products. However, this may be required during investigational sampling, for example if spoilage problems are encountered. Also, general cleanliness of the processing environment may be assessed by swab sampling and testing for aerobic colony count.

### 10.10.2.4 Shelf Life

The semi-preserved fish products have relatively long shelf life. Shelf life dating may be validated by storage trials using sensory assessment as a measurement.

### 10.10.2.5 End Product

Sampling and microbiological testing of end products is not useful for ensuring safety or quality, and thus routine sampling is not recommended. If spoilage problems arise, testing for lactic acid bacteria (LAB) and yeasts should be considered. Yeast counts above $10^4$ CFU/g or LAB above $10^7$ CFU/g can indicate that spoilage is of microbial origin. Note that histamine levels can be higher than that recommended for fresh products because this is formed naturally during the ripening of sardines. For histamine testing, see Sect. 10.2.2.5 for current recommendations (Table 10.9).

**Table 10.9** Testing semi-preserved fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Consider parasites and histamine according to description in Table 10.1 if confidence in the supplier is low |
| In-process | Low | Routine in-process samples are not necessary |
| Processing environment | Low | Routine sampling of equipment and the environment is not recommended. Sampling may take place during investigational sampling |
| Shelf life | Low | These products have a relatively long shelf life. Shelf life may be validated using storage trials and sensory assessment |
| End product | Low | Routine sampling is not recommended. If application of GHP and HACCP is in question, sampling for histamine may be considered for lot acceptance of scombroid species |

## 10.11 Fermented Fish Products

This section considers typical Southeast Asian products that are truly fermented; i.e., where microbial growth and acid production has taken place. These are products where low levels of salt (2–6%) are added to raw fish and fermentation takes place at ambient temperature. Autolyzed fish sauces and pastes containing 6–25% salt are addressed in Chap. 14.

### *10.11.1 Significant Organisms*

#### 10.11.1.1 Hazards and Controls

The use of raw fish makes parasites a significant hazard. Due to the anaerobiosis during fermentation, growth of *C. botulinum* should be considered. Careful removal of gut and washing of the cavity is critical to control *C. botulinum*. While naturally present *Vibrio* spp. from marine fish are not eliminated by processing, they do not proliferate during fermentation. Pathogens associated with the processing environment or with human handling may be present as a result of cross-contamination. Pond reared fish are often used for these products and the use of animal or human fertilizers in the pond can be a source of enteric pathogens such as *Salmonella* or human enteric viruses. The addition of low levels of NaCl inhibits pathogen growth until the LAB, which are the main fermenting microorganisms, become dominant.

#### 10.11.1.2 Spoilage and Controls

Despite the fermentation process and the high level of LAB in the final product, these products do not have long shelf life. Little is known about the spoilage process but it may be caused by LAB.

### *10.11.2 Microbial Data*

Table 10.10 summarizes useful testing for fermented fish products. Refer to the text for important details related to specific recommendations.

**Table 10.10** Testing fermented fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Parasites should be considered in raw fish as outlined in Table 10.1 |
| In-process | Medium | Measuring pH during the process assures that the fermentation is proceeding as expected |
| Processing environment | Low | Routine testing of processing environment is not recommended |
| Shelf life | Low | The products have relatively short shelf lives. Microbiological testing is not useful in determining shelf life limits |
| End product | Low | Routine sampling of the end product is not necessary (see text). If product is eaten raw, testing for specific pathogens or indicator microorganisms may be useful. If application of GHP and HACCP is in question, sampling for *Salmonella* may be considered for lot acceptance |

| | | | | Sampling plan & limit/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Fermented fish products | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 10.11.2.1 Critical Ingredients

Rice or other starchy ingredients may be added but neither is critical for microbiological safety or quality.

#### 10.11.2.2 In-Process

Product should be sampled during fermentation to validate pH decrease, which should decrease below 4.5 in 1–2 days.

#### 10.11.2.3 Processing Environment

Routine sampling of the processing environment is not recommended. In several small scale processes, back-slopping is used and the presence of fermenting microorganisms in the processing environment is required as starter culture.

#### 10.11.2.4 Shelf Life

If fermented properly, shelf life need not be limited for safety reasons. Determination of shelf life is done by sensory assessment.

#### 10.11.2.5 End Product

End product testing is not recommended for either safety or for quality. Emphasis should be on ensuring rapid fermentation through measurement of pH and NaCl in the water phase. If product is eaten raw,

testing for specific pathogens or indicator microorganisms may be useful. In case of investigational sampling in relation to botulism, sampling and testing for *C. botulinum* may be done. If fish from integrated farms are used, enteric pathogens, such as *Salmonella,* may be a concern.

## 10.12 Fully Dried or Salted Products

Fully dried or salted fish products are shelf-stable because they contain low levels of water. The only safety issue is the potential growth of mycotoxigenic fungi. Rapid drying and storage under dry conditions can control this risk. The products are shelf-stable if kept dry. They may spoil due to fungal growth.

## 10.13 Pasteurized Seafood Products

These products receive a heat treatment similar to pasteurization. Typical products are hot-smoked fish (60°C for 30 min) or sous-vide cooked products. Crab meat may be packed and pasteurized after cooking and peeling. Also, in some countries, surimi-based products are cooked (in-pack) and distributed as refrigerated products. Pasteurized mollusks were discussed in Sect. 10.7.

### *10.13.1 Significant Organisms*

#### 10.13.1.1 Hazards and Controls

Some of the hazards of raw fish carry over into the pasteurized products, i.e., aquatic toxins and histamine. Parasites are eliminated by pasteurization. If the products are handled after heat treatment, cross contamination with *L. monocytogenes* and enteric pathogens is a potential risk. If vacuum-packaged, potential growth and toxin production by *C. botulinum* should be controlled by a combination of NaCl and low temperature. In sous-vide products, a cooking temperature of 90°C for 10 min will eliminate spores of psychrotrophic *C. botulinum*. Viral pathogens may also emerge as a concern in certain products as information on heat resistance advances.

#### 10.13.1.2 Spoilage and Controls

Microbial growth can cause spoilage of these products. Thus, if aerobically packed, fungal growth occurs on hot-smoked fish. Some packs of sous-vide products may spoil due to germination and growth of spore formers.

### *10.13.2 Microbial Data*

Table 10.11 summarizes useful testing for pasteurized fish products. Refer to the text for important details related to specific recommendations.

#### 10.13.2.1 Critical Ingredients

Salt is typically added to these products. In some, such as hot-smoked fish, it is a critical ingredient with respect to prevention of growth of *C. botulinum* type E. Levels in the final product above 3% should be reached.

**Table 10.11** Testing pasteurized fish for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Raw fish | Medium | Parasites are destroyed by the cooking processes. If a supplier program is not in place, testing for histamine in scombroid species may be useful. Fish from waters with algal blooms should not be used |
| Critical ingredients | Low | If brine injection is used for salted products, the brine should be prepared freshly for each batch. If this is not the case, the brine should be checked for presence of *L. monocytogenes* even if subsequent heat treatment is believed to destroy the bacterium |
| In-process | Low | In-process samples are not normally collected but should be considered for investigational sampling |
| Processing environment | High | Swab product contact surfaces and close surfaces, and test for aerobic colony count and *L.* monocytogenes. Typical levels encountered after cleaning and disinfection:<br>• *L. monoc\ytogenes* – absent |
| Shelf life | Medium/high | Sensory evaluation may be useful for products with longer shelf life. The potential for growth of *L. monocytogenes* during shelf life should be determined. Products such as sous-vide cooked products should have a shelf-life limit that controls *C. botulinum* |
| End product | Medium | Routine sampling for pathogens is not necessary. If application of GHP and HACCP is in question, sampling for *L. monocytogenes* may be considered for lot acceptance |

| | | | | Sampling plan & limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Hazard | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Pasteurized fish, RTE No growth | *L. monocytogenes* | ISO 11290-2 | NA[c] | 5 | 0 | $10^2$ | – |
| | | | | Sampling plan & limit/25g[b] | | | |
| | | | | *n* | *c* | *m* | *M* |
| Growth supported | *L. monocytogenes* | ISO 11290-1 | NA[c] | 5[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] NA not applicable due to use of Codex criteria
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

### 10.13.2.2 In-Process

Microbiological testing of product during normal processing is not recommended. In case of investigational sampling, the fish may be sampled during processing to determine site of contamination. The pasteurization is a bactericidal step and measurement of heat treatment temperatures should be part of the HACCP program. Bactericidal effect can be verified by testing swabs of the fish before and after this processing step.

### 10.13.2.3 Processing Environment

The postprocessing environment has little importance for the microbiological quality and safety if the product is packaged before pasteurization. However, if the product is handled after heat treatment, the processing environment becomes crucial. It is the most common source of contamination

with *L. monocytogenes* and an environmental monitoring program may be helpful in controlling this microorganism. The frequency and extent of sampling depends on the potential for growth relative to the shelf life. If products are stabilized (e.g., *Listeria* cannot grow), less frequent sampling is required. The general cleanliness of the processing environment may be determined by swab sampling and testing for aerobic colony counts.

#### 10.13.2.4 Shelf Life

The shelf life of these products varies. Hot-smoked fish can be stored for 2–3 months if vacuum-packaged; refrigerated, pasteurized crabmeat may have a shelf life of up to 18 months; whereas sous-vide products have a much shorter refrigerated shelf life. Safety considerations should ensure that *C. botulinum* and *L. monocytogenes* do not grow to hazardous levels. This may involve a combination of measuring growth in naturally contaminated products or in inoculated products, as well as using predictive models. In terms of eating quality, the shelf life of these product types may vary dramatically, also between processors. Sensory assessment is used for this purpose and may be used when validating code dates.

#### 10.13.2.5 End Product

Application of GHP and HACCP should ensure prevention of cross-contamination. If the conditions of manufacture are not known or if the reliable application of GHP and HACCP is in question, sampling for *L. monocytogenes* may be appropriate in products that are not heat treated by the consumer before consumption. Depending on the potential for growth during storage, either the microorganism should be absent in 25 g or its presence in low levels is tolerable.

Sampling for *C. botulinum* is not recommended as control of this microorganism should be ensured by NaCl-levels, low temperature, short storage time and/or heat treatment of the product before consumption. For scombroid fish, testing for histamine should be considered and the reader is referred to Sect. 10.2.2.5 for current testing recommendations.

## 10.14 Canned Seafood

### *10.14.1 Significant Organisms*

#### 10.14.1.1 Hazards and Controls

The significant hazards of microbial origin in fully retorted seafood products are *C. botulinum* (only when under processed), some aquatic toxins and histamine. Histamine is heat stable and if formed preprocessing, it will be present in the finished canned product. Controlling time and temperature of the raw material in a chilled state is important to reduce risk from histamine poisoning. Refer to Chap. 24, for general controls for canned products.

#### 10.14.1.2 Spoilage and Controls

Spoilage of canned seafood products rarely occurs and is controlled by an appropriate heat treatment and container integrity.

### *10.14.2 Microbial Data*

#### 10.14.2.1 Critical Ingredients

Parasites are destroyed by the cooking processes. If a supplier program is not in place, testing for histamine in scombroid species may be useful. Fish or shellfish from waters with algal blooms should not be used.

#### 10.14.2.2 In-Process

In-process testing are not recommended; however, monitoring critical parameters of the thermal process is essential for safety and stability of the final product (see Chap. 24).

#### 10.14.2.3 Processing Environment

Environmental samples are not recommended.

#### 10.14.2.4 Shelf Life

Products produced under existing commercial sterilization programs based on GHP and HACCP should not experience microbial spoilage.

#### 10.14.2.5 End Product

If scombroid fish species have been used as raw material, testing for histamine may be recommended for lot acceptance if knowledge of the supplier program is not known. Follow the criteria for histamine testing recommended for pasteurized scombroid species in Table 10.11.

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# Chapter 11
# Feeds and Pet Food

## 11.1 Introduction

Feed is an important element of the food chain as it may contribute to the introduction of pathogens such as *Listeria monocytogenes* or *Salmonella* in the human food chain (Crump et al. 2002; Sapkota et al. 2007). Although only low levels and prevalence have been reported, feed has also been proposed as vector contributing to the presence of *Escherichia coli* O157:H7 in livestock (Davis et al. 2003; Dodd et al. 2003; Hutchinson et al. 2006; Sanderson et al. 2006). In this book, the microbiology of feeds and pet foods is only discussed in the light of its importance for human health and not in relation to the health of animals.

The origin of many cases and outbreaks of human disease has been linked to the contamination of animal feed with pathogens. *Salmonella* is the most widely known example. In the 1990, feed components were identified as sources of bovine spongiform encephalopathy (BSE) in cattle, for which epidemiological links were established with the variant Creutzfeld–Jacobs disease in humans.

Recommendations or regulations on the application of Good Hygiene Practices for animal feeding have been published by the Codex Alimentarius Commission (CAC 2004), the European Commission (2005) or the US FDA (2010).

Pet foods may also be a source of human disease, and contamination of different types of raw or processed pet food with *Salmonella* is well established (Finley et al. 2006, 2007; CDC 2008a, b). Such contamination leads to the direct or indirect exposure of persons in contact with pets, in particular of infants and children. Direct transmission of pathogens from pets such as cats, dogs, turtles and other reptiles are well established and excretion of human pathogens in pet environments contributes to human exposure. Consult ICMSF (2005) for more background on the microbial ecology and control measures appropriate to feeds and pet foods.

## 11.2 Processed Feed Ingredients

Feed ingredients are manufactured from animal and plant by-products which represent cheap sources of proteins and other elements such as fibers. They include meat and bone meal, fish meal, citrus pulp pellets, oilseed meal, corn gluten, corn fiber, soybean meal and flakes, etc. (see e.g. Bampidis and Robinson 2006; Lefferts et al. 2006; Sapkota et al. 2007; Thompson 2008; Berger and Singh 2010).

Such by-products are typically heat treated and dried before they are used as complete feed or included in compounded feed.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_11,

## *11.2.1 Significant Organisms*

### 11.2.1.1 Hazards and Controls

*Salmonella* is a recognized pathogen in animal and plant by-products. For salmonellae, heat treatment and prevention of post-process contamination are the most important control measures.

The presence of *Salmonella* in heat treated by-products is due to recontamination as shown by several authors (e.g., Jones and Richardson 2003; Nesse et al. 2003; EFSA 2008; Vestby et al. 2009; Davies and Wales 2010). This can be prevented through the application of GHP, especially strict separation of processing areas for raw and rendered material to avoid presence of the pathogen in the processing environment.

BSE was recognized as a major hazard in the 1990 and it became soon evident that heat treatments applied to destroy vegetative microorganisms such as *Salmonella* are insufficient to adequately control BSE. In order to prevent or reduce transmission of BSE, several authorities have taken regulatory measures to prohibit or restrict the use of animal by-products such as meat, bone meal and cerebrospinal tissues (Denton et al. 2005). When properly implemented, these measures led to a drastic reduction of BSE-cases. For more detailed information concerning these control measures the reader is referred to ICMSF (2005).

The contamination of raw materials of agricultural origin used to manufacture feed ingredients with mycotoxins such as aflatoxins, deoxynivanelol, fumonisins, zearalenone, T-2 toxins, ochratoxin and certain ergot alkaloids is widespread and has been discussed (Binder et al. 2007; Richard 2007). The occurrence of these mycotoxins not only represents a direct threat to animals but also to the food chain through the contamination of foods of animal origin such as milk, meat and eggs. Contamination risk and management options have been discussed (Kabak et al. 2006; Binder 2007; Kan and Meijer 2007; Coffey and Cummins 2008; Magnoli et al. 2010).

Selection of the ingredients, especially grains, is the control method of choice and testing of incoming raw materials can be useful as verification or monitoring, especially when using simple and inexpensive rapid screening methods. Testing for acceptance has limitations because of frequent heterogeneous contamination and associated limitations of sampling. Further discussions of this topic can be found in Chap. 15.

Raw materials and feed ingredients themselves stored in silos must be held under appropriate conditions to prevent mold growth and subsequent mycotoxin formation. Specific considerations to control temperature and humidity include material of construction, appropriate ventilation and insulation where necessary. Use conditions to prevent development of mycotoxins include:

- Regulation of flow to avoid coating and feed deposits.
- Complete evacuation of feeds.
- Thorough cleaning after emptying.
- Disinfection at regular intervals.
- Monitoring temperature and humidity.
- Periodic examination for visible mold.

Routine testing for molds and mycotoxins is not recommended in stored products. Monitoring storage parameters such as temperature and relative humidity are much more effective in demonstrating control, especially when done on a continuous basis.

### 11.2.1.2 Spoilage and Controls

Mold growth can also lead to spoilage of stored raw materials and end products. Control of spoilage is achieved through appropriate preparation and storage conditions discussed above.

## *11.2.2 Microbial Data*

Table 11.1 summarizes useful testing for processed feed ingredients. Refer to the text for important details related to specific recommendations.

### 11.2.2.1 Critical Ingredients

All animal offal and by-products as well as carcasses of diseased or deceased animals can potentially be contaminated with *Salmonella*. This is also true for plant by-products. However, heat-treatments are designed to destroy these vegetative microorganisms, thus testing of such raw materials for *Salmonella* is not recommended.

With respect to prions, an effective feed ban is measured by the estimation of BSE prevalence rates over a number of years. This is accomplished through BSE surveillance aiming at detecting

**Table 11.1** Testing of processed feed ingredients for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | It is not recommended to test animal or plant by-products for *Salmonella* which will be submitted to a heat-treatment<br>Recommendations for mycotoxins can be found in Chap. 15 |
| In-process | High | Testing of product residues from product contact surfaces after a kill-step for *Salmonella* and Enterobacteriaceae is essential during normal operation to verify control of the process. Typical levels encountered:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g<br>• Aerobic mesophilic counts – internal limits |
| Processing environment | High | Testing of residues and dust is essential during normal operation to verify control of the process. Test for *Salmonella* and Enterobacteriaceae in relevant areas. Typical levels encountered:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g or sample |
| Shelf life | Low | For products able to support growth of molds when there is moisture uptake, monitoring of the relative humidity or water activity is more relevant than testing for molds |
| End product | High | Testing for indicators of processed products is essential to verify control of process |

| | | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Processed feed ingredients | Enterobacteriaceae | ISO 21528-1 | 2 | 5 | 2 | $10^2$ | $10^3$ |

| | | Low/ High | Testing for *Salmonella* is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. Test for pathogens only when other data indicate potential for contamination |
|---|---|---|---|

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Processed feed ingredients | *Salmonella* | ISO 6579 | 10 | 5[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Chap. 7, Sect. 7.5.2 for compositing)

infected animals with a high degree of confidence and thus to eliminate them from the food chain (EFSA 2004; USDA 2006). Implementation of BSE surveillance testing of healthy slaughter cattle depends on the outcomes of a risk assessment which account for a country's risk factors and risk management actions.

Studies on the inactivation of BSE in rendering processes have shown that some of them were more effective than others in inactivating prions (Taylor 1998; Acheson et al. 2000; Taylor 2000; Grobben et al. 2005; Giles et al. 2008).

#### 11.2.2.2 In-Process

Testing residues from critical product contact surfaces located after kill-steps where presence or growth of *Salmonella* may occur is useful to detect contamination originating from the processing environment. For BSE agents, where presence would be related to inadequate heat-processing of contaminated raw material, testing of in-process samples is not relevant.

#### 11.2.2.3 Processing Environment

Testing samples such as dust or scrapings of residues from the processing environment for *Salmonella* is important in providing information as to the effectiveness of preventive measures, such as separation of different processing areas. Testing for microbial indicators such as Enterobacteriaceae represent a useful complement to verify adherence to GHP in the dry areas. Typically absence of *Salmonella* in any of the samples and levels of Enterobacteriaceae ranging between $10^2$–$10^3$ CFU/g is expected in such samples.

#### 11.2.2.4 Shelf Life

No issues are encountered if the products remain dry.

#### 11.2.2.5 End Product

Analysis of finished animal by-products for the presence of *Salmonella* can be used as a verification of the effectiveness of the combined preventive measures. It has been used for many years as an import control measure or as an obligatory requirement for commercializing such products. See Table 11.1 for sampling plan recommendations.

## 11.3 Unprocessed Feeds

This section discusses feeds based on plant material that are not or only minimally processed such as roughages, silage, cracked corn etc.

### *11.3.1 Significant Organisms*

#### 11.3.1.1 Hazards and Controls

Roughages are plant material and are highly variable in physical composition and nutritional quality. They range from very good nutrient sources, such as lush young grass, legumes and high-quality silage, to very poor sources such as straw, hulls and some browse (Kundu et al. 2005). They are used to feed grazing and browsing animals such as ruminants and horses.

Drying of grass does not inactivate most microorganisms including vegetative forms, thus pathogens such as pathogenic *E. coli* or spore formers such as *Clostridium botulinum* may be present.

Large quantities of herbage are converted into silage through anaerobic fermentation. When production of silage is not properly controlled, *L. monocytogenes* can grow. This may lead to direct infection of farm animals, particularly cattle, or indirect contamination of agricultural materials, such as raw milk, through fecal material. This can subsequently lead to human infection through consumption of raw milk or raw milk products (Czuprynski 2007; Antognoli et al. 2009). Appropriate fermentation conditions for the roughage used to prepare silage are important for control of *L. monocytogenes* and this was extensively covered previously (ICMSF 2005). These conditions can be summarized as follows:

- Do not use grass or other raw material on which animals with listeriosis were kept.
- Assure proper fermentation, limit air exposure, add fermentable carbohydrates, acids and/or starters.
- The pH should be 4.2 for silage with 25% dry matter.

Checking the effectiveness of the control measures is best done by visual inspection of the silage, including its smell, as well by measuring the pH. Microbiological testing for *L. monocytogenes* could be done if the adequacy of fermentation is in doubt, but routine testing is not recommended.

The occurrence of mycotoxins in silage has been reviewed by Storm et al. (2008) and discussions on other pathogens in raw milk which may originate from feed can be found in Chap. 23, and in ICMSF (2005).

#### 11.3.1.2 Spoilage and Controls

Spoilage of roughages such as hay is primarily caused by molds. Control is achieved by appropriate drying and subsequent storage to achieve and maintain low water activity (<0.6). Abnormal fermentation conditions and associated slow or insufficient drop in pH will permit growth of spoilage microorganisms such as yeasts and clostridia. *Clostridium* species typically associated with silage are saccharolytic species such as *Clostridium tyrobutyricum*, which may then contaminate milk and lead to spoilage of cheese (see Chap. 23).

### *11.3.2 Microbial Data*

Table 11.2 summarizes useful testing for roughages and silage. Refer to the text for important details related to specific recommendations.

#### 11.3.2.1 Critical Ingredients

Raw materials used to prepare roughages and silage should be selected to avoid introduction of high levels of pathogens originating from infected or shedding animals or from the use of contaminated manure. Prevention is ensured by appropriate Good Agricultural Practices but no testing is recommended.

For discussions on preventive measures related to manure and irrigation water, refer to Chap. 12.

#### 11.3.2.2 In-Process

Testing of in-process samples during silage preparation is not recommended. However, appropriate fermentation of silage can be checked through indirect means such as inspection of the wrapping

**Table 11.2** Testing of roughages and silage for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Apply Good Agricultural Practices for raw materials used to prepare roughages or silage. Avoid use of starting material that is heavily contaminated with pathogens of concern |
| In-process | Low | Microbiological testing is not recommended<br>Parameters such as visual inspection and checking pH to determine the appropriate drop can be used to verify whether fermentation is done well |
| Processing environment | Low | Not relevant |
| Shelf life | Low | For dry products, such as hay, that support growth of molds when moisture uptake occurs, monitoring of the relative humidity is relevant |
| End product | Low | Visual inspection, smell and, to a lesser extent, pH can be used to verify the appropriate fermentation conditions<br>No routine testing is recommended for indicator microorganisms or pathogens |

material for damage to avoid air-ingress, the smell of the silage and pH checks to determine whether the decrease of the pH occurs correctly.

#### 11.3.2.3 Processing Environment

Not relevant to roughages and silage.

#### 11.3.2.4 Shelf Life

Prolonged shelf life of dry roughages is ensured by appropriate conditions, including temperature and relative humidity. For silage, relevant trials may be conducted if starter cultures are used to enhance fermentation (e.g., Muck 2010).

#### 11.3.2.5 End Product

Smell and visual inspection of silage is useful for those familiar with silage to verify whether the process went well. Determination of the pH is less reliable as it depends on factors such as dry matter content. Microbiological testing under routine conditions is not recommended but may be useful for investigations.

## 11.4 Compounded Feeds

Compounded feeds are manufactured from both processed and unprocessed feeds described in Sects. 11.2 and 11.3, with addition of micronutrients such as vitamins or minerals to provide an adequate diet to animals. They are manufactured by feed compounders as powders, pellets or crumbles.

### *11.4.1 Significant Organisms*

#### 11.4.1.1 Hazards and Controls

*Salmonella* is the main hazard of concern for compounded feeds. Widely used processes such as pelletizing (Furuta et al. 1980; Cox et al. 1986; Himathongkham et al. 1996) have been shown to kill

salmonellae. Appropriate conditions should be validated and managed as a CCP. Alternative preservation techniques such as chemical decontamination have been discussed in ICMSF (2005). However, the main cause of contaminated compounded feeds is post-process recontamination, which needs to be controlled. This is primarily achieved by avoiding the use of contaminated ingredients and post-process contamination in the manufacturing plant. The microbiological quality of ingredients added after kill-steps have an important impact on finished products. This should be reflected in requirements defined in buyer-supplier agreements. Suppliers need to adopt appropriate preventive measures (GHP and HACCP) when manufacturing ingredients. Consult relevant chapters in ICMSF (2005) and this book for appropriate tests for these ingredients.

The main sources of mycotoxin found in compounded feeds are, as discussed in the previous sections, the ingredients. However, mycotoxins may also form during storage under inappropriate conditions that allow molds to grow. Appropriate control measures are identical to those described in Sect. 11.2.1.1.

#### 11.4.1.2 Spoilage and Controls

Mold growth can also lead to feed spoilage. Control of spoilage is achieved through appropriate storage conditions discussed above.

### *11.4.2 Microbial Data*

#### 11.4.2.1 Critical Ingredients

As outlined in the previous sections, processed and unprocessed feeds used as raw materials to manufacture compounded feeds may be contaminated with *Salmonella* and other pathogens such as pathogenic *E. coli*. It is therefore important to assess the risks associated with individual ingredients.

Testing for pathogens in incoming raw materials is not an effective control measure and supplier selection programs as outlined above should be favored. Monitoring of samples can be adapted to the level of confidence one has with a given supplier.

Moldy raw materials should not be used because mycotoxins will usually not be inactivated during further processing unless recently developed alternative strategies such as enzymatic or microbial detoxification of certain mycotoxins are applied (after appropriate validation) (Kabak et al. 2006; Binder 2007). When feeds are dry-mixed, selection will be critical and testing may be necessary, even when the safety of the ingredients cannot be assured in this manner.

#### 11.4.2.2 Other Production Stages

Considerations for in-process, processing environment, shelf life and end product microbial data are similar to those for processed feeds or pet food. Refer to Sect. 11.2 or 11.5.2 and Tables 11.1 or 11.3 for guidance.

## 11.5 Pet Foods, Chews and Treats

*Pellets*, also called kibbles, of dry pet food, mainly for dogs and cats, are manufactured by extrusion or by baking and subsequently coated by spraying with vitamins, fats and oils, or any other ingredients that are not heat-tolerant.

**Table 11.3** Testing compounded feeds (from processed feed ingredients), pet food, chews and treats for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | Confidence in supplier determines need for *Salmonella* and indicator testing in ingredients added *without* previous kill step. For low confidence suppliers, testing is essential to verify that ingredient specifications are met |
| In-process | High | Testing of product residues from product contact surfaces after a kill-step for *Salmonella* and Enterobacteriaceae is essential during normal operation to verify control of the process. Typical guidance levels:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g<br>• Aerobic mesophilic counts – internal limits |
| Processing environment | High | Testing is essential during normal operation to verify control of the process. Test for *Salmonella* and Enterobacteriaceae in relevant areas. Typical guidance levels:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g or sample |
| Shelf life | Low | For products able to support growth of molds when there is moisture uptake, monitoring of the relative humidity or water activity is more relevant than testing for molds |
| End product | High | Testing for indicators of processed products is essential to verify control of process |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Compounded feeds, dry pet foods, treats and chews | Enterobacteriaceae | ISO 21528-1 | 2 | 5 | 2 | $10^2$ | $10^3$ |

| | | Low to High | Testing for *Salmonella* is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. Test for pathogens only when other data indicate potential for contamination |
|---|---|---|---|

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Compounded feeds, dry pet foods, treats and chews | *Salmonella* | ISO 6579 | 10 | 5[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Chap. 7, Sect. 7.5.2 for compositing)

*Treats* are normally small, hard, shaped products that are colored to reflect the flavor. They are manufactured in a similar manner as pellets. Traditional flavors include beef, chicken, lamb, turkey, liver, cheese and bacon, as well as more unusual flavors such as raisin, spinach or peanut butter.

*Pet chews* are made from different parts of food animal bodies, such as raw hides, leg bones, intestine, snouts, pizzles or ears. They are commercialized in a variety of forms (twisted, curled) or molded in different shapes. After forming and shaping, chews are dried to obtain low moisture shelf-stable products; however, drying cannot be considered as a control step.

Canned (retorted) pet foods are not different from canned foods for human consumption and detailed discussions can be found in Chap. 24.

## *11.5.1 Significant Organisms*

### 11.5.1.1 Hazards and Controls

For dry pet foods, treats and chews the relevant pathogen is *Salmonella* as illustrated by several publications on outbreaks and surveys (e. g., Clark et al. 2001; Wong et al. 2007; Behravesh et al. 2010) as well as recalls of products. Although the direct or indirect transmission of *Salmonella* from dry pet food to humans, especially children, is recognized (CDC 2008a, b), no specific risk assessment is, to our knowledge, readily available to evaluate impact in more detail.

Mycotoxins also represent a significant hazard for dry pet foods, and control measures are the same as those described for compounded feed products. The prevalence of mycotoxins in pet foods and toxicological impact on animals have been discussed by Leung et al. (2006) and Boermans and Leung (2007).

### 11.5.1.2 Spoilage and Controls

Spoilage of dry pet foods by molds represents a major issue and is frequently due to insufficient drying of kibble, filling containers with hot product and subsequent formation of condensation in packed products. Application of appropriate GHP is necessary to control spoilage. Microbiological testing for molds is not recommended as contamination can be very heterogeneous. Alternatives, such as determination of the water activity of kibble, may be useful monitoring to prevent such issues.

## *11.5.2 Microbial Data*

Table 11.3 summarizes useful testing for pet food, chews and treats. Refer to the text for important details related to specific recommendations.

### 11.5.2.1 Critical Ingredients

Different ingredients used to manufacture dry pet food, and treats and chews represent a risk for the presence of *Salmonella.* However, extrusion and baking applied to manufacture pet foods and treats are designed to destroy these vegetative microorganisms, thus testing of such raw materials for *Salmonella* is not recommended.

If no killing step is applied during further processing, as for example in the case of chews, then the application of appropriate preventive measures at the supplier level represent the most effective control measures (see previous sections). Testing at receiving may be considered as monitoring if confidence in the supplier is low.

### 11.5.2.2 In-Process

Testing of residues from critical product contact surfaces after extrusion or baking (or any other biocidal step applied) where presence or even growth of *Salmonella* may occur is useful to detect contamination originating from the processing environment.

### 11.5.2.3 Processing Environment

See sections above.

#### 11.5.2.4 Shelf Life

Microbiological testing for molds is not recommended as contamination can be very heterogeneous. Alternatives such as determination of the water activity of kibbles may prove a useful monitoring tool to prevent such issues.

#### 11.5.2.5 End Product

Sampling dry pet food and treats follows the same rationale as discussed in Sect. 11.2.2.5. Proposed limits for *Salmonella* only reflect adherence to GHP as the products represent an indirect treat to human health. In the case of Enterobacteriaceae, limits in Table 11.3 reflect what is achievable when GHP and HACCP are applied during manufacture and are similar to those of EC (1990).

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# Chapter 12
# Vegetables and Vegetable Products

## 12.1 Introduction

Vegetables include products derived from the roots, leaves, tubers, bulbs, flowers, fruits and stems of many plant species. Certain foods are botanically considered to be fruits but are often referred to as vegetables (e.g., tomatoes, olives, green beans). Tomatoes are included in Chap. 13. The processes used to make vegetable products and their impact on the microbial populations of the final product were previously described (ICMSF 2005). Plant varieties; cultivation methods; and harvesting, packing, processing, distributing and final preparation techniques vary substantially. Regional and seasonal differences also occur.

This chapter covers microbiological testing for primary production, fresh and fresh cut, cooked, frozen, canned, dried, fermented and acidified vegetables, sprouts and mushrooms.

## 12.2 Primary Production

Primary production of vegetables involves the period from planting through harvest of the commodity. The cultivation of vegetables is carried out under a variety of diverse conditions and commodity specific methods. Traditional cultivation occurs in open fields, which can vary in size from small plot cultivation to large scale production. In addition, many vegetables are cultivated in green houses, which offer a higher degree of environmental control. Primary production of a limited number of vegetables is conducted using hydroponic techniques.

### *12.2.1 Significant Organisms*

The microbiota of vegetables during cultivation reflects those of the environment, seed sources, soil amendments and irrigation water. A wide variety of bacteria, molds, yeast and viruses are significant, including those linked to "market diseases" that contribute to spoilage. While primarily a quality issue, market diseases, insect damage, bruising and other quality defects may increase the potential for the presence of human pathogens.

#### 12.2.1.1 Hazards and Controls

Human pathogens are generally not among the normal microbiota of vegetables; rather they represent contamination of the primary production environment from human or animal sources. Once introduced

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_12,

into the agricultural environment, human pathogens can persist for extended periods. For example, enterohemorrhagic *Escherichia coli* O157:H7 can persist in manure-amended soils for months depending on the temperature and moisture content of the soil. There are exceptions to the transitory nature of human pathogens in the primary production environment. For example, *Listeria monocytogenes* is commonly associated with root crops such as radishes. Interestingly there are no documented cases of listeriosis associated with this vegetable. Additionally, zoonotic microorganisms such as *E. coli* and *Salmonella* may become established in soils and watersheds, particularly in warmer climates. An association between specific vegetables and specific human pathogens has been observed in some regions. For example the following associations have been observed in different regions of the world:

- Enterohemorrhagic *E. coli* (EHEC) O157:H7 with lettuce and spinach.
- *Salmonella* with cantaloupes, tomatoes and leafy greens.
- *Yersinia pseudotuberculosis* with shredded carrots.
- *Cyclospora cayatenensis* with basil.
- Hepatitis A virus with green onions.

Sometimes it is not clear how crops become contaminated. Contamination may originate directly or indirectly from the environment (water, wind, soil, animals or equipment) or humans during cultivation or harvesting. Contamination is thought to be primarily on the surface of the vegetable. However, under some study conditions, pathogens can be internalized during cultivation, harvesting or processing. The extent to which pathogens are internalized will affect the efficacy of postharvest control measures that are based on treating the surface of the vegetable.

Pathogens in the Enterobacteriaceae group are most common in frequency of contamination and incidence of foodborne disease, including *Salmonella* spp., *Shigella* spp. and EHEC. Viruses of primary concern are hepatitis A and norovirus. The most common protozoan parasites are *C. cayatenensis* and *Cryptosporidium parvuum*. Other protozoan parasites (e.g., *Entamoeba histolytica*, *Giardia* spp., *Toxoplasma gondii*) and nonprotozoan parasites (e.g., *Ascaris lumbricoides*, *Enterobius vermicularis*, *Taenia* spp., *Toxocara* spp.) can be transmitted via fresh produce in regions where these are endemic.

Understanding the mode of transmission and normal niche of these pathogens is necessary to perform a meaningful hazard analysis and select appropriate control measures. For example, humans are the primary source of *Shigella flexneri*, so primary control should focus on farm workers and sewage. Similarly, EHEC and *C. parvuum* are typically associated with herbivores, thus control is often focused on animal intrusions, soil amendments, adjacent land use and irrigation water.

The principal means for controlling contamination during primary production is through the implementation of Good Agricultural Practices (GAP). General guidance (FDA 1998, 2008) and specific guidance (e.g., Western Growers Association (2010) for leafy green vegetables, UF and NATTWG (2008)) for tomatoes have been developed by national governments, trade organizations and private standards-setting organizations (e.g., Global GAP). The focus of these programs is to limit the introduction of pathogenic microorganisms into the primary production environment. A key factor is the location of the cultivation site in relation to potential contamination sources (e.g., proximity to an animal rearing facility, large populations of wildlife, sources of irrigation and other agricultural water, and off-site contamination risks that may be carried onto the field by wind, runoff or during flooding). Irrigation water and application method is another potential source of contamination. Surface waters may be contaminated if they serve as a water source for domestic or feral animals, or as a stopover site for large numbers of water birds. Irrigation water from deep wells is less likely to be contaminated by pathogenic microorganisms, but broken well casings and seals or the lack of check values can lead to the infiltration of microorganisms from surface soils into the well water. Contaminated water sources may require water treatment or filtration prior to use, particularly if the irrigation water comes into direct contact with the edible portion of the plant (e.g., spray irrigation). Use of reclaimed water for agricultural purposes is encouraged for environmental benefits but its use for irrigation of vegetable crops may require at least secondary treatment of the water.

Use of manure as a soil amendment converts potential pollutants into an asset for sustainable agriculture. However, control is needed to prevent the manure from becoming a source of pathogenic microorganisms. For example, cattle manure could serve as a source of EHEC and chicken manure as a source of *Salmonella* if improperly composted. This is of particular concern with vegetables that are consumed raw. The primary means for controlling human pathogens in soil amendments is through adequate composting or pasteurization. The potential for the re-introduction and subsequent re-growth of pathogens may need to be considered.

During harvest, contact with equipment and humans, and stresses associated with harvesting make many vegetables particularly vulnerable to contamination. Harvest equipment should be clean and sanitized as one would for any food processing equipment and the hygienic practices used by harvest personnel should be the equivalent of any food worker. For some vegetables (e.g., head lettuce, bunch spinach, green onions), sometimes the only "processing" that the product receives is during harvest in the field, thus contamination that occurs in the field can be transmitted to the consumer.

#### 12.2.1.2 Spoilage and Controls

Both the quality and spoilage of vegetables are influenced by events that occur during cultivation. Most vegetables have an array of plant pathogens that may infect the plant and affect product quality (ICMSF 2005). The primary plant pathogen controls include selecting resistant plant varieties, rotating crops, disinfecting soil, minimizing insect damage and controlling postharvest temperature and respiration rates.

Events occurring during cultivation and harvesting can also affect the shelf life of vegetable products. Physical injury (e.g., puncture wounds, abrasions, bruising) during harvesting and transport can change vegetable metabolism and provide an avenue for contamination. Control of postharvest temperature and respiration rates can retard microbiological spoilage. Sorting to remove spoiled vegetables is also important to prevent the spread of contamination and thus extend the shelf life of vegetables.

### *12.2.2 Microbial Data*

For primary production, microbiological testing may be useful for irrigation water and soil amendments, preplanting evaluation (especially for plant pathogens) and during investigation to identify the source of an identified contaminant.

#### 12.2.2.1 Irrigation and Other Agricultural Waters

WHO and national governments have guidelines for reclaimed water used to irrigate vegetables. WHO guidelines (1989) recommend a tiered approach based on the intended use of the irrigation water (Table 12.1). The criteria balance the need for water for agricultural purposes, the risk of spraying crops

**Table 12.1** 1989 WHO guidelines for the use of reclaimed (treated) water in agriculture

| Category | Reuse conditions | Intestinal nematodes | Fecal coliforms |
|---|---|---|---|
| A | Irrigation of crops likely to be eaten raw ("salad vegetables"), sports parks, public parks | ≤1 eggs/L | 3.0 log CFU/100 mL |
| B | Irrigation of cereal crops, industrial crops, fodder crops, pasture, trees | ≤1 eggs/L | No standard recommended |
| C | Localized irrigation of crops in category B: exposure of workers and the public does not occur | Not applicable | Not applicable |

with water contaminated with low levels of fecal material and the technical and economic feasibility of treating the water prior to use. This balance of needs is of particular concern for developing countries where secondary or tertiary water treatment may not be available. In some developed countries, criteria for irrigation water also focus on use of reclaimed water; however, a combination of microbiological criteria and required treatments is used. For example, the US Environmental Protection Agency (EPA) guidelines for unrestricted use of reclaimed water for irrigation of crops to be eaten raw (category A) specify an absence of fecal coliforms/100 mL, absence of pathogenic microorganisms, and ≤200 fecal coliforms/100 mL for commercially processed and fodder crops (category B) (EPA 2004). The specific criteria can vary substantially among countries within the same geographical region. For example, Mexico, which supplies fresh vegetables to the US, has guidelines of ≤5 nematode eggs/L and a daily and monthly mean for fecal coliforms of ≤3.3 log CFU/100 mL and ≤3.0 log CFU/100 mL, respectively (Blumenthal et al. 2000). In 2009, the leafy greens industry in California implemented a moving window criterion for irrigation water, where the geometric mean of ≤126 MPN *E. coli*/100 mL for the five most recent water samples (Western Growers Association 2010).

The difference between the 1989 WHO guidelines and those of developed countries has been controversial, with the developing countries indicating that there is little epidemiological evidence that the more stringent requirement reduces the incidence of gastrointestinal disease in their countries. Furthermore, there has been ongoing discussion on the adequacy of any of these standards in relation to viral diseases such as hepatitis A. However, segments of the fresh produce industry attribute their water quality monitoring practices for the reduction in the number of outbreaks associated with their products. Several risk assessments and risk profiles related to the impact of reclaimed water standards on the transmission of human disease via produce are available (Gale 2001; Hamilton et al. 2006; Steele and Odumeru 2004; Steele et al. 2005; Stine et al. 2005). Blumenthal et al. (2000) evaluated studies and risk assessments, and recommended modification of 1989 WHO guidelines to differentiate use groups and exposed populations (Table 12.2).

The 1989 WHO guidelines for treated reclaimed water in agriculture were replaced in 2006 with risk-based consideration of conditions of use (WHO 2006). However, these new approaches provided little clear guidance on how to use these analyses to develop easily interpretable and implementable internationally-harmonized microbiological criteria for irrigation water that would be useful for verifying the application of GAP to the cultivation of vegetables that will be introduced into international trade.

**Table 12.2** Proposed revisions to the 1989 WHO guidelines for the use of reclaimed (treated) water in agriculture that have been recommended to WHO (Blumenthal et al. 2000)

| Category | Use conditions | Exposed group | Irrigation method | Intestinal nematodes (eggs/L) | Fecal coliforms (log CFU/100 mL) |
|---|---|---|---|---|---|
| A | Unrestricted irrigation: (for use with vegetable and salad crops to be eaten uncooked, sport fields, public parks) | Workers, consumers, public | Any | ≤0.1 | ≤3.0 |
| B | Restricted | Workers (but not children ≤15 years, nearby communities) | Spray or sprinkler | ≤1 | ≤5.0 |
| | | | Furrow | ≤1 | ≤3.0 |
| | | | Any | ≤0.1 | ≤3.0 |
| C | Localized irrigation of crops in category B if exposure of workers or the public does not occur | None | Trickle, drip, or bubbler | Not applicable | Not applicable |

The purpose of microbiological testing of irrigation water is to periodically verify that the water source has not become contaminated with a microbiological hazard. The frequency of testing of irrigation water should be based on the risk that the water source is contaminated. Accordingly, irrigation water derived from surface water sources are likely to require more frequent testing than water obtained from deep wells. In general, the likelihood that a water source is contaminated is as follows: raw or inadequately treated wastewater > surface water > groundwater from shallow wells > groundwater from deep wells > potable or rain water. The frequency of testing should be adjusted according to the source's history of contamination; i.e., the frequency of testing should be increased if previous testing has indicated that there is an unacceptable level of contamination.

The specific microorganisms evaluated depend, in part, on a risk evaluation of the water source and its surrounding environment and region. As a hypothetical example, surface water from an area with a high beaver population (a feral animal in certain regions of North America that is often host to *Giardia* spp.), might require *Giardia* to be included for that location. However, *Giardia* would not be universally considered a target hazard for irrigation water testing. In general, the focus of such testing would be to determine if the water source has been contaminated with fecal material (Table 12.3). For most zoonotic concerns, the use of one or more indicator microorganisms is likely to be more effective than examining the water for specific pathogens, though this would depend on an initial risk evaluation. Traditional indicator microorganisms, such as *E. coli*, are most pertinent. Other indicators such as fecal coliforms are less effective since many members of this class are not specifically associated with fecal material and may be part of the normal agricultural environment including surface water sources (e.g., *Klebsiella* spp. and *Enterobacter* spp. are often associated with plant material). In those instances where reclaimed water from human sewage treatment is used for irrigation, particularly for vegetables likely to be eaten raw, acceptable waters should be limited to waste water that has received at least a tertiary treatment. In such instances, the use of a viral indicator (e.g., male-specific coliphages) or a pathogenic virus (e.g., hepatitis A) should be considered in

**Table 12.3** Testing of irrigation and other agricultural water for safety and quality of vegetables

| Intended use | Relative importance | Microorganism | Analytical method[a] | Case | Sampling plan and limits/100 mL[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *n* | *c* | *m* | *M* |
| Irrigation water (surface, shallow well, deep well, or reclaimed): | | | | | | | | |
| • For vegetables that are likely to be eaten raw | High[c] | *E. coli*[d, e] | ISO 9308-1 | NA | 3[f] | 1 | 10 | $10^2$ |
| • For vegetables that are eaten only after cooking | Moderate | *E. coli*[d, e] | ISO 9308-1 | NA | 3[f] | 1 | $10^2$ | $10^3$ |
| Water for diluting pesticides, cleaning of harvesting equipment, etc. | High | *E. coli*[d, e] | ISO 9308-1 | NA | 5[f] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Relative importance of testing depends on method of irrigation, with foliar application having the highest priority. Consider increasing sampling frequency if evidence of unacceptable levels of contamination are found, the source has a history of sporadic contamination or if events (e.g., flooding) are likely to increase the risk of contamination
[d] For reclaimed water from human waste water treatment or water sources likely to be contaminated by human source, consider including a viral indicator of fecal contamination (see text)
[e] For reclaimed water or other treated water that are likely to be contaminated with nematodes or protozoan parasites, consider including tests for appropriate oocysts (see text)
[f] Individual 100 mL analytical units

addition to bacterial indicators of fecal contamination because viruses are more likely to survive water treatment than bacteria. Protozoan parasites (e.g., *C. cayatenensis*, *C. parvuum*) are highly resistant to water treatment and may need to be considered. However, protozoan and nonprotozoan parasites can be avoided by a filtration systems or settling basins to remove cysts and eggs prior to the use of the water for irrigation.

In addition to the evaluation of irrigation waters for human pathogens, there may be instances where the water is also evaluated for its overall microbiological load or for the presence of specific pathogens. This would be most pertinent when the primary producer is concerned with specific plant pathogens that may be waterborne.

Water is also used on farm in a variety of other ways such as the dilution of pesticides, the cleaning of cultivation and harvesting equipment, sanitizing solutions for use during harvesting and handwashing water for farm workers. Water that meets the microbiological criteria for drinking water is generally considered to be needed for these and similar applications (see Chap. 21).

Since the goal of testing agricultural waters is to determine the continuing control of this potential source of contamination, adapting testing of agricultural waters to "processing control" microbiological criteria may be useful (see Chap. 3). This sampling approach was recommended for irrigation water used for lettuce and other leafy greens (Western Growers Association 2010), with the microbiological criterion based on testing water samples at least once per month. Irrigation water for foliar applications was considered unacceptable if any single sample exceeded a generic *E. coli* count of 235 MPN/100 mL or if the "rolling geometric mean" of the five most recent samples was ≥126 MPN/100 mL.

#### 12.2.2.2 Soil Amendments

Soil amendments derived from animal waste (manure), human waste (sewage sludge or tea) or plant waste (green manure) are important resources for the production of vegetables in both developing and developed countries. However, inappropriate use can affect the quality and safety of vegetables and vegetable products. This is controlled through adequate composting or pasteurization (heat treatment) of the soil amendment. The composting of animal or plant "manures" is generally effective due to the heat generated during fermentation, but composting is often an uncontrolled process. Microbiological testing can be useful to verify the effectiveness of treatment processes, in some instances (e.g., composted manures) on a lot-by-lot basis and in other instances (e.g., heat treated manures) on a process verification basis. Such testing is often required by primary producers or purchasers of vegetables as part of GAP certification programs. It is particularly important for vegetables that may be eaten without cooking by the consumer or not subjected to bactericidal treatments by a processor.

The microorganisms surviving in composted or pasteurized soil amendments can also influence the quality of vegetables if the soil amendment is a source of specific plant pathogens. The microorganisms of concern are likely to be vegetable- and region-specific and the usefulness of the microbiological testing depends on hazard assessments performed by the primary producer.

US industry (Western Growers Association 2010), US government (FDA 1998) and intergovernmental organization (Codex Alimentarius 2003) guidance recommends that raw or inadequately treated (composted or pasteurized) manures, biosolids or green waste not be used in fresh vegetable production unless there is an extended period between application and rearing of crops. In the case of leafy green vegetables, industry guidelines (Western Growers Association 2010) recommend recording the temperature profile of organic soil amendments during composting and subsequent verification by microbiological testing. The latter includes fecal coliforms as an indicator microorganisms, plus *Salmonella* and *E. coli* O157:H7. The use of fecal coliforms may have limitations if the manure has a substantial percentage of plant material or uses plant material as a cover. For this reason, the ICMSF recommendations rely on generic *E. coli* as a more direct indicator of the survival of pathogenic enteric bacteria (Table 12.4).

**Table 12.4** Testing of composted or pasteurized soil amendments for the safety and quality of vegetables

| Intended use | Relative importance | Microorganism | Analytical method[a] | Sampling plan and limit/g[b] | | | |
|---|---|---|---|---|---|---|---|
| | | | | *n* | *c* | *m* | *M* |
| Composted manures: for vegetables likely to be eaten raw | High | *E. coli* | ISO 16649-2 | 5 | 2 | $10^2$ | $10^4$ |
| | | | | Sampling plan and limit/10 g[b] | | | |
| | | EHEC[c] | ISO 16654 | 5[d] | 0 | 0 | – |
| | | *Salmonella* | ISO 6579 | 5[d] | 0 | 0 | – |
| Pasteurized manures: for vegetables likely to be eaten raw | Moderate | *E. coli* | ISO 16649-2 | 5[d] | 1 | 0 | – |
| | | EHEC[c] | ISO 16654 | 5[d] | 0 | 0 | – |
| | | *Salmonella* | ISO 6579 | 5[d] | 0 | 0 | – |
| Composted manures: for vegetables not likely to be eaten raw | Low | EHEC[c] | ISO 16654 | 5[d] | 0 | 0 | – |
| | | *Salmonella* | ISO 6579 | 5[d] | 0 | 0 | – |
| | | | | Sampling plan and limit/g[b] | | | |
| | | *E. coli* | ISO 16649-2 | 5 | 2 | $10^3$ | $10^5$ |
| Pasteurized manures: for vegetables not likely to be eaten raw | Routine microbiological testing not recommended. Periodic testing to verify effectiveness of process may be beneficial | | | | | | |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] EHEC appropriate for ruminant manures and may not be relevant for poultry manures
[d] Individual 10 g analytical units (see Sect. 7.5.2 for compositing)

Assuming a standard deviation of 0.8, the recommended sampling plans for generic *E. coli* would provide 95% confidence of detecting 48 CFU/g for composted manure used for vegetables likely to be eaten raw, 1 CFU/8 g of pasteurized manure for vegetables likely to be eaten raw and 478 CFU/g for composted manure used for vegetables likely to be cooked. The sampling plans for EHEC and *Salmonella* would provide 95% confidence of detecting 1 CFU/22 g of manure, also assuming a standard deviation of 0.8. See Appendix A for performance of these sampling plans for other standard deviations.

## 12.3 Fresh and Fresh-Cut, Minimally Processed Vegetables

In some cultures (e.g., in Asian cuisines), consumption of vegetables without cooking is not a traditional practice, while in others (e.g., North America and Europe) this is a common practice. Microbiological safety concerns for fresh and fresh-cut produce intensified in the 1980s after a number of outbreaks were associated with the consumption of certain fresh fruits and vegetables in several countries (NACMCF 1998; FAO/WHO 2008). The increase in produce associated foodborne disease involves a number of different factors including the increased availability and consumption of fresh produce, the globalization of the food industry, advances in preservation and transportation systems that allow a broader range of produce to be marketed as fresh or fresh-cut products, and the centralization of primary production. It also reflects advances (e.g., PulseNet; SalmNet) in the ability to link diffuse cases into single source outbreaks.

Improvement in the production, packaging, processing, packaging, distribution and marketing practices has led to an increasing portion of total vegetable consumption being fresh and fresh-cut products. Fresh vegetable products are generally restricted to products that retain the vegetable's essential form and appearance as encountered at harvest. Fresh-cut products are vegetables that have

been processed for increased convenience without substantially altering the fresh character of the vegetable. Typical processes employed include peeling, coring, cutting, slicing, shredding, dicing, and packaging. Different vegetables may be combined to provide products such as preprepared salads. While some treatments can extend fresh-cut vegetable shelf life, these products are highly perishable.

## *12.3.1 Significant Organisms*

The microorganisms associated with fresh and fresh-cut vegetables are those associated with primary production (see Sect. 12.2) plus additional microorganisms acquired as a result of harvesting, packing and processing. This can include microorganisms associated with farm workers, harvesting, transportation equipment, and the production and harvesting environments. Many vegetables support the growth of bacteria, including human pathogens, particularly at cut surfaces. Control of bacterial growth is critical for quality and safety. There are significant opportunities for cross contamination particularly when water flumes are used during processing. This can lead to the extensive spread of initial spot contamination. The microbial load on vegetables can be reduced to some degree (i.e., typically 1–2 logs) by washing and disinfection. However, this is generally restricted to microorganisms on the surface of the vegetable and internalization of contamination decreases the effectiveness of surface antimicrobial treatments. Thus, care must be taken that processes do not foster such uptake of microorganisms into the vegetable tissues. No chemical treatments can assure complete destruction of contaminating microflora on vegetable surfaces. The primary purpose of antimicrobials added to wash or flume water is to prevent cross-contamination.

### 12.3.1.1 Hazards and Controls

Fresh and fresh-cut vegetables have been associated with outbreaks and sporadic cases caused by a variety of microorganisms (see Sect. 12.2.1.1) of both zoonotic and human origin. The risk of disease can be amplified by the ability of most vegetables to support bacterial growth. The specific hazards and control measures depend on the type and source of the vegetable, the location of initial processing, the extent of processing and hygiene programs. For example, head lettuce is often field-packed, with initial trimming, overwrapping and boxing taking place within minutes of harvest, and then the product is transported to a facility for cooling. Alternatively, vegetables such as green peppers are transported to a "packing shed" where they are sorted, cleaned, packed and cooled. The same can occur with fresh-cut products where some initial processing may take in place in the field. For example, head lettuce destined for the fresh-cut market is often cored and the outer wrapper leaves removed in the field prior to being sent to a processing facility for further cooling, washing, slicing and packaging.

The control of microbiological hazards typically involves four activities: prevention of contamination during harvesting and postharvest processing and handling (e.g., hygienic practices by food workers and hygienic equipment and contact surfaces), prevention of the cross contamination (e.g., use of antimicrobials in flume water), treatments to reduce the levels of contamination (e.g., washing of vegetables with water containing an antimicrobial) and inhibition of bacterial growth (e.g., maintenance of the cold chain until consumption). In general, control measures are designed to control enteric bacteria (e.g., *Salmonella*, EHEC); however, in certain instances control may be focused on other microorganisms (e.g., *L. monocytogenes* in shredded cabbage; hepatitis A virus in green onions).

#### 12.3.1.2 Spoilage and Controls

Spoilage of fresh and fresh-cut vegetables is predominately associated with bacterial soft rot, which results from pectolytic capability of a number of bacterial species. Predominate species encountered are *Erwinia carotovora* and pectolytic fluorescent *Pseudomonas* spp. (e.g., *P. fluorescens*) (Liao 2006; Barth et al. 2009). The former grows poorly below 10°C and can be controlled through adequate refrigeration. The latter are psychrotrophic and the primary cause of soft rot in refrigerated vegetables. Their growth is retarded by refrigeration at 1–4°C and through the use of modified atmosphere packaging. In addition, the prevention of cross contamination and the removal of spoiled or damaged vegetables are important to prevent the spread of these microorganisms. Avoidance of bruising, cuts and internalization of bacteria is also important for control of spoilage (Liao 2006; Bartz 2006).

### *12.3.2 Microbial Data*

The perishable nature of fresh and fresh-cut vegetables and the low frequency of contamination of the products with human pathogens makes the use of routine microbiological testing as a means of separating safe and unsafe product impractical. However, occasional microbiological testing and related analysis can be useful to verify process control, i.e., the effectiveness of steps to reduce existing contamination and prevent new contaminants and cross contamination (ICMSF 2002). In addition, the use of microbiological testing of the processing environment and food contact surfaces can provide an objective means of verifying the effectiveness of sanitation programs and hygienic practices.

#### 12.3.2.1 Critical Ingredients

Fresh vegetables are typically the only ingredient for this category of products whereas fresh-cut vegetables may be a single vegetable, a combination of vegetables or vegetables in combination with other salad components (e.g., croutons, grated cheese). Fresh vegetables are typically the critical ingredient in both sets of products. The quality and safety of these products is highly dependent on events occurring during their cultivation, and GAP is essential (see Sect. 12.2).

#### 12.3.2.2 In-Process

While vegetables may be subjected to processes that may reduce the risk of contamination (e.g., antimicrobial rinses), these treatments cannot ensure the elimination of pathogenic microorganisms. Furthermore, the effectiveness of these treatments depends highly on maintaining antimicrobial treatment concentrations, and in many instances, the pH of the treatment carrier, the organic load and possibly other factors (e.g., turbidity). However, once validated, control of these steps is typically monitored through chemical or physical analyses of the conditions of use.

Lack of attention to in-process conditions can lead to increased food safety risks and loss of product quality. Of particular concern are pathogenic bacteria whose growth is supported by fresh or fresh-cut vegetables. The primary control (i.e., controlled temperature storage at the appropriate temperature) is critical and its maintenance from harvest to consumption is probably the single most critical factor after cultivation for most fresh and fresh-cut vegetables. The proper temperature for holding intact vegetables is commodity specific. For some vegetables, storage at too cold a temperature leads to chill damage. Fresh-cut vegetables should be consistently stored at refrigeration temperatures. Physical damage can also detract from the safety of fresh and fresh-cut vegetables by providing additional nutrients and points of entry leading to internalization.

#### 12.3.2.3 Processing Environment

The processing environment for fresh vegetables represents a significant challenge since many vegetables receive their initial, and sometimes only processing in the field at the time of harvest. Furthermore, most packaging operations are open to the surrounding environment or have only rudimentary environmental controls. These challenges are exacerbated by the typically seasonal nature of the work force and the corresponding limited hygiene training they receive. Periodic microbiological testing of food contact surfaces and the packing facility environment can serve as an important tool for verifying the effectiveness of cleaning operations and hygienic practices. This is generally limited to tests for indicator microorganisms (e.g., aerobic plate counts, *E. coli*) or other indicators (e.g., ATP); however, in certain instances, analysis for specific pathogens or indicator tests may be warranted based on an assessment of potential contamination sources (e.g., monitoring the environment for *Salmonella* in a packing facility that has had past concerns with birds or vermin, *Listeria* spp. in fresh-cut facilities).

Fresh-cut vegetables typically represent a transition from a raw agricultural commodity to a ready-to-eat product, and many of the same environmental challenges noted above for fresh vegetables exist. For example, most initial processing of leafy vegetables designed for the fresh-cut market is carried out in the field, and many other vegetables are obtained from the same packing facilities used for fresh vegetables. Once in the fresh-cut manufacturing facility the environment is generally easier to control but effective control of safety and quality depends on adequate sanitation programs and adherence to good hygienic practices. Microbiological verification of cleaning procedures can be an effective means of verifying the effectiveness of hygiene programs. Again, these will generally be limited to indicator microorganisms. Such sampling programs are most effective when designed to provide a quantitative measure of process control (ICMSF 2002) that can be monitored via trend analysis and corrective actions taken before the occurrence of a process failure. In addition to food contact surface and general environmental sampling, there are specific steps, such as transport within a plant by fluming or hydrochillers, where monitoring of flume water for sufficient levels of antimicrobials is critical for the control of cross-contamination. Such analyses are typically chemical or physical in nature, with microbiological testing limited to occasional sampling to verify continuing efficacy or evaluation when monitoring of antimicrobial treatments indicate a process deviation.

#### 12.3.2.4 Shelf Life

Shelf life duration for fresh and fresh-cut vegetables may be determined through a series of trials, which may include microbiological testing. These should be conducted in a manner that takes into account the conditions that are likely to be encountered during distribution, marketing and consumption. Packaging can influence the potential growth of different bacteria, in some instance allowing the growth of microorganisms that would normally be suppressed. For example, Gimenez et al. (2003) reported that certain packaging films extended the shelf life of artichokes but allowed the growth of anaerobic bacteria without loss of sensory properties. Challenge studies with bacteria that are pathogenic for humans may be beneficial where systems for extending shelf life could lead to the growth of the pathogens to high levels before a product spoiled. In such instances, a secondary barrier may need to be established to control pathogen growth. Predictive models have been introduced for estimating the shelf life of fresh-cut vegetables (Corbo et al. 2006).

Once shelf life duration is established, routine microbiological testing to determine a product's shelf life is not warranted. Where shelf life is limited by microbiological activity, occasional microbiological studies may be beneficial to verify that shelf life expectations continue to be valid, and investigative testing is warranted when there are complaints of shelf life failures without apparent errors in handling (e.g., loss of temperature control).

#### 12.3.2.5 End Product

Enterobacteriaceae, coliforms and fecal coliforms are part of the normal microbiota found on fresh vegetables produced using GAP, therefore these groups do not reflect the sanitary status of raw vegetables. Furthermore, some species of these groups grow under refrigeration conditions; therefore they are generally poor indicators of the hygienic status or storage or handling practices used for fresh and minimally processed vegetables. Since psychrotrophic fluorescent pseudomonads are the predominate spoilage microorganism in fresh-cut vegetables (Liao 2006; Barth et al. 2009), periodic testing for this group may be helpful in ensuring adequate shelf life after the product enters the distribution/marketing system. Typically, the levels of psychrotrophic fluorescent pseudomonads would be expected to be <100 CFU/g using the standard culture method, i.e., Fluorescent Pseudomonas Agar (McFeeters et al. 2001).

Fresh and fresh-cut vegetables that are likely to be eaten without any further microbiocidal treatment (e.g., cooking) should be free of infectious pathogens to a degree needed to ensure a low risk of foodborne disease. The specific level of control required depends on the specific vegetable, its conditions of use and the microbiological hazards associated with the vegetable. In general these products are classified as high risk foods. Depending on the public health consequence of specific pathogens, fresh and fresh-cut vegetables would be classified as ICMSF cases 8, 11 and 14 for microorganisms whose growth is not supported by the vegetable, and cases 9, 12 and 15 for microorganisms that are capable of growth.

The direct testing of fresh and fresh-cut vegetables may be necessary when there is no information available on the lot of food in question. However, in most instances the defect rates (i.e., the percentage of individual vegetables within a lot that are contaminated) encountered, even within a lot of food, are so low that end product testing is impractical. Additionally, the time associated with testing may make testing impractical for short shelf life products.

When information on the product and how it was processed and handled is available, microbiological testing for process verification using an appropriate indicator microorganism (e.g., *E. coli* for fecal contamination) may be more effective than pathogen testing. This would provide a means for process control charting that would allow corrective actions to be taken prior to reaching the point of process failure. Similar process control (cross-lot) testing for mesophilic or psychrotrophic aerobic colony counts may also be useful for assessing maintenance of control of key spoilage microorganisms.

The diversity of vegetables in this category prevents recommendations of specific aerobic colony counts because the level of indicators can vary considerably. For example, root crops (e.g., onions, radishes, etc.) would be expected to have high bacterial loads than the interior portions of tightly nested leaf crops (e.g., cabbage, iceberg lettuce, etc.). Climatic conditions at the time of harvest may also alter microbial loads (e.g., rain vs. dry conditions). Baselines for specific process would have to be established to determine if these criteria would be relevant in specific situations. Routine end product testing for pathogens in fresh and fresh cut vegetables is not recommended. Test for pathogens only when other data indicate potential for contamination, using recommended sampling plans in Table 12.5. As methods become available for other EHEC strains the sampling plan for *E. coli* O157:H7 would apply.

## 12.4 Cooked Vegetables

Many vegetables are traditionally consumed as cooked foods, such as green beans, potatoes, broccoli, squash, sweet corn, etc. (ICMSF 2005). A variety of cooking methods are used, such as boiling, steaming, baking and frying. In some instances these vegetables are commercially prepared and marketed as refrigerated precooked products. In other instances these vegetables are prepared in food service establishments or the home and stored under refrigeration. While canned products are cooked,

**Table 12.5** Testing of fresh and fresh-cut vegetables (to be eaten without cooking) for safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Initial contamination is highly dependent on implementation of good agricultural practices (see Sect. 12.2) |
| In-process | High | Monitoring antimicrobial concentration is recommended to prevent cross contamination via wash water, flume water, etc. |
| | Low | Periodic microbiological testing of paired (i.e., before and after) produce samples may be useful to assess effectiveness of these controls |
| Processing environment | Medium | Periodic testing of food contact surfaces and processing environments are recommended to verify adequacy of cleaning and sanitization protocols. Potential assays include aerobic colony counts and *E. coli*<br>Consider environmental testing for *Salmonella* in environments with a history of issues with birds or vermin<br>Consider environmental testing for *Listeria* spp. or *L. monocytogenes* for refrigerated fresh-cut vegetables when growth may occur within usable shelf life |
| Shelf life | Low | Where shelf life of fresh-cut vegetables is limited by microbiological activity, validate shelf life after major change in process technologies. Periodic verification through microbiological analysis for spoilage species may be beneficial for such products |
| End product | Medium | Routine testing is not recommended but periodic testing for specific indicators using internal standard or those below may be useful to verify process control and trend analysis |

| Product | Microorganism | Analytical method[a] | Case | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| | | | | *n* | *c* | *m* | *M* |
| Fresh-cut vegetables | *E. coli* | ISO 7251 | 6 | 5 | 1 | $10^1$ | $10^2$ |

Routine microbiological testing for pathogens is not recommended. Test for pathogens only when other data indicate potential for contamination

| Relative importance | Product | Microorganism | Analytical method[a] | Case | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *n* | *c* | *m* | *M* |
| Low | Fresh-cut vegetables | *Salmonella* | ISO 6579 | 12 | 20[c] | 0 | 0 | – |
| Low | | *E. coli* O157:H7 | ISO 16654 | 15 | 60[c] | 0 | 0 | – |
| Low | | *L. monocytogenes* | ISO 11290-1 | NA[d] | 5[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[d] *NA* not applicable; used Codex criterion for RTE foods supporting *L. monocytogenes* growth

they are considered separately (see Sect. 12.6). Cooked vegetables that are distributed as a frozen product are addressed in Sect. 12.5.

Cooking inactivates vegetative cells of most microbial species present in raw vegetables, but would not inactivate most spores. Cooking induces biochemical and structural changes that impact the ability of vegetables to support growth of bacteria. Recontamination of cooked vegetables or germination of surviving bacterial spores can lead to growth due to changes that make nutrients and entry sites more available, and elimination of competing microorganisms. Cooking typically decreases the oxygen content and redox potential of vegetables, increasing their potential to support the growth of anaerobic and microaerophilic species. Boiling has been reported to be sufficient to

inactivate norovirus and hepatitis A (Koopmans and Duizer 2004); however, cooking at milder times and temperatures may not be sufficient to fully inactivate these viruses.

## *12.4.1 Significant Organisms*

The microbiota of cooked vegetables reflect the microorganisms that survive the cooking step (primarily spore formers), any microorganism re-introduced from the postcook environment, the care and hygienic practices of food workers, and the microbiological ecology of other ingredients added to the final product. A diverse group of potential pathogens and spoilage microorganisms can be introduced.

### 12.4.1.1 Hazards and Controls

Of particular concern are specific enteric bacteria (e.g., *Salmonella*, *Shigella*) and viruses that are commonly associated with food service operations (e.g., norovirus, hepatitis A virus). The outgrowth of *Clostridium botulinum* spores has been associated with a limited number of outbreaks associated with potato salad, sautéed onions and lotus root (ICMSF 2005; CDC 1984). The potential growth of nonproteolytic *C. botulinum* in sous-vide processed products has been a potential concern for nonproteolytic *C. botulinum*; however, there is little evidence that cases have actually occurred with these products. *L. monocytogenes* is a potential microorganism of concern due to its ability to grow in refrigerated ready-to-eat foods and at least one outbreak of *Listeria* gastroenteritis has been associated its growth in a cooked vegetable, i.e., canned sweet corn (Aureli et al. 2000). This outbreak demonstrates the need for care, as contamination must have occurred during preparation because *Listeria* cannot survive the canning process.

The primary means of control is through maintaining the integrity of the cold chain. Even with psychrotrophic *L. monocytogenes* and nonproteolytic *C. botulinum*, the primary control measure is to maintain the product at 1–4°C. Where there is a significant potential for temperature abuse of significant duration during storage, distribution, marketing or use, additional barriers may have to be considered such as acidification or antimicrobials.

### 12.4.1.2 Spoilage and Controls

Spoilage of cooked vegetables depends on the microbiota re-introduced postcooking and the spore formers that survived the heat treatment. Refrigeration for extended periods of time fosters spoilage by psychrotrophic microorganisms (i.e., bacteria, yeasts, molds), with the specific genera influenced by the packaging systems used selecting for aerobes, facultative anaerobes and microaerophiles, or anaerobes (e.g., sous-vide). Refrigeration in combination with controlled atmosphere packaging will retard the growth of psychrotrophic fluorescent pseudomonads, the primary cause of spoilage in fresh vegetables. Various *Bacillus* spp. can spoil pasteurized vegetable purees, depending on the temperature of storage (Guinebretiere et al. 2001). Spoilage is largely controlled through maintaining temperatures between 1 and 4°C.

## *12.4.2 Microbial Data*

Table 12.6 summarizes useful testing for cooked vegetables. Refer to the text for important details related to specific recommendations.

**Table 12.6** Testing of cooked vegetables for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low | Routine microbiological testing would have limited benefit | | | | | | | |
| In-process | Low | Periodic testing to verify sanitation programs and hygienic practices. Possible indicators include aerobic colony counts, *E. coli* or Enterobacteriaceae using internally developed standards | | | | | | | |
| Processing environment | Low to high | Periodic testing to verify sanitation programs and hygienic practices for potential *L. monocytogenes* harborage if the potential for recontamination exists. *Listeria* spp. is a possible indicator microorganism | | | | | | | |
| Shelf life | Low | Validated through microbiological testing before initiation of a new product line and re-validated after any major change in process technologies. Verification testing after complaints of shelf life failures | | | | | | | |
| End product | Low | Routine testing is not recommended. Periodic testing for indicators may be useful for verifying process control and trend analysis | | | | | | | |
| | | | | | | Sampling plan and limits/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Low | Cooked vegetables | Aerobic colony count[c] | ISO 4833 | 3 | 5 | 1 | $10^4$ | $10^5$ |
| | Low | | Enterobacteriaceae[d] | ISO 21528-1 | 6 | 5 | 1 | 10 | $10^2$ |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | Low | RTE cooked vegetables supporting growth | *Listeria* spp. | ISO 11290-1 | NA[e] | 5[d] | 0 | 0 | – |
| | Low | Routine microbiological testing for specific pathogens is not recommended. Test for specific pathogens only when other data indicate potential for contamination | | | | | | | |
| | | | | | | Sampling plan and limits/g[b] | | | |
| | | RTE cooked vegetables supporting growth | *L. monocytogenes* | ISO 11290-1 | NA[e] | 5[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Incubate at 20–28°C to allow for growth of psychrotrophic microorganisms
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[e] *NA* not applicable due to use of Codex criteria

#### 12.4.2.1 Critical Ingredients

In general, the microbiological quality and safety of cooked vegetable products is independent of the raw vegetables and other ingredients unless the ingredients are added after the cooking step. A potential exception is vegetables containing an excessive level of spore forming bacteria. Microbiological testing is of limited benefit except for investigating incidences of unacceptable spoilage.

#### 12.4.2.2 In-Process

In-process microbiological testing would have limited benefits. Microbiological studies to validate the efficacy of the cooking process are desirable when a new product is being introduced or when there is a significant change in technologies or ingredients.

#### 12.4.2.3 Processing Environment

Since re-introduction of microorganisms is the primary source of contamination, control of the processing environment and hygienic practices is particularly important. Microbiological testing can be an effective means for verifying sanitation and hygiene programs. The focus will generally be indicator microorganisms such as aerobic colony counts or *Enterobacteriaceae* or *E. coli*. Testing for pathogens would be typically limited to *L. monocytogenes*, though its indicator, *Listeria* spp., may be equally effective.

#### 12.4.2.4 Shelf Life

Shelf life duration for cooked vegetables may be determined through a series of microbiological testing trials. These should take into account conditions likely to be encountered during distribution, marketing and consumption. Typically such trials focus on growth of psychrotrophs. In some instances inoculated pack studies with a psychrotrophic pathogen such *L. monocytogenes* or nonproteolytic *C. botulinum* may be conducted to ensure that pathogens do not achieve a high level of growth before spoilage occurs. Choice of microorganisms to study depends on the packaging system (e.g., aerobic, vacuum, modified atmosphere, etc.), filling process (e.g., hot filled, ambient fill, etc.) and other conditions (e.g., pH, water activity, preservatives, etc.).

#### 12.4.2.5 End Product

The perishable nature and low defect rates associated with cooked vegetables limit the usefulness of routine microbiological sampling of end products. End product testing would be largely limited to a sampling rate sufficient to verify the continuing efficacy of the controls designed into the food manufacturing and distribution system. In general, analysis of products for specific indicator microorganisms such as aerobic colony counts, *E. coli* or Enterobacteriaceae may be useful. The location of sampling (after production, after chilling, in distribution, end of shelf life, etc.) on the magnitude of decision criteria must be considered. For example, the level of psychrotrophic microorganisms at retail is expected to be greater than that immediately after final packaging. This might have to be reflected in the m and M values selected. In those instances where refrigerated cooked vegetables have a history of association with *L. monocytogenes*, periodic testing of end products for this pathogen may be beneficial for verifying the effectiveness of control measures, unless filling procedures (e.g., hot fill) are monitored to eliminate this concern.

## 12.5 Frozen Vegetables

Freezing provides a means for the long term storage of many vegetables in a state that retains many of the characteristics of fresh vegetables. Frozen storage prevents the growth of microorganisms. In addition, the blanching step that is generally required to inactivate the vegetable's enzyme system

also inactivates vegetative bacterial cells from 1 to 5 log cycles (ICMSF 2005). While freezing should not be considered a microbiocidal treatment, it injures a variety of microorganisms, particularly Gram-negative bacteria.

Especially in temperate regions, vegetables to be frozen are grown as seasonal crops and the time of harvesting and processing is very intense. To achieve the highest quality product, fields may be harvested around the clock, 7 days a week and processing lines can run for extended periods of time. The hot, wet environment and readily available nutrients from vegetable material presents a very suitable environment for microbial growth.

## *12.5.1 Significant Organisms*

The microbiota is largely a function of the microorganisms that can survive the blanching step and those that are acquired from the postblanching environment. The microbial population is diverse, and typically includes Gram-positive bacteria such as lactic acid bacteria, enterococci, and spore formers. If frozen vegetables are thawed, the microbial considerations are similar to those for cooked vegetables (see Sect. 12.4).

### 12.5.1.1 Hazards and Controls

Frozen vegetables generally present minimal risk in regard to foodborne pathogens, though this depends on the hygienic practices between blanching and freezing. Gram-positive pathogens such as *L. monocytogenes* are likely to survive extended periods of frozen storage, whereas Gram-negative species such as *Salmonella* are more susceptible to cold shock. Both protozoan and nonprotozoan parasites are inactivated by extended frozen storage. Control is achieved through use of quality vegetables grown under GAP, maintenance of hygienic practices and processing environment, timely freezing and maintenance of frozen storage temperatures.

### 12.5.1.2 Spoilage and Controls

Microbiological spoilage of frozen vegetables is rare, but spoilage will proceed as soon as the product is thawed. Long term storage should be at ≤−16°C. Growth of psychrotrophic microorganisms begins when temperatures approach 0°C. Control is achieved using the same factors identified above for microbiological hazards.

## *12.5.2 Microbial Data*

Microbiological testing, using indictor microorganisms, is a common industrial practice to verify process control and hygienic status of frozen vegetable manufacture. This is particularly useful when extended production runs are used. Data to demonstrate control of *L. monocytogenes* may be considered if the product is likely to be thawed, held under refrigeration for extended periods and consumed without further cooking. Table 12.7 summarizes testing used for microbiological safety and quality of frozen vegetables.

### 12.5.2.1 Critical Ingredients

Routine testing is not recommended; however, ingredients should be produced using GAP.

**Table 12.7** Testing of frozen vegetables for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low | Routine testing is not recommended. Vegetables should be grown using GAP | | | | | | | |
| In-process | High | Testing in-process samples to verify postblanching sanitation programs and hygienic practices (see text). Typical levels encountered include<br>• Aerobic colony count – $<10^4$ CFU/g<br>• Enterobacteriaceae – $<10^2$ CFU/g<br>• *E. coli* – absent | | | | | | | |
| Processing environment | High | Periodic testing to verify sanitation programs and hygienic practices for potential *L. monocytogenes* harborage. *Listeria* spp. is a possible indicator microorganism | | | | | | | |
| Shelf life | – | Not relevant for frozen vegetables | | | | | | | |
| End product | – | Test for indicators for verification of control and trend analysis. If criteria for indicators are exceeded, test for pathogens to determine disposition of lot | | | | | | | |
| | | | | | | Sampling plan and limits/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | High | Frozen vegetables | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $10^4$ | $10^5$ |
| | High | | Enterobacteriaceae | ISO 21528-1 | 5 | 5 | 2 | 10 | $10^2$ |
| | High | | *E. coli*[c] | ISO 16649-2 | 5 | 5 | 2 | <10 | – |
| | | Relative importance of testing for pathogens under routine situations is low. If indicators or in-process testing exceeds expected levels, testing for pathogens is high | | | | | | | |
| | | | | | | Sampling plan and limits/g[b] | | | |
| | Low–high | Frozen vegetables | *L. monocytogenes* | ISO 11290-2 | NA[d] | 5 | 0 | $<10^2$ | – |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | Low–high | | *Salmonella* | ISO 6579 | 11 | $10^e$ | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Detection of *E. coli* above m should trigger pathogen testing as it is typically absent during production under GHP. No value is specified for *M* because *E. coli* is rarely detected at levels above 10/g in frozen vegetables
[d] *NA* not applicable due to use of Codex criteria
[e] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 12.5.2.2 In-Process

Periodic verification of blanching temperatures and times may be warranted to avoid quality defects and ensure a degree of control over vegetative cells of bacteria. Testing in-line samples at various points in the process (e.g., post blanch, de-watering stages, freezer entrance and exit, etc.) for indicators, such as aerobic colony counts, Enterobacteriaceae and *E. coli*, is useful for trend analysis and verification of process control. Levels encountered may vary depending on the vegetable and processing conditions, therefore internally developed standards may be necessary. Typically, aerobic colony counts are $<10^4$–$10^5$ CFU/g, Enterobacteriaceae are $<10^2$ CFU/g and *E. coli* is typically absent.

#### 12.5.2.3 Processing Environment

Sufficient microbiological testing of the environment should be conducted to verify the effectiveness of sanitation programs and hygiene practices. Enterobacteriaceae may be useful postblanching but

would be of limited utility before this heating step. A potential indicator for fecal contamination is *E. coli.* Testing for *Listeria* spp. can be used as a means to periodically verify removal of harborage sites for *L. monocytogenes.*

#### 12.5.2.4 Shelf Life

Shelf life testing is not relevant for frozen vegetables.

#### 12.5.2.5 End Product

Because of the extended run times used for processing many frozen vegetables, testing of finished product for indicators is beneficial to verify that the overall process continues to function as intended. When in-process or environmental testing indicates concerns related to fecal contamination or harborage of *Listeria* spp., a period of end product testing for enteric pathogens (e.g., *Salmonella*, EHEC) or *L. monocytogenes* may be warranted.

## 12.6 Canned Vegetables

Canning is a mature technology for the long-term, shelf-stable preservation of vegetables. This requires the vegetables to be heat treated to achieve commercial sterility. See Chap. 24, for additional information on canned foods.

## 12.7 Dried Vegetables

Dehydration of vegetables is a traditional preservation system that is used for vegetables such as peas, onions, garlic, potatoes, carrots, etc. The reduction in water activity to levels that do not support microbial growth yields an inherently shelf-stable product. Once dried, the microbiological stability of the product depends on maintaining the dry state through appropriate bulk storage or product packaging.

### *12.7.1 Significant Organisms*

The microbiota of these products reflects on the microorganisms associated with the primary production of the raw vegetables and those acquired during processing and handling before and after drying. For vegetables that require blanching before drying, the levels of vegetative microorganisms are likely to be reduced by several orders of magnitude. Drying generally has a minimal effect on microbial levels. However, drying and dry storage foster the survival of microorganism that tolerate extended exposure to dry conditions. Dry products are generally hydroscopic and storage in high humidity conditions or temperature fluctuations that can produce “wet spots” can lead to the local rehydration of the product. Once rehydrated above minimal $a_w$ values, most microorganisms will resume growth if the vegetable is capable of supporting it.

#### 12.7.1.1 Hazards and Controls

While the microbiota of dried vegetables is diverse, the extended dry storage of these products favors the survival of spore formers, including pathogenic species such as *Bacillus cereus*, *C. botulinum* and

*C. perfringens*. Blanching eliminates most vegetative cells but these can be reintroduced if sound hygienic practices are not followed. Thus, it is possible that dried vegetables could contain low levels of pathogens such as *Staphylococcus aureus*, *L. monocytogenes* and *Salmonella*; however, these appear to be uncommon in a well controlled process. Primary control measures include selection of quality raw ingredients; adequate blanching where appropriate; timely drying to target $a_w$ values and effective packaging or storage conditions to maintain dry conditions.

#### 12.7.1.2 Spoilage and Controls

A variety of potential spoilage microorganisms can be present on dry vegetables, with lactic acid bacteria being common. The specific microbial profile depends on the characteristics of individual vegetables and the conditions of cultivation and storage. Blanching reduces vegetative cells levels but not spores. Bacterial spoilage of dry vegetables is uncommon though possible if there is sufficient rehydration. Spoilage by molds is more likely. The microorganisms in dry vegetables will reinitiate growth when the product is used as an ingredient in high moisture foods or after the consumer or food service worker has rehydrated the vegetable. Control of spoilage microorganisms is the same as that indicated above for pathogens.

### *12.7.2 Microbial Data*

Microbiological data for dry vegetables provides confidence in processes, ingredients and hygiene programs, and thus is focused on verification instead of routine testing for release. Table 12.8 summarizes useful testing for dried vegetable products. Refer to the text for important details related to specific recommendations.

#### 12.7.2.1 Critical Ingredients

The quality and safety of dry vegetables will largely be a function of the raw vegetables used and the hygienic practices used during manufacturing, particularly for vegetables that are not blanched.

**Table 12.8** Testing of dried vegetables for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low | Routine testing is not recommended. | | | | | | |
| In-process | Low | Routine testing is not recommended | | | | | | |
| Processing environment | Medium | Test periodically to verify effectiveness of hygienic practices using internally developed standards. Potential microorganisms include yeast and molds, Enterobacteriaceae or *Salmonella* | | | | | | |
| Shelf life | – | Routine testing is not recommended | | | | | | |
| End product | Low | Routine testing is not recommended but periodic testing for specific indicators may be useful to verify process control and to conduct trend analysis. Specific indicators and level is product dependent | | | | | | |
| | Low | Routine testing for pathogens not recommended unless conditions of manufacture indicate potential contamination. | | | | | | |
| | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

Microbiological testing for verification is beneficial to build confidence in suppliers and periodic testing for appropriate indicator microorganisms may be appropriate. However, because of the perishable nature of the raw ingredients and the nonperishable nature of the finished product, it may be more effective to focus verification testing on finished product. Increased testing would be warranted if there are concerns regarding the ability of a supplier to provide consistently sound ingredients.

#### 12.7.2.2 In-Process

In-process microbiological testing is generally of limited value and routine testing is not recommended. Inoculation pack and related studies may be needed to validate blanching, dehydration and packaging systems.

#### 12.7.2.3 Processing Environment

Since the contamination of dry vegetables depends on hygienic practices before and after dehydration, periodic sampling of the processing environment may be useful to verify the effectiveness of sanitation programs and hygienic practices.

#### 12.7.2.4 Shelf Life

Microbiological testing is not relevant for dried vegetables.

#### 12.7.2.5 End Product

The nonperishable nature of dry vegetables makes end product testing feasible from the standpoint of acquiring the results before release of the product. However, the low level of contamination would generally make routine testing unnecessary. It is possible that use of the dried vegetables for special products or special populations might require testing for specific pathogens. The periodic testing of end product can provide a means of verifying the integrated effectiveness of process controls. Specific indicators that would be most effective will vary on an individual product basis but might include lactic acid bacteria, yeasts and molds and spore forming bacteria.

## 12.8 Fermented and Acidified Vegetables

Preservation of vegetables through acidification is used for traditional products in many regions of the world. It has also been used to extend the shelf life of minimally processed vegetables. Sauerkraut, kimchi and pickles are examples of well known vegetable products that are preserved through fermentation; however, many other vegetables such as beets, green tomatoes, peppers, etc. are also preserved in this manner. In addition, some vegetables, such as “fresh pack” pickles, are acidified through the direct addition of vinegar and spices.

While the fermentation of specific vegetables varies, the general process involves adding salt and restricting the amount of available oxygen (ICMSF 2005). This results in the sequential growth of a series to lactic acid bacteria (e.g., *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Pediococcus acidilactici*, *L. plantarum*, *P. pentosaceus*) that ferment available carbohydrates and decrease the pH.

## *12.8.1 Significant Organisms*

Successful fermentation of vegetables depends on the proper sequence of lactic acid fermentation. This is largely controlled through proper selection of fermentation conditions.

#### 12.8.1.1 Hazards and Controls

If properly fermented or acidified, the acidity of fermented vegetables should ensure elimination of pathogenic microorganisms.

#### 12.8.1.2 Spoilage and Controls

The specific microorganisms associated with the spoilage of properly fermented vegetables depend on factors such as salt content, acid type and concentration, and oxygen content. High salt content, salt-stock pickles tend to spoil by yeasts, obligate halophiles and coliforms if acidity is insufficient. Softening of pickles is associated with various yeast and *Bacillus* spp.

Spoilage is prevented through proper control of the fermentation process and proper refrigeration or pasteurization of the finished product (ICMSF 2005). Increasingly, starter cultures are used to help ensure the adequacy of the fermentation process. Preventing carry over of contamination between batches of fermented or acidified vegetables is important.

## *12.8.2 Microbial Data*

In general, microbiological testing is limited to investigation of product defects. Routine testing is generally restricted to chemical attributes (e.g., pH, titratable acidity, carbohydrate levels, salt concentrations) that either determine or measure the adequacy of fermentation or acidification process. Table 12.9 summarizes useful testing for fermented and acidified vegetable products. Refer to the text for important details related to specific recommendations.

#### 12.8.2.1 Critical Ingredients

Routine microbiological testing of raw vegetables is not recommended. Other ingredients may be periodically evaluated to ensure that they are not a source of contamination. For example, use of recycled brine requires adequate treatment to ensure it is not a source of contamination that can contribute to spoilage, particularly if there is a history of quality defects.

**Table 12.9** Testing of fermented and acidified vegetables for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Routine microbiological testing not recommended |
| In-process | Low | Routine microbiological testing not recommended. Monitoring fermentations for specific chemical attributes (e.g., pH, % acidity) is important for on-going process control and trend analysis. |
| Processing environment | Low | Sufficient periodic testing to validate effective of sanitation programs and hygiene practices |
| Shelf life | Low | Routine testing not recommended |
| End product | Low | Routine microbiological testing not recommended |

#### 12.8.2.2 In-Process

Routine microbiological testing of in-process activities is generally not recommended; the adequacy of the fermentation is more effectively monitored through testing of chemical attributes. Evaluation of starter cultures for identity and effectiveness should be conducted with sufficient frequency to ensure effective maintenance of ferment capability.

#### 12.8.2.3 Processing Environment

Routine microbiological testing not recommended; however, periodic microbiological testing can be effective for verifying the ongoing efficacy of sanitation programs and hygiene practices.

#### 12.8.2.4 Shelf Life

Routine testing for shelf life is not recommended, though analysis of retained samples may be beneficial if spoilage problems are at an unacceptable rate.

#### 12.8.2.5 End Product

Routine analysis of end products is not recommended unless there is a history of spoilage problems.

## 12.9 Sprouted Seeds

Originally a traditional part of the cuisine of many Asian countries, sprouted seeds has become a common salad vegetable worldwide. This includes the seeds of a wide variety of plants such as alfalfa, chick peas, soy bean, lentils, radish, broccoli, mung beans, fenugreek, cress, clover and sunflower. While some may be consumed primarily after cooking (e.g., mung bean sprouts), many are consumed without cooking. During the 1990s, several national and international outbreaks associated with various sprouted seeds brought attention to these vegetables as a source of foodborne disease (NACMCF 1999).

The specific production methods used to produce sprouts depends on the species being produced (ICMSF 2005). In general, the process involves an initial soaking of the seeds, incubation for 3–8 days at 20–30°C with periodic wetting, washing to remove seed hulls, dewatering, packaging and refrigerated distribution. The conditions for optimal sprouting favor bacterial growth and there are generally no microbiocidal treatments employed after production.

### *12.9.1 Significant Organisms*

Sprouting seeds supports the growth of a wide variety of bacteria including human and plant pathogens, providing an ideal environment in terms of moisture, temperature and available nutrients. The microbiota of sprouts reach aerobic colony count levels of $10^8$–$10^9$ CFU/g, psychrotroph levels of $10^7$ CFU/g and coliform levels of $10^6$–$10^7$ CFU/g (ICMSF 2005; Palmai and Buchanan 2002a, b). *Klebsiella pneumoniae* and *Enterobacter aerogenes* were the predominant coliforms isolated from mung beans (Splittstoesser et al. 1983).

#### 12.9.1.1 Hazards and Controls

Epidemiologically, sprouts have been implicated in outbreaks of salmonellosis and EHEC infections, including the largest EHEC outbreak recorded (MHWJ 1997). The sprouting of different seeds have been shown experimentally to support the growth to high levels of various pathogens including *Salmonella*, *L. monocytogenes*, *B. cereus* and *Vibrio cholerae*. The source of pathogens can be varied, but epidemiological investigations of several international outbreaks suggest that low level contamination of the seeds may be the predominant source for *Salmonella* and EHEC. Cultivating the seeds using GAPs and screening of seed lots for contamination can help to prevent contamination.

Unlike most vegetables, sprouted seeds are cultivated under environmentally controlled conditions, so increased control of primary production is possible. Primary control of contamination is through a combination of good hygienic practices, seed treatment and microbiological testing. A presoak with hyperchlorinated water is generally the means to reduce the levels of enteric pathogens on seeds. Decreases in *Salmonella* and *E. coli* are typically in the range of $10^2$–$10^4$ CFU/g. The efficacy of the treatment is thought, in part, to be determined by the degree to which the pathogenic bacteria have been internalized in the seed, which makes them unavailable to the antimicrobial. Other antimicrobials have been evaluated, but have generally been less effective (Fett 2006). More aggressive treatments (e.g., irradiation) have been explored but tend to decrease viability of the seeds at levels that are effective for inactivating pathogens. Testing of incoming seeds can identify batches that are heavily contaminated but a substantial level of false-negative results should be expected due to the low level nature of the contamination. Better results may be obtained by testing the sprouted seeds or the spent irrigation water. If these tests are performed relatively early in the sprouting process, the result can be used to prevent contaminated lots from being released into commerce. The implementation of seed treatment and in-process testing of sprouting seeds or spent irrigation appears to be major contributing factors to the reduction in sprout-associated outbreaks during the late 1990s.

The postsprouting washing of sprouts can help reduce pathogen levels but this is generally restricted to a 1–2 log cycle reduction even when an antimicrobial is added to the wash water. Other control measures have been explored with limited success. The introduction of a competitive microorganism has been explored with limited success in suppressing the growth of *Salmonella* (Fett 2006) and *L. monocytogenes* (Palmai and Buchanan 2002a, b). Colicins (Nandiwada et al. 2004) and bacteriophage (Pao et al. 2004) treatments have also been investigated. The thermal characteristic of enteric pathogens suggest that brief blanching in hot water (≥90°C) could be used by consumers can reduce the likelihood that enteric pathogens are on sprouts (Fett 2006).

#### 12.9.1.2 Spoilage and Controls

The high respiration rates of sprouts requires postharvest storage at refrigeration temperature to prevent enzymatic and microbial spoilage. Relatively little data are available on the spoilage of sprouts but they are likely to be susceptible to psychrotrophic fluorescent *Pseudomonas* spp. and mold growth. Control of spoilage is achieved through the application of rigorous sanitation programs and hygienic practices, adequate dewatering of the product and maintenance of the cold chain.

### *12.9.2 Microbial Data*

The general lack of effective postgermination, microbiocidal treatments requires a high reliance on general hygienic controls and in some instances targeted acquisition of microbial data. Table 12.10 summarizes useful testing for sprouted seeds. Refer to the text for important details related to specific recommendations.

**Table 12.10** Testing of sprouted seeds (sprouts) for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | High | Test seed lots for *Salmonella* and *E. coli* O157:H7 particularly if confidence in the supplier is low | | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Seeds | *Salmonella* | ISO 6579 | 12 | 20[c] | 0 | 0 | – |
| | | | *E. coli* O157:H7 | ISO 16654 | 15 | 60[c] | 0 | 0 | – |
| In-process | High | Test either spent irrigation water or immature sprouted seeds in-process | | | | | | | |
| | | Spent irrigation water | | | | Sampling plan and limits/100 mL[b] | | | |
| | | | | | | *n* | *c* | *m* | *M* |
| | | | *Salmonella* | ISO 6579 | 12 | 5[d] | 0 | 0 | – |
| | | | *E. coli* O157:H7 | ISO 16654 | 15 | 15[d] | 0 | 0 | – |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | | | | | *n* | *c* | *m* | *M* |
| | | Sprouted seeds | *Salmonella* | ISO 6579 | 12 | 20[c] | 0 | 0 | – |
| | | | *E. coli* O157:H7 | ISO 16654 | 15 | 60[c] | 0 | 0 | – |
| Processing environment | Medium | Routine environmental testing not recommended. Periodic testing for *E. coli* or *Listeria* spp. may be appropriate to monitor hygienic conditions or if harborage is a concern. Extensive environmental testing should be conducted as part of the response to the production of contaminated sprouts to ensure return to control | | | | | | | |
| Shelf life | Low | Routine testing not recommended | | | | | | | |
| End product | Low | Routine end product testing is not recommended but periodic testing for indicators (*E. coli* or *Listeria* spp.) may be useful to verify process control and conduct trend analysis. Test for pathogens only when other data indicate potential for contamination or when history is not known | | | | | | | |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[d] Individual 100 mL analytical units reduces number of samples to achieve same total volume tested for cases 12 and 15

#### 12.9.2.1 Critical Ingredients

The use of high quality seeds that are free from contamination with *Salmonella* and EHEC is an important control measure for microbiological safety of sprouts. Particularly when there is a history of contamination from a growing region, testing for the presence of these pathogens may be beneficial for diverting contaminated seed lots to other uses. Testing for generic *E. coli* may serve as an alternative to testing for specific pathogens but its use needs to be weighed against the potential lack of a clear association between generic *E. coli* and the two pathogens at low contamination rates. This is most effectively done at the seed distributor level and may require the sprouting of sample batches if there are concerns about the ability of available methods to detect low level contamination. Availability of certified pathogen-free seeds would be highly beneficial to the sprout industry.

#### 12.9.2.2 In-Process

In process sampling of either the sprouted seeds or the spent irrigation water can be a useful tool for screening lots for the presence of specific pathogens, particularly *Salmonella* and EHEC. This is particularly beneficial when there is little history with the seed supplier or there are concerns about the effectiveness of seed sanitization treatments. Due to the diverse, abundant microbiota of most types of sprouted seeds, in-process testing for spoilage microorganism is not recommended.

Microbial challenge studies may be warranted to validate and periodically verify the effectiveness of the treatments used to sanitize the seeds.

#### 12.9.2.3 Processing Environment

The control of microbiological contamination is important for assuring the safety of sprouts that will be eaten without cooking. Periodic environmental sampling for indicator microorganisms (e.g., *E. coli*) can be used to verify the effectiveness of sanitation programs and hygienic practices. Testing for Enterobacteriaceae is likely to be of limited usefulness due to their common occurrence in sprouting seeds. Environmental testing for *Listeria* spp. may be warranted if harborage sites for *L. monocytogenes* are a concern.

#### 12.9.2.4 Shelf Life

Routine testing to determine shelf life is not recommended. However, retaining samples to conduct storage studies may be warranted to periodically confirm the appropriateness of prior shelf life determinations.

#### 12.9.2.5 End Product

The highly perishable nature of sprouted seeds generally makes routine microbiological testing of end product ineffective. Certification of seed lots and in-process testing are more effective. However, periodic testing of end product for *E. coli* or *Listeria* spp. may have benefit for evaluating the overall effectiveness of hygiene practices and postsprouting treatments (e.g., final rinse).

## 12.10 Mushrooms

While botanically not a true plant, mushrooms are traditionally grouped with vegetables due to similarities in characteristics, processing technologies and consumer uses. Mushrooms are the aerial fruiting bodies (sexual reproductive organs) of mycelial fungi. Most cultivated mushrooms belong to the sub-kingdom Basidiomycotina (e.g., *Agarius bisporus* (button mushrooms), *Lentinula edodes* (shiitake mushrooms), *Pleurotus ostreatus* (oyster mushrooms)), with a few species within the sub-kingdom Ascomycotina (e.g., truffles, morels) traded commercially. Mushrooms are cultivated on decomposed organic matter which typically is a mixture of manure (horse or chicken) hay, corn cob, cocoa seed hull, brewer's grain, hay, cotton seed and water (Chikthimmah and Beelman 2006). Mushrooms are sold in a number of forms including fresh, dried, marinated and canned. For the latter three forms, the concerns and controls are similar to other vegetables previously described for those types of vegetable products (see Sect. 12.6, 12.7, and 12.8). This section discusses fresh and minimally processed mushrooms.

### *12.10.1 Significant Organisms*

Details of mushroom cultivation vary from species to species, however, commercial cultivation generally involves initial composting of the growth substrate, the inoculation of the mycelial starter culture, incubation under specific conditions, harvesting of the mushrooms, and postharvest handling and processing. Successful production, both in terms of safety and quality, depends on controlling contamination during the cultivation.

#### 12.10.1.1 Hazards and Controls

Fresh and fresh-cut mushrooms and mushroom products have been associated with a limited number of documented microbiological hazards, including *C. botulinum*, *S. aureus*, *Campylobacter jejuni*, *L. monocytogenes* and *Salmonella*. The ability of mushrooms to support the growth of a number of pathogenic bacterial species and the extensive handling that mushrooms encounter provide general concerns regarding contamination with a variety of pathogenic, enteric bacteria.

Like sprouted seeds, the commercial cultivation of mushrooms typically occurs under environmentally controlled conditions that provide increased control of primary production. Since fresh mushrooms support the growth of bacteria, yeast and molds, do not undergo any postharvest steps that ensure elimination of pathogenic microorganisms and are often consumed in the raw state, control of cultivation, careful handling to prevent bruising, strict adherence to hygienic practices and maintenance of the cold chain are critical for ensuring product safety. Preparation of the growth substrate is particularly important. This is generally a two phase process that involves initial aerobic composting of the material for 15–25 days, when temperatures can reach as high as 80°C as a result of microbial activity (Chikthimmah and Beelman 2006). The substrate is then transferred to control atmosphere for further microbial action and nutrient conversion. This second phase is completed with a pasteurization step at 60–63°C for at least 2 h to inactivate spoilage organisms, human pathogens, weeds and insects (ICMSF 2005; Chikthimmah and Beelman 2006).

The rapid respiration rate of fresh mushroom combined with the use of plastic film packaging has led to concerns about the potential germination and outgrowth of *C. botulinum* spores in fresh mushrooms if they are held for any significant period without refrigeration. The use of packaging with sufficient openings to maintain an aerobic environment has been used to prevent spore germination; however, the primary barrier is strict control of refrigeration temperatures. Cases of staphylococcal enterotoxin intoxications associated with canned mushrooms have led to substantial investigations of the conditions for toxin production and inactivation in mushrooms and mushroom products. The use of brine solutions to store mushrooms prior to processing potentially allows growth and toxin production by *S. aureus* if refrigeration is not adequately maintained (Bennett, personal communication). The growth of *L. monocytogenes* may also be favored by brining and a sporadic case of listeriosis was attributed to brined mushrooms (Junttila and Brander 1989). A number of treatments have been investigated to control both spoilage microorganisms and pathogens. None has been universally used for fresh or fresh-cut mushrooms. Most other applications (e.g., freezing, canning) require the mushrooms to be blanched and treated to prevent enzymatic browning. These treatments reduce the levels of vegetative microorganisms.

#### 12.10.1.2 Spoilage and Controls

When freshly harvested, mushrooms contain a diverse microbiota including bacteria, yeasts and molds. Aerobic colony counts can range from $10^6$ to $>10^7$ CFU/g (Doores et al. 1986), and yeast and molds counts of $10^6$ and $10^3$ CFU/g, respectively are observed (Chikthimmah and Beelman 2006). The predominant bacterial species are fluorescent pseudomonads, with flavobacteria, chryseobacterium,

coryneform bacteria and lactic acid bacteria also present. The primary spoilage of mushrooms is enzymatic browning as a result of the fungi's own tyrosinase. *Pseudomonas* spp. and *Flavobacterium* spp. can reach levels of 7.3–8.4 log CFU/g and yeasts reaching levels of 6.9–8.0 log CFU/g (Chikthimmah and Beelman 2006). *Pseudomonas tolaasii*, *P. putida* and *P. fluorescens* appear to be particularly important in the spoilage of *A. bisporus* mushrooms.

The source of spoilage microorganisms appears to be the cultivation environment and the production personnel. The initial control of quality is use of properly composted growth substrate (see above). The incidence of spoilage is increased by over-watering the fungi during cultivation. Common methods for controlling spoilage microorganisms during cultivation is the addition of calcium salts or antimicrobial treatments (e.g., chlorine dioxide, electrolyzed oxidizing water, hydrogen peroxide) to the irrigation water. Maintenance of effective refrigeration is critical to delay spoilage and this can be extended further with the appropriate use of modified atmosphere packaging (2.5–5.0% $CO_2$ and 5–10% $O_2$) (Lopez-Briones et al. 1992). Potential postharvest treatments to delay spoilage include washing with antimicrobials, irradiation and pulsed ultraviolet light (Chikthimmah et al. 2005; Chikthimmah and Beelman 2006).

## *12.10.2 Microbial Data*

Since the principal controls for the microbiological safety and quality of mushrooms is during primary production, the most useful testing is targeted to ensure the effectiveness of composting processes, sanitation programs and hygienic practices.

Table 12.11 summarizes useful testing for mushrooms. Refer to the text for important details related to specific recommendations.

### 12.10.2.1 Critical Ingredients

The control of growth substrate is best monitored through routine measurement of the time and temperatures reached during the initial composting and during the pasteurization step prior to inoculation of the spawn. Periodic sampling for Enterobacteriaceae or other indicators may be beneficial to verify the continue effectiveness of these controls and prevention of recontamination. Periodic testing to assess the level of spore forming bacteria may be useful if there are concerns that excessive levels of spores are surviving the pasteurization process.

**Table 12.11** Testing of mushrooms for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Routine testing not recommended. Periodic testing to verify effectiveness of growth substrate pasteurization and control of re-contamination may be beneficial using Enterobacteriaceae and spore forming bacteria |
| In-process | Low | Routine testing is not recommended |
| Processing environment | Medium | Periodic testing to verify effectiveness of sanitation programs and hygiene practices includes testing for *E. coli* and *Listeria* spp. |
| Shelf life | Low | Routine testing is not recommended |
| End product | Low | Routine testing for assessing microbiological quality is not recommended. Periodic testing for indicators for on-going process control and trend analysis may be considered for psychrotrophic fluorescent pseudomonads, *Listeria* spp., yeast and molds, and *E. coli* |
| | Low | Routine microbiological testing for specific pathogens is not recommended. Test for specific pathogens only when other data indicate potential for contamination or when production conditions and history are not known |

#### 12.10.2.2 In-Process

Routine in-process microbiological testing is of little benefit if control of the environment and the growth substrate is effectively managed. Investigational testing for specific microorganisms may be needed when quality defects or the presence of pathogens are observed.

#### 12.10.2.3 Processing Environment

The safety and quality of the product depend on maintaining sanitary production and processing environments and good hygienic practices, therefore periodic microbiological testing to determine the effectiveness of these programs is useful. This is complicated by the nonsterile nature of the environment, which negates the usefulness of general indicators such as aerobic colony counts or Enterobacteriaceae. *E. coli* may be more effective as an indicator of fecal contamination. Since fresh and fresh-cut mushrooms are refrigerated ready-to-eat foods, testing for *Listeria* spp. in the environment could be beneficial. More intensive investigational testing for specific microorganisms may be necessary to address quality defects and identify harborage sites.

#### 12.10.2.4 Shelf Life

Routine testing for shelf life is generally not useful. Microbiological studies for establishing shelf life duration and for identifying likely spoilage microorganisms are beneficial after a significant change in technologies or facilities.

#### 12.10.2.5 End Product

The highly perishable nature of fresh and fresh-cut mushrooms makes routine testing of mushroom lots difficult and generally not pertinent. This would only be useful if there is no information on the safety of the product lot or if there is a history of concern with the manufacturer. However, periodic testing of end product for specific microbial indicators may be beneficial for assessing the overall performance of the food safety and food quality system. Potential indicators could include psychrotrophic fluorescent pseudomonads, *Listeria* spp., *E. coli* and yeast and mold counts.

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# Chapter 13
# Fruits and Fruit Products

## 13.1 Introduction

Fruits are defined in general terms as "the portions of plants which bear seeds." This definition includes true fruits such as citrus, false fruits such as apples and pears, and compound fruits such as berries. The definition includes tomatoes, chilies, capsicum, eggplant, okra, peas, bean, squash and cucurbits such as cucumbers and melons although for culinary purposes a number of these fruits are classified as vegetables. For the purposes of this chapter, tomatoes and melons will be considered fruits, whereas cucumber, egg plant, okra, peas, beans, squash, chilies and capsicum will be considered as either vegetables or spices.

Most fruits are high in organic acids, and hence have a low pH (ICMSF 2005). However, melons and some tropical fruits such as durian (*Durio* spp.) have a pH near neutrality. The principal acid in citrus fruits and berries is citric acid, malic acid in pome and stone fruits, and tartaric and malic acids in grapes and carambola. Because the pH varies within the product, care must be exercised in interpreting the pH values cited for most fruits. The pH values for fruits are typically determined by homogenizing an intact fruit and determining the pH of the expressed juice or pulp. This is not the microenvironment that a microorganism experiences when invading an intact fruit. For example, in an intact orange the acidic juice is maintained within the juice-sac whereas the surrounding tissue has pH values closer to neutrality. The traditional interpretation of the acidity of many fruits is being modified as research with apples, tomatoes, and oranges has demonstrated the growth of pathogenic enteric bacteria within intact or wounded fruit (Asplund and Nurmi 1991; Wei et al. 1995; Janisiewicz et al. 1999; Dingman 2000; Liao and Sapers 2000; Shi et al. 2007).

Most fruits are more susceptible to damage from molds and yeasts rather than from bacteria because of their low pH. This low pH means that most fruit-based products require only pasteurization to be microbiologically stable. Examples of exceptions include cucumbers, melons and some varieties of tomatoes.

Fruits may be processed by cutting, canning, freezing, sun-drying or dehydration, reducing its water activity through concentration or removal of water or the addition of salt or sugar. The pH of tomatoes can be reduced to below 4.5 by adding acids during processing, while chilies and durian are often pickled or fermented with lactic acid bacteria to produce microbiologically stable products that no longer need a low-acid canning process to retard spoilage.

For further information on the microbial ecology and control of fruits and fruit products related to food safety management principles, the reader is referred to *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005) and other texts (James 2006; Fan et al. 2009).

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_13,

## 13.2 Primary Production

The microbiota of fruits during cultivation is diverse, and reflects the cultivation environment, seed sources, soil amendments, irrigation water sources, host-adapted fruit pathogens and commensal microorganisms. A wide variety of bacteria, parasites, molds, yeast, and viruses are significant. For further details related to primary production of fruits and vegetables, see Sect. 12.2 of Chap. 12.

Human pathogens are generally not among the normal microbiota of fruits, but represent contamination occurring at some point of the supply chain, including from the primary production environment. The primary production environment includes water sources for irrigation and fruit spray applications, soil and soil amendments (e.g., manure, compost or manure teas), animals (e.g., mammals, birds, reptiles, insects), production and harvesting utensils and equipment, human handling, and nearby areas that may contain hazards that can be vectored onto the field or orchard by wind, run-off water or flooding.

Once introduced into the agricultural environment, human pathogens can persist for extended periods. As an example, large outbreaks of *Cyclospora cayatenensis* occurred for a number of years in North America due to raspberries imported from Guatemala. Although the original source of contamination was never verified, contaminated pesticide spray water was highly suspected to be the source (Herwaldt and Beach 1999).

### *13.2.1 Significant Organisms*

#### 13.2.1.1 Hazards and Controls

A wide range of potentially pathogenic microorganisms can be introduced into the primary production environment and ultimately be transmitted to harvested fruits and vegetables. A detailed description of these can be seen in Sect. 12.2.1.1 of Chap. 12. The principal means for controlling contamination during primary production is through the implementation of Good Agricultural Practices (GAP) programs, which are described in more detail in the chapter on vegetables (Chap. 12, Sect. 12.2.1.1).

#### 13.2.1.2 Spoilage and Controls

Both the quality and spoilage of fruits can be influenced by microbiological events that occur during cultivation. Most fruits may contain a wide variety of fruit pathogens that infect the fruit and cause visual and sensory changes in product quality (ICMSF 2005). Insect damage to picked fruits may increase the likelihood of spoilage. The primary control of fruit pathogens is through the selection of resistant fruit varieties, effective crop rotations and soil disinfestations, control of insect damage and effective control of temperature and respiration rates post-harvest. As a living tissue fruits undergo enzymatic browning, texture decay, microbial contamination, and undesirable volatile production, highly reducing their shelf life, especially if they are wounded. Edible coatings can be used to help in the preservation of whole and fresh-cut fruits (Olivas and Barbosa-Cánovas 2005).

The lower pH and the natural acid content of fruits often inhibit the growth of bacteria. As a result, fungi are frequently the dominant microorganisms in many fruits. However, there are several important bacterial causes of market diseases, particularly bacterial soft rots that are caused by *Erwinia carotovora*. Predominant molds occurring on fruits include both spoilage and innocuous fungi. A complete listing can be found in Table 6.2 in *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005). Yeasts occurring on fruits are evenly divided between ascosporogeneous and non-ascosporogeneous species.

### *13.2.2 Microbial Data*

The primary microbiological data needed to help control microbiological contamination during primary production of fruits is to provide assurances that the potential for the introduction of human pathogens is minimized. Microbiological testing for human pathogens is most likely to be important in two areas, the verification of microbiological quality of irrigation waters and the evaluation of soil amendments. Additional investigational testing may be employed if a primary producer is attempting to identify the source of an identified contamination. Please refer to Chap. 12, for a detailed discussion on irrigation waters and soil amendments, as well as suggested microbiological sampling plans.

## 13.3 Fresh Whole Fruits

Fresh whole fruits are commonly sold after minimal processing and packaging treatments and may be chilled or refrigerated. Common processing steps for fresh fruits may include washing, dipping, waxing, or wrapping in paper impregnated with preservatives against mold (ICMSF 2005).

### *13.3.1 Significant Organisms*

The microorganisms associated with fresh fruits consist of the microbiota acquired as a result of primary production (see Sect. 13.2), plus any additional microorganisms acquired as a result of harvesting, packing, processing, and transporting. This can include a diverse variety of microorganisms associated with farm workers and harvesting, processing and transportation equipment, and handlers. There are a number of fruits, including tomatoes, mangoes and oranges, but melons in particular, that can support the growth of bacteria, including human pathogens. The control of bacterial and fungal growth is critical both for quality and safety. There are significant opportunities for cross-contamination, particularly for those fruits that are transported during processing by fluming. The microbial load on fruits can be reduced to some degree (i.e., typically 1–2 logs) as a result of treatments such as hot or cold water washing, surface pasteurization (Annous et al. 2004), gaseous chlorine dioxide (Sy et al. 2005; Popa et al. 2007) and disinfection (Bastos et al. 2005). However, this is generally restricted to microorganisms on the surface of the fruit, and internalization of contamination decreases the effectiveness of surface antimicrobial treatments. Thus, care must be taken to ensure that processes do not foster such uptake of microorganisms into the fruit tissues or spread point source contamination throughout a batch.

#### 13.3.1.1 Hazards and Controls

Fresh whole fruits have been associated with outbreaks and sporadic cases caused by a variety of microorganisms of both zoonotic and human origin. In particular, *Salmonella* spp. have been associated with a large number of melon and tomato outbreaks, viruses such as norovirus and hepatitis A with strawberries and raspberries, and *Cyclospora* with raspberries (ICMSF 2005). The risk of disease can be amplified in the case of pathogenic bacteria by the potential ability of some whole fruits (e.g., oranges, mangoes, tomatoes and cantaloupes) to support bacterial growth (Wade and Beuchat 2003; Eblen et al. 2004; Richards and Beuchat 2005). The specific hazards and control measures depend on the type and source of the fruit, the location of initial processing, the extent of processing, and hygiene programs. For the most part, there are no steps to inactivate microorganisms during the processing of whole fruits. However, research on the use of hydrogen peroxide (Ukuku 2004), different combinations of nisin/EDTA/sodium lactate/potassium sorbate (Ukuku and Fett 2004), lactic acid (Alvarado-Casillas et al. 2007), and surface pasteurization (Annous et al. 2004) has shown promise in terms of the

inactivation of salmonellae on the surface of melons. Practices that are thought to increase the risk of melon-associated outbreaks include soil and irrigation water contamination of melons (Materon et al. 2007), the holding of cut melons at room temperatures, failing to wash melon rinds before cutting, and the misapplication of insecticides (Sivapalasingam et al. 2004).

## *13.3.2 Microbial Data*

The perishable nature of fresh fruits in combination with the low frequency of contamination of the products with human pathogens makes the use of microbiological testing as a means of separating safe and unsafe product impractical. However, microbiological testing and related analysis can be a useful means of verifying process control, i.e., the effectiveness of steps to reduce existing contamination and prevent new contaminants and cross-contamination (ICMSF 2002). In addition, the use of microbiological testing of the environment and food contact surfaces can provide an objective measure of hygienic practices. Table 13.1 summarizes useful testing for fresh fruit. Refer to the text for important details related to specific recommendations.

### 13.3.2.1 Critical Ingredients

There are no critical ingredients for this category of products, as the whole fresh fruit is the only ingredient. The quality and safety of these products is highly dependent on events occurring during their cultivation.

### 13.3.2.2 In-Process

While fruits may be subjected to processes that may reduce the risk of contamination (e.g., antimicrobial rinses), these treatments cannot ensure the elimination of pathogenic microorganisms. Furthermore, the effectiveness of these treatments is highly dependent on maintaining proper water temperatures, antimicrobial treatment concentrations, and, in many instances, the pH of the treatment carrier and the organic load. Once validated, control of these steps is typically monitored through chemical or physical analyses of the conditions of use.

In addition to food contact surface and general environmental hygiene sampling, there are specific steps, such as the use of dump or wash tanks, or the transport within a plant by fluming or hydrochillers, where monitoring of the transport medium for sufficient levels of antimicrobials is important for the control of cross-contamination. Such analyses will typically be chemical or physical in nature. A lack of attention to in-process conditions can lead to increased food safety risks and loss of food quality. Of particular concern are pathogenic bacteria that are able to grow on the fresh fruit being processed. Physical damage of fresh fruits can provide additional nutrients and cause points of entry, leading to internalization.

### 13.3.2.3 Processing Environment

The processing environment for fresh fruits represents a significant challenge since many fruits receive their initial, and sometimes only processing in the field at the time of harvest. Furthermore, most packaging operations are open to the surrounding environment or have only rudimentary environmental controls. These challenges are even greater when the typically seasonal nature of the work force and the corresponding limited hygiene training they may receive, are considered. Microbiological testing of food contact surfaces and the packing facility environment can serve as an important tool for verifying the effectiveness of cleaning operations and hygienic practices.

**Table 13.1** Testing of fresh fruits for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Monitoring or verifying that GAPs were followed during production is recommended to minimize the risk of contamination prior to further processing. Refer to Chap. 12 for guidance on growing conditions |
| In-process | Medium | Periodic or continual testing of antimicrobial levels in flume, wash water, etc., may be necessary; however, this is typically performed using chemical or physical analyses |
| Processing environment | Medium | Periodic testing of food contact surfaces and processing environments may be appropriate for certain types of fruits to verify adequacy of cleaning and sanitization protocols. Visual hygiene inspections are recommended |
| Shelf life | Low | Testing is not relevant |
| End product | Low | Routine testing for specific pathogens is not recommended. Testing may be warranted when information indicates a potential for contamination or when production conditions and history are not known |

| | | | | Sampling plan and limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Fresh fruits | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |
| | *E. coli* O157:H7 | ISO 16654 | 14 | 30 [b] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 of Chap. 7 for compositing)

This will be generally limited to indicator organisms (e.g., aerobic plate counts, Enterobacteriaceae). However, in certain instances, the analysis for specific pathogens may be warranted, based on an assessment of potential contamination sources (e.g., monitoring the environment for *Salmonella* in a facility that has had past concerns with birds or vermin).

Microbiological verification of cleaning operations by testing for indicator organisms can be an effective means of ensuring the effectiveness of hygiene programs. Such sampling programs are most effective when designed to provide a quantitative measure of the extent of control so that process control (ICMSF 2002) can be monitored via trend analysis and corrective actions taken before the occurrence of a process failure.

#### 13.3.2.4 Shelf Life

Establishment of shelf life values for whole fresh fruits depends on the type of fruit, and is typically determined by conditions encountered during production and harvesting and expected to be encountered during further handling in distribution, marketing and consumption.

#### 13.3.2.5 End Product

Fresh fruits are ready-to-eat (RTE) foods that are likely to be eaten without any further microbiocidal treatment and thus should be free of microbial pathogens to a degree needed to ensure a low risk of foodborne disease. The specific level of control required depends on the specific fruit, its conditions of use and the microbiological hazards associated with the fruit.

The direct testing of fresh fruits may be necessary in instances where there is no information available concerning the lot of food in question. However, in most instances, the defect rates (i.e., the

percentage of individual fruits within a lot that are contaminated) observed, even within a lot, are so low that end product testing is impractical.

*Escherichia coli* may be an indicator of fecal contact somewhere in the production system, but is not a good indicator of fecal or pathogen contamination of the fruit. Microbiological levels of fresh produce are not useful for process control charting. Total plate counts of fresh produce, regardless of commodity or how grown, can vary by as much as 5 logs, lot to lot, even item to item, without an impact on quality or safety. The normal range of coliform or *E. coli* levels will be smaller (e.g., 3 logs), but the initial variability would still be too great for process charting. If using microbiological testing for process control, the testing would likely only be useful if performed on the same lot of produce, i.e., counts at the beginning of the handling process versus counts at the end of the process.

In those instances where information on the product and how it was processed and handled is available, microbiological testing for process verification using an appropriate indicator microorganism (e.g., *E. coli* for fecal contamination) may be much more effective and provide a means for process control charting that would allow corrective actions to be taken prior to reaching the point of process failure. Similar process control (cross-lot) testing for mesophilic or psychrotrophic aerobic plate counts may also be useful for assessing maintenance of the control of key spoilage microorganisms.

## 13.4 Fresh-Cut, Minimally Processed Fruits

Fresh-cut fruits include RTE, pre-cut and lightly processed fruits. Minimally processed refrigerated fruits meet consumer demands for convenient, like-fresh fruit products, while at the same time ensuring food safety and maintaining nutritional and sensory quality. Typical processes used for different fresh-cut fruits include cutting, slicing, shredding, peeling, dicing, coring, and packaging. This also includes combining different fresh-cut fruits to provide pre-prepared fruit mixes. Fresh-cut fruits are sold under refrigerated storage in supermarkets, retail food stores and restaurants or chilled on ice in roadside fruit stalls in many countries.

### *13.4.1 Significant Organisms*

#### 13.4.1.1 Hazards and Controls

The main pathogens of concern are *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes*, as these microorganisms have been involved in foodborne outbreaks with fresh-cut fruit. Details on the ecology and epidemiology of these organisms have been previously published (Herwaldt et al. 1994; Ooi et al. 1997; Sewell and Farber 2001; CDC 2002; Johannessen et al. 2002; Sivapalasingam et al. 2004; ICMSF 2005; Bowen et al. 2006; Varma et al. 2007).

Starting with high quality fruit is critical to the successful production of safe fresh-cut fruit. An approved supplier program should be developed for the fresh fruit suppliers to ensure that GAPs and proper handling are being followed to meet food safety requirements. Upon receipt, the fruit should be thoroughly washed and then inspected to ensure that the level of defective fruit is low. Windfalls or dropped fruit should not be used to produce fresh-cut products.

Effective cleaning of the fruit surfaces prior to cutting and the maintenance of high sanitation throughout processing and packaging is very important. Typically, the fruit undergoes extensive washing before and after cutting with water containing chlorine or other antimicrobials to prevent cross-contamination from contaminated to uncontaminated fruit. Although a number of disinfectants have been evaluated for their effectiveness against various pathogenic enteric bacteria, including hypochlorite, acidified sodium chlorite, peroxyacetic and mixed peracid products, hydrogen peroxide, chlorine dioxide, lactic acid and hot water (Pao and Brown 1998; Sapers et al. 1999; Liao and Sapers 2000; Pao et al. 2000; Wisniewsky et al. 2000; Fleischman et al. 2001; Du et al. 2002; Ukuku

and Fett 2002; Bastos et al. 2005; Alvarado-Casillas et al. 2007), such treatments have limited effectiveness, with microbial reductions generally in the range of 1–3 log cycles. It is important to validate the systems being used, understanding the importance of temperature, organic load etc., on the effectiveness of the antimicrobial treatment.

The general approach to controlling pathogens in a fresh-cut fruit operation involves separation of raw from cut produce, managing the sanitation of the manufacturing environment where product is exposed and subject to contamination and, where applicable to the commodity, washing in antimicrobial-treated water to reduce surface contamination and prevent cross-contamination. The low temperature typically maintained in fresh-cut operations (<12°C in Europe, <4°C in U.S.), also reduces the risk of harborage of mesophilic pathogens like *Salmonella* spp. and *E. coli* O157:H7 in the processing environment.

#### 13.4.1.2 Spoilage and Controls

The type and importance of fresh-cut fruit spoilage reflects the intended use of the product and the adequacy of the cold chain. For street venders, where the shelf life of the product is a few hours and the product is generally not refrigerated or packaged for extended storage, spoilage is not an issue. As the shelf life of the product becomes increasingly extended, the shelf life of the fresh-cut fruit is increasingly dependent on adequate refrigeration. With a product that has a 7–14 day shelf life, the microorganisms of concern in fresh-cut fruits are psychrotrophs that are capable of growing at 2–4°C, and typically have optimal growth at temperatures between 20 and 30°C (Brackett 1994). In addition, modified atmosphere packaging (MAP), which combines modified atmospheres and chilling temperatures to retard microbial spoilage and delay fruit senescence, e.g., the use of ethylene to control the ripening of apples, can be used. Microbial growth can be affected by the amounts of oxygen and carbon dioxide present in the package (Day et al. 1990). Care must be taken in selecting the MAP to be employed since fresh-cut fruit is an actively respiring system and certain gas combinations will adversely affect the fruit metabolism and thus its shelf life. For further details on spoilage and controls, the reader is referred to ICMSF (2005).

### *13.4.2 Microbial Data*

The perishable nature of fresh-cut fruits in combination with the low frequency of contamination of the products with human pathogens makes the use of microbiological testing as a means of separating safe and unsafe product impractical. However, microbiological testing and related analysis can be a useful means of verifying process control, i.e., the effectiveness of steps to reduce existing contamination and prevent new contaminants and cross-contamination (ICMSF 2002). In addition, the use of microbiological testing of the environment and food contact surfaces can provide an objective measure of hygienic practices. Table 13.2 summarizes useful testing for fresh-cut fruits. Refer to the text for important details related to specific recommendations.

#### 13.4.2.1 Critical Ingredients

No additional ingredients are added to fresh-cut fruit and the product is commercialized as such. Although not an ingredient in fresh-cut fruit, water and ice that may come into contact with the fruit during production and storage should meet, as a minimum, local requirements for potable water.

#### 13.4.2.2 In-Process

Testing is not applicable.

#### 13.4.2.3 Processing Environment

Microbiological testing of the processing environment is appropriate for pathogens reasonably able to become established. For example, testing for *Salmonella* spp. may be warranted in processing operations maintained above the organisms' minimum growth temperature. Monitoring the processing environment where fresh-cut product is exposed for *L. monocytogenes*, which can grow at refrigerated temperatures, is appropriate. The frequency of sampling should be related to the risk, and will be line and plant specific. Sampling of the environment should be focused on zones which are in the finished product area, and in close proximity to the processing lines. Detailed characterization of the strains by molecular typing could provide useful information in terms of pinpointing contamination niches within the plant, by source tracking. Tests for aerobic colony counts may also be useful to determine the general impact of processing and handling. Rapid methods, such as ATP measurement, can be a useful tool to assess equipment hygiene. Details on the establishment of environmental sampling programs are provided in ICMSF (2002) and Chap. 4.

#### 13.4.2.4 Shelf Life

The typical refrigerated shelf life of a fresh-cut fruit is very short, although manufacturers are aiming for products that have a longer shelf life. However, extension of shelf life could lead to the growth of pathogens to high levels before a product spoiled. This would mainly be the case for products such as fresh-cut mangoes (González-Aguilar et al. 2000), tomatoes (Das et al. 2006) and melons (Raybaudi-Massilia et al. 2008). Challenge studies with bacteria that are pathogenic for humans may be beneficial where systems for extending shelf life could lead to the growth of the pathogens to high levels before product spoilage. In such instances, a secondary barrier may need to be established to control pathogen growth.

From a spoilage standpoint, the microorganisms of concern in fresh-cut fruits are psychrotrophs and molds that are capable of growing at 2–4°C. There are no routine methods to evaluate the microbiological shelf life of fresh-cut fruits. There are also, at present no true microbial indicators of spoilage, except for the obvious presence of mold appearing on the product. Thus, sensory indicators of spoilage (e.g., taste, feel, texture) are used to evaluate shelf life of a product. Fresh-cut fruit operations may choose to conduct tests to evaluate whether their code-dating practices reflect the shelf life of the product. Such tests can consist of storing representative packages of product at one or more temperatures and durations that the product reasonably may be expected to experience during storage, distribution and display, and conducting a sensory evaluation on days that bracket the code date that is applied In addition, companies can conduct a survey of their product at the retail level. Sensory evaluation can be supplemented with microbiological tests for quality indicators (e.g., total counts or yeast/mold).

#### 13.4.2.5 End Product

The presence of enteric pathogens is the major food safety concern, but testing for all the possible pathogens mentioned above is not recommended. It may be appropriate to use *E. coli* as an indicator of the hygienic conditions of growing, harvesting, transporting and processing. Enterobacteriaceae, coliforms or "fecal coliforms" are not effective indicators because they occur naturally in the field and plant environment and may not be directly linked to the attributes being controlled to assure microbial safety and quality (ICMSF 2005).

Few countries have developed microbiological criteria for fresh-cut fruits. The EU published microbiological criteria for fresh-cut fruits and vegetables (EC 2005). For *L. monocytogenes*, $n=5$, $c=0$, $m=10^2$ CFU/g at the distribution level for all RTE foods that do not support growth. For those RTE foods that are able to support the growth of *L. monocytogenes*, there is an additional criterion of absence in $5\times25$ g at the manufacturing level. There is also a criterion for *Salmonella*, which is

an absence of Salmonella spp. in 5×25 g. In addition to the criteria for *Salmonella* and *Listeria*, there is also one for *E. coli* in pre-cut fruits of $n=5$, $c=2$, $m=10^2$ CFU/g, $M=10^3$ CFU/g. The Codex Alimentarius Commission guidelines for *L. monocytogenes* differ slightly from the EU regulations (CAC 2009). Canadian regulations stipulate an action level of $10^2$ CFU/g for *L. monocytogenes* if the product has a shelf life of less than or equal to 10 days.

The *E. coli* criteria do seem reasonable as an indicator of the hygienic conditions of growing, harvesting, transporting and processing. There should be a difference in approach between testing in a routine/monitoring situation versus investigational sampling. The recommended ICMSF limits for fresh-cut fruit are presented in Table 13.2.

**Table 13.2** Testing of fresh-cut fruit for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Monitoring or verifying that GAPs were followed during production is recommended to minimize the risk of contamination prior to further processing. Refer to Chap. 12 for guidance on growing conditions. Good quality fruit should be used to produce fresh-cut fruit |
| In-process | Medium | Periodic or continual testing of the pH of water or the antimicrobial levels in flume, wash water, etc., may be necessary |
| Processing environment | Medium | In addition to chemical tests (e.g., ATP), periodic testing of food contact surfaces and processing environments are recommended for verifying the adequacy of cleaning and sanitization protocols. Potential assays include aerobic colony counts, total psychrotrophs or yeast and mold. Consider environmental testing for salmonellae, *Listeria* spp. or *L. monocytogenes* in processing environments where fresh-cut fruits are exposed and temperatures are greater than the minimum growth temperature for the organism |
| Shelf life | Medium | Validated through microbiological testing or sensory analysis before initiation of a new product type and revalidated after any major change in process technologies. Periodic verification through microbiological analysis for spoilage species may be beneficial, when shelf life is limited by microbiological activity |
| End product | Low | Routine testing for specific pathogens is not recommended. Testing may be warranted when information indicates a potential for contamination |

| | | | | Sampling plan and limits/25g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Fresh-cut fruit, RTE | *Salmonella* | ISO 6579 | 12[c] | 20 [c] | 0 | 0 | – |
| Fresh-cut fruit, RTE, supporting growth | *L. monocytogenes* | ISO 11290-1 | – | 5 [c] | 0 | 0 | – |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Fresh-cut fruit, RTE, no growth | *L. monocytogenes* | ISO 11290-2 | – | 5 | 0 | $10^2$ | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] For fresh-cut fruit that do not support growth, e.g., fresh-cut pineapple, case 11 would apply
[c] Individual 25g analytical units (see Sect. 7.5.2 of Chap. 7 for compositing)

## 13.5 Frozen Fruits

Freezing provides a significantly extended shelf life and has been successfully employed for the long-term preservation of many fruits. Fruits to be preserved by freezing are sometimes pre-treated by blanching to inactivate enzymes. This effectively destroys the surface vegetative microbiota.

### *13.5.1 Significant Organisms*

#### 13.5.1.1 Hazards and Controls

The hazards in frozen fruits that have caused outbreaks include salmonellae, norovirus and hepatitis A. Contamination of frozen mamey with *Salmonella* Typhi has led to two outbreaks of typhoid fever in the US (Katz et al. 2002; CDC, 2010). Frozen strawberries have been linked to outbreaks of hepatitis A in the US (Ramsay and Upton 1989; CDC 1997), and frozen raspberries have been linked to norovirus outbreaks in Finland (Pönkä et al. 1999); France (Cotterelle et al. 2005), Denmark (Falkenhorst et al. 2005) and Sweden (Hjertqvist et al. 2006). Control is achieved through acquisition of quality fruits, maintenance of hygienic practices and processing environment, timely freezing, and maintenance of frozen storage temperatures.

#### 13.5.1.2 Spoilage and Controls

The normal microbiota of frozen fruit consists mainly of fungi, especially yeasts. Growth and spoilage is influenced by storage temperature; partial or complete thawing will frequently lead to yeast spoilage from gas production. However, if adequately maintained at frozen temperatures, spoilage is generally due to non-microbial attributes. Microbial populations on fruits to be frozen are best controlled by adequate washing, removal of obviously diseased fruit, careful handling to prevent bruises, frequent cleaning and sanitation of handling and conveying equipment and prompt freezing of the prepared fruit.

Time and temperature controls are needed before, during and after preparation, as well as during transportation, storage and sale. Fungi, especially yeasts, can proliferate on equipment used to prepare product for freezing. Some are killed or injured by the freezing process, and numbers slowly decline further in storage. Provided product is handled correctly after thawing, such contamination is of no consequence.

### *13.5.2 Microbial Data*

The acquisition of microbiological data as a control measure is generally not warranted for frozen fruits. However, periodic testing for the purposes of verifying the microbiological profile of raw ingredients and the effectiveness of sanitation and hygiene programs are desirable in ensuring continuing attention to factors that can affect the safety and quality, if not maintained. Process control verification testing for *L. monocytogenes* may be considered if the product is likely to be thawed and then held under refrigeration for extended periods of time and the product supports growth. Table 13.3 summarizes useful testing for frozen fruit. Refer to the text for important details related to specific recommendations.

#### 13.5.2.1 Critical Ingredients

In the case of frozen fruit, sugar may be added. If water or ice is used, it should, as a minimum, meet local requirements for potable water.

**Table 13.3** Testing of frozen fruit for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | GAPs should be followed in fruit production. Refer to Chap. 12 for guidance on growing conditions<br>Good quality fruit should be used to produce frozen fruit |
| In-process | Low | No specific testing recommended. Possible tests are<br>• Aerobic colony count may be used to monitor process control, potential temperature abuse and the effectiveness of equipment hygiene<br>• Periodic testing, appropriate for the product, may be considered and will vary depending on the product and processing conditions |
| Processing environment | Low | No specific testing recommended. Possible tests are<br>Aerobic colony counts to monitor process hygiene for product contact surfaces |
| Shelf life | - | Not applicable |
| End product | Low | Test for indicators for ongoing process control and trend analysis |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Frozen fruit | *E. coli* | ISO 16649-2 | 5 | 5 | 2 | 10 | $10^2$ |

| | | |
|---|---|---|
| | Low | Routine testing for specific pathogens is not recommended. Testing may be warranted when information indicates a potential for contamination or when production conditions and history are not known |

| | | | | Sampling plan and limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Frozen fruit | *Salmonella* | ISO 6579 | 11 | 20[c] | 0 | 0 | – |

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to s A for performance of these sampling plans
[c]Individual 25 g analytical units (see Sect. 7.5.2 of Chap. 7 for compositing)

#### 13.5.2.2 In-Process

For frozen fruits, no specific testing of the processing lines is recommended.

#### 13.5.2.3 Processing environment

Similar to above, no specific testing is recommended, although environmental testing for *L. monocytogenes* or indicators could monitor the potential for contamination if the product is likely to be thawed and then held under refrigeration for extended periods of time and the product supports growth.

Tests for aerobic colony counts may also be useful to determine the general impact of processing and handling. Rapid methods, such as ATP measurement, can be a useful tool to assess equipment hygiene.

#### 13.5.2.4 Shelf Life

The shelf life of frozen fruit can be several months. Frozen storage below −10°C will prevent all microbial growth, but does not necessarily lead to inactivation of microorganisms. Microbial spoilage of frozen fruit is not an issue. Sensory indicators of spoilage (e.g., taste, feel, texture) are the only means at present of evaluating the remaining shelf life of a product. Frozen food operations may choose to conduct tests to evaluate whether their code dating practices reflect the sensory shelf life of the product.

#### 13.5.2.5 End Product

Routine microbiological testing of frozen fruit is not recommended. Some countries have recommended general hygiene criteria such as the absence of coliforms, molds, yeasts and *Staphylococcus aureus* in 10 or 100 g of product. For generic *E. coli*, one country recommends an absence of the organism in 10 g of product. In terms of microbiological criteria for pathogens, a couple of countries have criteria of an absence of salmonellae in 20 or 25 g of product, and one country has a criteria of an absence of *Shigella* spp. in 25 g. In general, it does not make sense to have microbiological criteria for a low-risk product such as frozen fruit which would normally has a very low incidence of product contamination.

## 13.6 Canned Fruits

For information on canned fruits, please see Chap. 24.

## 13.7 Dried Fruits

Drying of fruits is an important method of preservation and includes production of a wide variety of products. Drying changes the physical and biochemical form of the fruit leading to shrinkage and change of color, texture and taste. If the water activity is reduced to appropriate levels, the dried product can have a shelf life exceeding 1 year if properly packaged (Ratti and Mujumdar 2005). Some fruits, such as apricots, peaches, pears and bananas, are dried after addition of $SO_2$, and most microorganisms will be eliminated. Dried prunes, figs and most vine fruits, however, are not processed with $SO_2$, and are susceptible to spoilage by xerophilic fungi (Pitt and Hocking 2009). Dehydrated fruits are often added to RTE products (e.g., breakfast cereals, chocolate, fruit and nut mixes) without a kill step.

### *13.7.1 Significant Organisms*

#### 13.7.1.1 Hazards and Controls

Survival of pathogenic bacteria on dried fruits is usually poor, and limited to a few weeks. Relatively long storage periods before sale, normal for such products, further minimizes risks. However, *E. coli* O157: non-H7 has been isolated from one sample of conventionally grown raisin and one sample of organically grown apricot (Johannessen et al. 1999). In addition, salmonellae have been isolated from commercially available high-moisture dried prunes in South Africa (Witthuhn et al. 2005). Most countries now permit the addition of weak acid preservatives such as sorbate or benzoate to high moisture prunes, figs and other similar products.

Toxigenic species of *Aspergillus* can occur in figs, and cause spoilage and form mycotoxins. Dried figs lots entering into the processing plant should be sampled and analyzed for moisture (moisture content ≤24% and $a_w$ ≤0.65) and screened for bright greenish yellow fluorescence (BGYF). Dried figs contaminated with aflatoxins will fluoresce under long wave (360 nm) ultra violet light (Steiner et al. 1988), and should be removed to obtain lower aflatoxin content in the lot. A Codex Alimentarius Commission Code of Practice currently exists for the prevention and reduction of aflatoxin in figs (Codex Alimentarius 2008). Infection of *Aspergillus carbonarius, Aspergillus niger* and related species in dried vine fruits is common, and the presence of ochratoxin A may occur (Pitt and Hocking 2009).

Reduction in fruit damage, by reducing insect infestation, disease control, and careful handling before drying are important. General control measures would include frequent and thorough cleaning of equipment, prompt drying to low $a_w$, either by sun drying or dehydration, appropriate loading of the product into the dryer to achieve even drying, hygienic handling of the dried product and storage of the dried product to preclude entry of moisture. Moisture control is an important factor to minimize the risk of recontamination of dried fruits. One should also minimize the time of storage of the cleaned, cut fruits before drying. Blanching, when applicable, will reduce the microbial load. Recommended International Code of Hygiene Practice for dried fruits (Codex Alimentarius 1969) exists and should be follow for all dried fruits. The Grocery Manufacturers Association published practical information on control of salmonellae in all low-moisture foods (GMA 2009).

#### 13.7.1.2 Spoilage and Controls

Fruits that are not treated with preservatives such as $SO_2$ are susceptible to spoilage by xerophilic fungi. However, if fruits are properly dried and stored, the extent of damage should be slight. Poor factory hygiene may result in contamination of dried-fruit during packaging. In particular, the extreme xerophile *Xeromyces bisporus*, which is able to grow quite rapidly at 0.70–0.75 $a_w$, may build up on conveyers and other equipment, be transferred to the fruit and then cause spoilage of product that is safe from all other fungi (Pitt and Hocking 1982, 2009). Mature figs are always contaminated in the seed cavity by yeasts (Miller and Phaff 1962). Spoilage of dried figs sometimes occurs if these contaminant yeasts include xerophilic species. Partially prepared glacé pineapple may spoil due to the growth of the yeast *Schizosaccharomyces pombe*. Frequent and careful cleaning of processing and filling lines and equipment is essential to prevent the build up of fungi, especially *X. bisporus* and xerophilic *Chrysosporium* species (Pitt and Hocking 2009). Insect damage can also occur during the storage of dried fruit products.

### *13.7.2 Microbial Data*

Microbiological data for dry fruits is acquired to provide confidence in processes, ingredients and hygiene programs and, as such, is focused on verification instead of routine testing for release. Table 13.4 summarizes useful testing for dried fruit products. Refer to the text for important details related to specific recommendations.

#### 13.7.2.1 Critical Ingredients

There are no critical ingredients in the production of dried fruits. The quality and safety of these products depends highly on the status of the fruit before drying. Good quality, sound fruit should be used. Moldy fruits should not be used.

#### 13.7.2.2 In-Process

Testing is not applicable.

#### 13.7.2.3 Processing Environment

Testing of the environment for pathogens is not recommended. Control of the environment is necessary to prevent the ingress of spoilage organisms, in particular heat resistant fungal spores. In facilities where this has become an ongoing problem, monitoring of the environment should be considered.

**Table 13.4** Testing of dried fruit for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | GAPs should be followed in fruit production. Refer to Chap. 12 for guidance on growing conditions<br>Good quality fruit should be used to produce dried fruit |
| In-process | – | Not applicable |
| Processing environment | Medium | • In plants with periodic problems with molds, monitoring of the environment for fungal spores should be done<br>• Periodic sampling of the processing environment to verify effective application of hygienic practices. This will typically require the establishment of an "in-control" baseline for the manufacturing facility. Potential microorganisms include yeast and molds, Enterobacteriaceae, or *Salmonella* |
| Shelf life | – | Not applicable |
| End product | Low | Routine testing for specific pathogens or mycotoxins is not recommended. Testing may be warranted when information indicates a potential for contamination or when production conditions and history are not known<br>Test for indicators for ongoing process control and trend analysis |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Dried fruit | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $10^3$ | $10^4$ |
| | *E. coli* | ISO 16649-1 or 2 | 5 | 5 | 2 | $10^2$ | $10^3$ |

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to Appendix A for performance of these sampling plans

#### 13.7.2.4 Shelf Life

Dried fruits may spoil due to the growth of filamentous fungi. Microbiological shelf life testing is not relevant for these products.

#### 13.7.2.5 End Product

Aerobic colony count (ACC) is a useful measure of hygiene and process control; however, the ACC will vary for different fruits and conditions of growing and processing. The presence of coliforms is not a useful indicator of fecal contamination; however, the presence of *E. coli* may indicate a cause for concern. The current Codex Alimentarius Commission standard for dried fruit was written in 1969 and does not provide any specific guidance on microbiological criteria.

## 13.8 Tomatoes and Tomato Products

Other than fresh product, many tomato products are canned foods such as whole, peeled or diced tomatoes with or without added juice or tomato puree; tomato concentrates including tomato juice and tomato pastes; tomato powder; and formulated products such as salsa, tomato sauce (catsup or ketchup), soup and chili sauce (ICMSF 2005). This section deals with fresh and fresh-cut tomatoes. For the canned tomato products, see Chap. 24.

## *13.8.1 Significant Organisms*

### 13.8.1.1 Hazards and Controls

*Salmonella* is the primary pathogen of concern in tomatoes. There have been a number of tomato-associated salmonellosis outbreaks in the USA. In the 14-year period between 1990 and 2004, nine outbreaks affecting an estimated 60,000 people occurred in the USA (CDC 2005). During 2005–2006, four large multistate outbreaks of *Salmonella* infections associated with eating raw tomatoes at restaurants occurred in the US (Greene et al. 2008). Diced and whole tomatoes can also support the growth of *Salmonella* spp. at 20°C or higher (Zhuang et al. 1995). As a result, the USA considers cut tomatoes as a potentially hazardous food that requires time and temperature control for safety (FDA 2009). In addition, a large multiple-restaurant tomato-associated outbreak due to *Shigella flexneri* serotype 2a occurred in the USA in 2001 (Reller et al. 2006).

The key critical control point includes the regular changing and maintenance of water quality in packing houses and processing facilities. Water temperatures should be maintained at a temperature around 6.6°C above the incoming tomatoes to prevent the ingress of pathogens into the fruit. For example, salmonellae can enter tomatoes through the stem scar, small cracks in the skin or through the plant itself (Guo et al. 2001). The porosity of the stem-end scar increases with fruit pulp temperatures so the potential for infiltration is greatest in the summer months. The organism was shown to grow in the pulp tissue and stem scar of tomatoes stored at 12 and 21°C (Beuchat and Mann 2008). Infiltration can also occur by pressure if tomatoes are submerged too deeply in a wash tank. Immersion or spraying of *Salmonella* surface-inoculated tomatoes with chlorinated (200 mg/L) and ozonated water (1 and 2 mg/L), for 120 and 30 s, respectively, can cause a 2 to 3-log reduction in the viable counts (Chaidez et al. 2007). Use of antimicrobial treatments in wash and flume water varies by country and should follow local regulations.

### 13.8.1.2 Spoilage and Controls

With an internal pH of 4.0–4.5, tomatoes can be affected by fungal and bacterial market diseases. The primary spoilage bacterium is *E. carotovora* subsp. *carotovor*a, which causes bacterial soft rot. *Alternaria* is important in rots of tomatoes. Other fungi important in spoilage include *Cladosporium herbarum*, *Botrytis cinerea*, *Rhizopus* spp. and *Geotrichum candidum*.

## *13.8.2 Microbial Data*

The perishable nature of tomatoes and tomato products in combination with the low frequency of contamination of the products with human pathogens makes the use of routine microbiological testing as a means of separating safe and unsafe product impractical. However, occasional microbiological testing and related analysis can be a useful means of verifying process control, i.e., the effectiveness of steps to reduce existing contamination and prevent new contaminants and cross-contamination (ICMSF 2002). In addition, the use of microbiological testing of the processing environment and food contact surfaces can provide an objective means of verifying the effectiveness of sanitation programs and hygienic practices.

### 13.8.2.1 Critical Ingredients

There are no critical ingredients.

#### 13.8.2.2 In-Process

No microbiological testing is recommended. However, monitoring of the pH, water temperature and antimicrobial levels, if allowed, in the dump tank and flume waters is recommended.

#### 13.8.2.3 Processing Environment

No microbiological testing for pathogens is recommended. See the section on fresh fruit and on fresh-cut fruit, as appropriate, for more information.

#### 13.8.2.4 Shelf Life

Microbiological shelf life testing is not relevant for these products.

#### 13.8.2.5 End product

Routine microbiological testing is not recommended for these products, unless data indicate the potential for contamination with Salmonella spp.

## 13.9 Fruit Preserves

Fruit preserves refers to fruit that have been heat treated, acidified, canned or jarred for long-term storage. The preparation of fruit preserves may involve the use of pectin. There are various types of fruit preserves made globally, and they can be made from sweet or savory ingredients.

### *13.9.1 Significant Organisms*

#### 13.9.1.1 Hazards and controls

Pathogenic bacteria are not normally associated with fruit preserves.

#### 13.9.1.2 Spoilage and Controls

Fruit preserves are heat treated food products, so spoilage agents are primarily heat-resistant fungi. Ascospores of *Byssochlamys fulva*, *Byssochlamys nivea, Talaromyces* species and *Neosartorya* species occur naturally in the soil and thus fruit that comes into contact with the soil or rain splash such as strawberries, pineapple and passion fruit, are more susceptible to contamination. Thus, it is very important to sort the bad quality fruits from the good fruit, and to wash the selected fruits well, before using them to make preserves. Additionally, lack of hygiene in the processing environment can lead to high levels of heat-resistant ascospores.

### *13.9.2 Microbial Data*

#### 13.9.2.1 Critical Ingredients

There are no critical ingredients, and routine microbiological testing of raw fruits is not recommended.

#### 13.9.2.2 In-Process

For fruit preserves, no specific in-process testing is recommended.

#### 13.9.2.3 Processing Environment

Similar to above, no specific testing is recommended, although indicators could monitor the potential for contamination. Tests for aerobic plate count may also be useful to determine the general impact of processing. Rapid methods, such as ATP measurement, can be a useful tool to assess equipment hygiene.

#### 13.9.2.4 Shelf Life

The shelf life of fruit preserves can be several months. Microbial spoilage during storage can be assessed by visual examination of the product. Routine microbiological testing is not recommended.

#### 13.9.2.5 End Product

Routine microbiological testing of fruit preserves is not recommended, as this category of foods is heat-processed for long-term storage and is a low-risk product that has a low incidence of product pathogen contamination.

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# Chapter 14
# Spice, Dry Soups and Asian Flavorings

## 14.1 Introduction

Spices, dry soups and Asian flavorings consist of variety of products with regard to their raw materials and types of processing. This category consists of (1) dry spices and herbs, (2) dry spice blends and seasonings (3) dry soup and gravy mixes, (4) soy sauce and (5) fish or shrimp sauces and paste. Dry spices and herbs may be produced by drying raw spices with or without kill steps such as irradiation, steaming etc. Spice blends or seasonings, produced with or without kill steps, are mixtures of dry spices with or without a carrier (salt, dextrose, maltodextrin or gum arabic) or a mixture of carriers with oleoresin or essential oils of spices. Dry soup and gravy are mixtures of dry seasonings with dried meats, poultry, seafood, vegetables, flour, starches or thickener, eggs, sugars, etc. Soy sauce is a seasoning made of soy, which undergoes mold and salt fermentation. Fish sauce and paste are obtained from fish hydrolysis by enzymes and microorganisms at high salt concentrations. These products are commonly applied as seasonings and condiments in Asian dishes.

Details on the different processing steps applied to the manufacture of these products and their impact on the microbiota of the final product have been described (ICMSF 2005). The number of spore forming bacteria in spices is especially important when the products are to be used as ingredients in thermally processed foods. Fresh herbs and fresh-frozen herbs have microbial ecology and processing similar to vegetables and they are addressed in Chap. 12.

## 14.2 Dry Spices and Herbs

This group consists of variety of dry products that may be used as ingredient by other manufacturers or used directly by consumers. Of the many types available, dry pepper is the most traded spice in the world, and accounts for 20% of the spice market (UNIDO and FAO 2005). Dry spices includes rhizomes (e.g., ginger), bark (e.g., cinnamon, cassia), leaves (e.g., basil) and seeds (e.g., nutmeg). Dried product processing generally involves cleaning, sorting, sometimes soaking, slicing or pulverizing, drying and on occasion grinding. Drying may be carried out by cabinet (tray) drier or under the sun for several days. When spices are sun-dried at small farms, it is important for manufacturers to build food safety practices to minimize contamination. Some dry spices are also treated after grinding to inactivate the non-sporeformers, either by gas treatment, irradiation or steaming. With increasing health concern on ethylene oxide, the last two types of processing have become technologies of choice for reducing the microorganisms in spices.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_14,

## *14.2.1 Significant Organisms*

### 14.2.1.1 Hazards and Controls

Spore forming bacteria, including pathogens such as *Bacillus cereus, Clostridium perfringens*, and *Clostridium botulinum*, as well as non-sporeforming vegetative cells such as *Escherichia coli* and Enterobacteriaceae can be found in dry spices or herbs (ICMSF 2005). *C. botulinum* has been reported as the causative agent for outbreaks linked to spices such as garlic in oil and mustard (ICMSF 2005). However, *B. cereus* or *C. perfringens* outbreaks associated with spices have not been reported. Although the above pathogens survive drying; due to the low $a_w$ and inhibitory characteristics of spices, spore germination in spices may not occur easily.

Presence of thermophilic spore forming bacteria in spices can be a problem when spices are used in canning process. An average of $9.2 \times 10^3$ CFU/g of thermophillic spore forming bacteria in black pepper has been reported (Richmond and Fields 1966), and several thermophillic spoilage *Bacillus* were also isolated from others spices such as turmeric, onion powder, garlic powder and mustard. These bacteria have been reported to cause flat sour spoilage in canned soup. However, they are not a problem in products that do not support growth.

*Salmonella* has been found in various spices (Guarino 1972; Satchell et al. 1989) and was the causative agent in outbreaks associated with paprika-powdered potato chips (Lechmaker et al. 1995), fresh cilantro (Campbell et al. 2001), etc. A multistate outbreak due to *S*. Montevideo associated with the use of contaminated red and black pepper in Italian-style-sausage was also reported (CDC 2010). Paprika was the most frequent spice recalled by the US FDA due to *Salmonella* contamination during 1970–2003 (Vij et al. 2006). Drying may reduce numbers but cannot eliminate vegetative pathogens. Eighteen strains of *Salmonella* were reported to survive drying in a disk model with pH of 4.0–9.0. Some strains survived for 22–24 months in the model (Hiramatsu et al. 2005). Gas treatment, irradiation, and heat may be used as a control step for some but not all products, depending on quality attributes and regulatory requirements (ICMSF 2005). *Salmonella* will also survive in many of these products if recontamination occurs.

Growth of mold prior to and after drying may result in mycotoxin production. Various spices have been reported to contain low concentrations of aflatoxin, with nutmeg and red pepper as the most sensitive (ICMSF 2005). Romagnoli et al. (2007) reported that 7% of 28 spice samples collected from Italian markets contained between 5–27 μg/kg aflatoxin B1, while none of 28 herb and 48 herb infusions contained aflatoxin. Proper drying and storage to achieve a water activity below 0.6 is adequate to prevent mycotoxin production (Muggeridge and Clay 2001).

### 14.2.1.2 Spoilage and Controls

There is little evidence of spoilage of dry spices, herbs or seasonings because of the low water activity of these products. However, inappropriate handling of raw materials may support the growth of several spoilage molds prior to drying. Banerjee and Sarkar (2002) reported that 97% of 27 types of retail spice in India contains mold. The drying may contribute to the reduction of the initial load of mold, but may leave spore forming bacteria capable of causing spoilage. Proper storage of raw material and the final products are critical to maintain low $a_W$.

## *14.2.2 Microbial Data*

Table 14.1 summarizes useful testing for dried spices. Refer to the text for important details related to specific recommendations.

**Table 14.1** Testing of dry spices for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | Herbs and spices should be grown using good agricultural practices. |
| In-process | Low–medium | Monitor time–temperature drying<br>Monitoring of Enterobacteriaceae and salmonellae to verify process control may be useful when a kill step is used in the process. Typical levels encountered when a kill step is used:<br>• Enterobacteriaceae – 10–$10^2$ CFU/g<br>• *Salmonella* – absent |
| Processing environment | Low | Routine testing of the processing environment is not recommended for processes without a kill step; however maintaining hygiene is essential |
| | Medium | Periodic testing on processing environment can be useful to verify the adequacy of cleaning and sanitation when a kill step is used to reduce the potential for recontamination. Typical levels encountered:<br>• *Salmonella* – absent |
| Shelf life | – | Not applicable |
| End product | Medium | Routine testing for pathogen is not recommended. However, where there is a question as to the conditions of manufacture, sources of ingredients, or in the event of a public health issues, the following is recommended |

| Product | Microorganism | Analytical method[a] | Case | Sampling plan and limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| | | | | *n* | *c* | *m* | *M* |
| Dry spices for direct consumption | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to Appendix A for performance of these sampling plans
[c]Individual 25 g analytical units (see Sect. 7.5.2 of Chap. 7 for compositing)

#### 14.2.2.1 Critical Ingredients

Dry spices and herbs are sold individually, blended or mixed with salts. Spices can be critical ingredients in other products, especially when no kill step is applied in the production of dry spices. The Codex Alimentarius Commission (1995) outlined Good Agricultural Practices for producing these raw materials.

#### 14.2.2.2 In-Process

Monitoring time and temperature of drying can be done to achieve low moisture content of dry spices. For dry pepper, for example, the desired moisture content is 8–10%.

#### 14.2.2.3 Processing Environment

Dried spices and herbs generally have a dry processing environment. Hygiene monitoring of the environment is desirable when kill steps are employed to prevent recontamination. Environment samplings for *Salmonella*, for example, can be useful as a caution of the possibility of recontamination. Evaluation of grinders for the presence of condensation is important since the presence of condensation may support the growth of spoilage or potentially pathogenic bacteria. *Salmonella* should be absent in all samples analyzed. Details on the establishment of environmental sampling programs are provided in ICMSF (2005) and in Chap. 4.

#### 14.2.2.4 Shelf Life

Microbiological shelf life testing is not applicable for these products.

#### 14.2.2.5 End Product

ICMSF (1986) considered spices as raw material. Therefore, sampling plans and appropriate microbiological criteria depends on the intended use of the product. When dried herbs and spices are to be consumed without a kill step, the absence of *Salmonella* is essential in 25 g samples (Codex Alimentarius 1995). Because of the natural inhibitory substances present in some spices, specific sample preparation may be needed. Andrews and Hammack (2009) recommended different sample preparation for three different groups of spices; i.e., (1) allspice, cinnamon, cloves, and oregano; (2) onion flakes, onion powder, and garlic flakes; and (3) black pepper, white pepper, celery seed or flakes, chili powder, cumin, paprika, parsley flakes, rosemary, sesame seed, thyme, and vegetable flakes.

When spices are used as ingredients in thermally-processed food, the number of thermophilic aerobic spore forming bacteria should to be evaluated. Microbial criteria for starches and sugars as recommended by the National Canners Association (NCA 1968) may be suitable for the purpose, and typically the concentration of heat resistant thermophilic spores in ingredients should be less than $10^2$ CFU/g. Table 14.1 suggests the relative importance of testing for these products.

## 14.3 Dry Spice Blends and Vegetable Seasonings

Dry spice blends and vegetable seasoning may be made by mixing several spices with or without a carrier (gum, rusk, starch, etc.) or mixing a carrier with oleoresin or essential oil through dry mixing. A kill step may or may not be applied after mixing. Examples of the products include meat seasonings, Italian seasonings, etc.

### *14.3.1 Significant Organisms*

#### 14.3.1.1 Hazards and Controls

*Salmonella* is the hazard of concern, although spore forming pathogens such as *B. cereus, C. perfringens* and *C. botulinum* can be found. Hazards found in the product mainly originate from the raw materials; i.e., dry spices as described above and carriers which are addressed in Chap. 15. For *Salmonella* control the reader is also referred to a guideline for *Salmonella* control in low moisture foods (GMA 2009).

#### 14.3.1.2 Spoilage and Controls

Microbial spoilage of dry spice blend or seasonings is not an issue due to the low water activity. However, inappropriate handling of raw materials may support the growth of several spoilage molds. Proper storage of raw materials and the final product is critical to maintain low $a_w$.

### *14.3.2 Microbial Data*

Table 14.2 summarizes useful testing for dried spice blends and vegetable seasonings. Refer to the text for important details related to specific recommendations.

**Table 14.2** Testing of dry spice blends and vegetable seasoning for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low–medium | When history of raw material is not known and no kill step will be applied after mixing, testing for *Salmonella* can be useful depending on intended use | | | | | | | |
| In-process | High | Testing to provide additional information in addition to material testing, relevance depends on intended use and recipe, is useful when condensation is likely to occur. Typical levels encountered:<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g<br>• *Salmonella* – absent | | | | | | | |
| Processing environment | Low–medium | Testing for hygiene indicators and *Salmonella* for product for direct consumption can be useful. Typical levels encountered:<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g or sample<br>• *Salmonella* – absent | | | | | | | |
| Shelf life | – | Not relevant | | | | | | | |
| End product | Medium | When history of product or supplier is not known, the following testing is recommended: | | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Dry spice blend and vegetable seasoning for direct consumption | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 of Chap. 7 for compositing)

#### 14.3.2.1 Critical Ingredients

Dry spice blends and seasonings are made of dry spices whose quality and safety depend on whether or not treatments (kill steps) have been applied prior to mixing. Testing for a hazard may be relevant when history of the raw material is not known or upon intended use of the products although this is commonly done due to specifications and customer demands. When products are to be consumed directly, and no kill step is applied after mixing, testing of *Salmonella* in ingredient is desirable.

#### 14.3.2.2 In-Process

Testing of in-process samples can provide information in addition to raw material testing. Depending on the intended use, testing of *Salmonella* can be useful.

#### 14.3.2.3 Processing Environment

Hygiene monitoring of the processing environment is useful to prevent recontamination especially when products are intended to be used directly for consumption.

#### 14.3.2.4 Shelf Life

Microbiological shelf life testing is not applicable for these products.

#### 14.3.2.5 End Product

When products are intended to be used for direct consumption, testing for *Salmonella* is recommended especially when history of product is unknown (Table 14.2).

## 14.4 Dry Soups and Gravy

Dry soup and gravy, including bouillon and consommé are processed by mixing dry seasonings with fat, dried meats, poultry, seafood, vegetables, flour, starches or other thickeners, eggs, sugars, etc. The dry seasonings were obtained as above, while other ingredients were also subjected to various drying (oven, vacuum oven, spray drying, freeze drying), agglomeration, milling, or fat coating prior to mixing. The products, powder or paste with low $a_W$ (0.1–0.35), may or may not need to be cooked prior to consumption.

### *14.4.1 Significant Organisms*

#### 14.4.1.1 Hazards and Controls

In addition to the hazards present in the spices, which was discussed above, potential pathogens present in the products depend on the other ingredients used. Hazard associated with each ingredient is discussed in Chaps. 8, 9, 10, 15, 18, 19, 22. Properly dried ingredients have low $a_W$ that is not favorable for pathogen growth. However, pathogen survival is possible and *Salmonella* is of the greatest concern.

Since no kill steps occur in the production of dry soup and gravy, raw materials are critical in determining the quality and safety of the end product. It is also important to prevent post-processing contamination through good GHP. For *Salmonella* control the reader is also referred to a guideline for *Salmonella* control in low moisture foods (GMA 2009).

#### 14.4.1.2 Spoilage and Controls

Spoilage of dry soup and gravy is uncommon due to the low $a_W$. In high humidity environments, the product may become damp and risk of mold contamination exists. In this case, impermeable packaging and proper storage are important.

### *14.4.2 Microbial Data*

Table 14.3 summarizes useful testing for dried soups and gravy. Refer to the text for important details related to specific recommendations.

#### 14.4.2.1 Critical Ingredients

Dried meat, poultry, seafood, egg, or flour added to spices can be critical ingredients, especially when the drying process is poorly controlled. A supplier quality assurance program is necessary to assure absence of pathogens, such as *Salmonella*, and mycotoxins. This is of particular importance when dry soup or gravy is not to be cooked prior to consumption.

**Table 14.3** Testing of dry soups and gravy for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low–high | Testing of *Salmonella* would apply for raw material without kill step | | | | | | |
| In-process | Low | The straight forward process generally does not benefit from in-process testing | | | | | | |
| Processing environment | Low | Test for *Salmonella* and Enterobacteriaceae. Typical guidance levels:<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g or sample<br>• *Salmonella* – absent | | | | | | |
| Shelf life | – | Not applicable | | | | | | |
| End product | Low | Test for indicators for on-going process control and trend analysis : | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Dry soups and gravy | *Salmonella* | ISO 6579 | 10[c] | 5[d] | 0 | 0 | – |
| | | | | | 11 | 10[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Intended to be boiled thoroughly
[d] Individual 25 g analytical units (see Sect. 7.5.2 of Chap. 7 for compositing)

#### 14.4.2.2 In-Process

Because production of dry soup and gravy is a straight forward process, involving mixing and packaging, evaluation of intermediate products is not relevant.

#### 14.4.2.3 Processing Environment

The processing environment is very important to assure that mixing and packaging are conducted such that recontamination is minimized. Sampling of environment is done to evaluate presence of Enterobacteriaceae and *Salmonella*. It is reasonable to target a level of $10^2$–$10^3$ CFU Enterobacteriaceae/g or sample and *Salmonella* should be absent.

#### 14.4.2.4 Shelf Life

Evaluation of microbiological quality for shelf life is not applicable.

#### 14.4.2.5 End Product

Dry soups and gravy have low moisture content (<7%) and $a_W$ (0.1–0.35) that render the product shelf-stable. They may be consumed with or without cooking. Table 14.3 suggests the relative importance of testing to be carried out for these products.

## 14.5 Soy Sauce

Soy sauce is a fermented soy seasoning commonly produced in Eastern and Southeast Asian countries although it can be found worldwide. ICMSF (2005) summarized the types and processing steps involved in soy sauce production. Industrial soy sauce production as practiced in Japan (shoyu)

includes mixing cooked soybean with roasted wheat; fermentation using *Aspergillus oryzae* or *Aspergillus sojae* to yield koji, koji fermentation in brine (*moromi*), which includes addition of lactic acid bacteria (mainly *Pediococcus halophilus*) and yeast (*Zygosaccharomyces rouxii*); pressing *moromi* to yield raw soy sauce; pasteurization and bottling. Traditional production uses mold cultures from previous batches without addition of *Pediococcus* or yeast. Blended soy sauce may be made by blending soy sauce with hydrolyzed vegetable proteins or chemically hydrolyzed soy. Soy sauce has a low pH (4.0–6.1, depending on the types) and high salt content. The salt content varies from 16–18% (Japanese shoyu) or 10–23% for most others. An exception is Indonesian soy sauce, which has only 6–7% salt but also contains 40% sugar (National Standard Agency of Indonesia 1999).

## *14.5.1 Significant Organisms*

### 14.5.1.1 Hazards and Controls

There is no report of foodborne illnesses due to consumption of soy sauce. During soy sauce production, heat treatment of raw materials prior to *koji* fermentation and pasteurization of raw soy sauce eliminate most of the non-spore forming pathogenic bacteria. Artificially inoculated *C. botulinum* types A and B survive in *shoyu* but do not grow at 30°C for 3 months (Steinkraus et al. 1983).

*A. sojae* and *A. oryzae* have a safe history for use in soy production. The high salt content and low pH of the product contribute to inhibition of pathogen growth. However, precaution must be made for soy sauce containing low salt (<10%). Maintaining hygienic conditions is important to prevent contamination from environment and raw materials, which will influence fermentation process.

### 14.5.1.2 Spoilage and Controls

Spoilage has to be controlled during processing of soy sauce. Soaking water needs to be changed every 2–3 h to avoid excessive number of spore forming *Bacillus* (Beuchat 1984). The presence of contaminants may result in failure of the fermentation process, leading to unacceptable product quality. Controlled temperature-time cooking and a maximum moisture content of 62% for steamed soybean are crucial to prevent spoilage. Post pasteurization recontamination may occur, especially by mold and yeast. Application of para-hydroxy benzoate or sorbate up to 1,000 mg/kg (Codex Alimentarius 2010) is commonly used to reduce mold spoilage. In Indonesian sweet soy sauce, addition of palm sugar to raw soy sauce prior to heating decreases the need for this preservative.

## *14.5.2 Microbial Data*

Table 14.4 summarizes useful testing for soy sauce. Refer to the text for important details related to specific recommendations.

### 14.5.2.1 Critical Ingredients

In addition to soybeans, wheat flour or crushed wheat, water, salt and mold inoculum are ingredients during soy sauce production. Soybeans and wheat flour generally contain fungi, which will be readily inactivated during cooking. Salt concentration is critical for preventing the growth of undesired microorganisms such as bacilli.

**Table 14.4** Testing of soy sauce for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Not applicable |
| In-process | Medium | Testing for yeast or osmophilic yeast, for in-process samples after pasteurization |
| Processing environment | – | Not applicable |
| Shelf life | – | Not applicable |
| End product | – | Not applicable |

#### 14.5.2.2 In-Process

In-process testing for osmophillic yeast after pasteurization is recommended to control spoilage.

#### 14.5.2.3 Processing Environment

Testing is not relevant, hygienic condition is maintained through GHP.

#### 14.5.2.4 Shelf Life

Growth of spoilage osmophilic yeast can have an undesirable impact on the sensory quality of the soy sauce, such as film or pellicle formation. However, testing of the yeast is not commonly done for shelf life.

#### 14.5.2.5 End Product

Soy sauce is used as seasonings prior to cooking or added into ready-to-eat food. With high salt content (>10%) and or high sugar content (>10%) in the case of sweet soy sauce, routine microbiological testing is not recommended (Table 14.4).

## 14.6 Fish and Shrimp Sauce and Paste

Fish and shrimp sauce and paste are seasonings or condiments commonly used in South-East Asian countries. There are various products throughout the regions but generally they are products of fish/shrimp protein autolysis by naturally occurring proteases and lactic acid bacteria in the presence of high salt concentrations. Traditionally, fish sauce is made by mixing coarse salt with raw fish at various ratios and placing the mixture in a tube for at least 6 months. The liquid is collected and filtered for further fermentation or sugar addition and may or may not be pasteurized before bottling. Fish and shrimp paste is made by mixing salt and raw fish or shrimp followed by sun-drying for 5–8 h. The partially dried fish is then minced and placed in a tube under anaerobic condition for 7 days. The paste is then minced, sun dried and placed in a tube for another anaerobic fermentation for 1 month and the processes are repeated until the desired texture and flavor are achieved (ICMSF 2005). The final salt contents of fish sauce or paste in Malaysia (*budu, belacan*), Philippine (*patis, bagoong*) and Indonesia (*bakassang, terasi*) are 13–15%, 20–25%, and 19–25%, respectively (Ijong and Ohta 1995).

## *14.6.1 Significant Organisms*

### 14.6.1.1 Hazards and Controls

Raw fish carries various hazards including pathogenic bacteria, virus, parasites, aquatic toxins and biogenic amine (ICMSF 2005). Addition of salts is the most critical step to assure the growth of lactic acid bacteria such as *Leuconostoc mesenteroides* subsp. *mesenteroides* or *Lactobacillus plantarum*. Salt concentration is also important when no kill step is applied, thus reducing salt concentration has to be conducted cautiously. Amano (1962) reported *C. botulinum* type E poisoning linked to reduced salt fermented fish product. The introduction of contaminants from flies during sun drying is also an issue and pest control should be practiced to minimize contamination.

### 14.6.1.2 Spoilage and Controls

The high salt content, and therefore low $a_w$, of these products is generally unfavorable for microbial growth. However, moderately halophilic *Bacillus* and *Staphylococcus* (Mabesa et al. 1986) and extremely halophilic strains of *Halobacterium salinarum* have been linked to spoilage of these products. Appropriate formula, salt content and fermentation process can control this.

## *14.6.2 Microbial Data*

Table 14.5 summarizes useful testing for fish and shrimp sauces and paste. Refer to the text for important details related to specific recommendations.

### 14.6.2.1 Critical Ingredients

The quality of fish as the main ingredient is important to yield quality products. Spoiled fish, especially those of Clupeidae, Scombridae, Scombresocidae, Pomatomidae and Coryphaenedae families should not be used because of the possible generation of high histamine content in the end product (see Sect. 14.6.2.5). Salt quality and concentration is critical for lactic acid fermentation to occur. Although the concentration may vary for different producers, the added salt has to be set such that the salt content of the end product inhibits pathogens as well as unwanted spoilage microorganisms.

### 14.6.2.2 In-Process

In-process samples may not be relevant because they are not related to the quality and safety of the products.

**Table 14.5** Testing of fish sauce and paste for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Visual examination for fish quality and testing for histamine are recommended |
| In-process | – | Not applicable |
| Processing environment | – | Not relevant |
| End product | Medium | Testing for histamine may be relevant (see text) |

#### 14.6.2.3 Processing Environment

Periodic testing for hygiene indicator such as Enterobacteraceae, coliforms and mold/yeast in the environment can be useful to evaluate compliance with GHP. Contamination during processing may lead to undesired microorganisms that cause fermentation to fail.

#### 14.6.2.4 Shelf Life

Microbiological shelf life testing is not relevant for these shelf-stable products.

#### 14.6.2.5 End Product

Fish sauce and fish paste is a shelf-stable product with risk of mold contamination when they are not properly packaged. Routine testing for microorganisms is not recommended for end product. If application of GHP and HACCP is in question, sampling for histamine may be considered for lot acceptance of product produced from scombroid species. Consistent with recommendations in Chap. 10, the product should not contain more than 20 mg of histamine per 100 mL using the methods of Malle et al. (1996) and Duflos et al. (1999).

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# Chapter 15
# Cereals and Cereal Products

## 15.1 Introduction

Cereals and cereal products, such as grains, flours, grits and meal, are basic source of human nutrition. Since they are the principal part of the diet for a large proportion of people in the world, their safety and quality for human consumption is a significant concern for both producers and regulatory bodies.

This chapter covers the major grains and flours and food products made from them. Major grains include wheat, rice, maize, barley, oats, rye, millet and sorghum. Corn and potato flour products such as bread, rolls and tacos and manioc flour products such as pão de queijo (Brazilian cheese rolls) are also covered in this chapter. Minor products are not covered in this chapter.

This chapter categorizes cereal products into seven groups according to processing characteristics, ingredients and storage forms. A detailed description and example foods are provided in each subchapter for the following groups:

- Dried, raw grains (rice, wheat, maize, oats, etc.), and their flour and flour-based mixes, which are stored, transported, traded and intended to be cooked.
- Raw dough products, which may be frozen or refrigerated.
- Dried cereal products including breakfast cereals, snack foods and rice cakes, which have long shelf life.
- Breads made of flour of various grains and tubers, heated at high temperatures after fermentation of dough, in many cases, including yeast.
- Pasta and noodles that may include eggs and other ingredients.
- Cooked grains such as rice, wheat and oats that are consumed fresh and moist.

Pastries and topped or filled products including bakery products and dumplings with various types of ingredients are addressed in Chap. 26. The microbial ecology and control measures for cereals and cereal products were previously described in detail (ICMSF 2005).

## 15.2 Dried, Raw Grains and Their Flour and Flour-Based Mixes

Many crops are cultivated and consumed in the world such as rice, wheat, maize, oats, barley, rye, millet, sorghum and others. Temperature and rainfall influence the grains grown and thus the dietary culture in a region. After the crops are harvested and dried, some grains are stored, shipped and internationally traded as raw grains. Others are milled to flour and, by adding other dry ingredients such as sugar, salt, baking soda, shortening, are converted to flour-based dry mixes.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_15,

## *15.2.1 Significant Organisms*

### 15.2.1.1 Hazards and Controls

When grains are harvested in good condition, dried rapidly to a moisture level that prevents microbial growth and stored under conditions that prevent excessive ingress of water, they present few microbiological risks. However, contamination with mycotoxigenic fungi and pathogenic bacteria may occur under unfavorable conditions. The microbial ecology and distribution of toxigenic fungi and mycotoxins, such as aflatoxins, fumonisins, nivelanol, deoxynivalenol (DON) and other trichothecenes, as well as salmonellae were previously described (ICMSF 2005). Flour and flour-based mixes produced from contaminated grain contain the same contaminants.

Under certain conditions, toxigenic fungi may invade grains before or after they are harvested and then produce toxins in the grains. The mold found is greatly influenced by climatic conditions. Once produced, mycotoxins are not completely reduced by processing and cooking procedures used for cereals, therefore they have the potential to be one of the most prevalent health problems in the world if not controlled.

The recommended control measures to prevent fungal growth in the crop are to harvest grains from areas with minimal crop stress, to visually check for fungal growth and insect infestation and to dry crops rapidly to a safe moisture content. Maintenance of low moisture during storage and transportation is also required to avoid acute temperature changes that can cause condensation. Pest control practices are needed to prevent contamination and reduce the potential for mycotoxin production in the raw grains and their flour. Additional control measures, such as fumigation, sealed storage and atmosphere control may also be used. Testing grains for mycotoxins, is appropriate especially in crops that are subjected to stress conditions during growth and harvest.

The Codex Alimentarius Commission (2003) adopted a code of practice for prevention and reduction of mycotoxin contamination in cereals, including ochratoxin A, zearalenone, fumonisins and trichotecenes. Integrated control programs incorporate HACCP principles to manage risks associated with mycotoxin contamination of foods (FAO 1999). The implementation of HACCP principles minimizes mycotoxin contamination through application of preventive controls in the production, handling, storage and processing of each cereal crop.

Salmonellae may occasionally contaminate grains and flour (Sperber and NAMA 2007). If uneven distribution of moisture in the products results in wet spots, salmonellae can grow. Storage of grains and flour under conditions that prevent mold growth will also control growth of salmonellae because molds can grow at a much lower water activity than salmonellae. Salmonellae are capable of surviving in dry flour for many months (Dack 1961). Storage of grains and flours at elevated temperatures under dry conditions has been shown to reduce the microbial population to varying degrees depending on the commodity, temperature and moisture level (van Cauwenberge et al. 1981). Storage at elevated temperatures has been used commercially to destroy salmonellae in bulk quantities of dry products. Pest control programs are appropriate for grain and flour storage to prevent contamination with salmonellae.

The milling process for grains may reduce the load of microorganisms by removing debris and the husk strips but the reduction is not very large. Washing and bleaching of grains prior to milling may contribute to microbial contamination if not controlled.

It is important to use dry cleaning methods for equipment in dry milling and processing environments for flours and dry mixes to prevent establishment of harborage sites. Water used in wet cleaning may support growth of enteric pathogens in cracks and crevices that collect water but are difficult to clean, thus wet cleaning is not recommended. Testing of grain and flour processing environments for *Salmonella* is appropriate to detect potential harborage sites (ICMSF 2005).

#### 15.2.1.2 Spoilage and Controls

Certain fungi and bacteria are pathogenic to plants and cause crop diseases, leading to spoilage of the harvested grains. Fungal growth may cause not only direct damage, but also physical (by spontaneous heating) or chemical (by enzyme or fatty acid production) damage in the grains. Deterioration of flour may be caused by inappropriate harvesting, processing and storage conditions; temperature abuse; and moisture control failure. Measures for controlling fungal and bacterial hazards are also recommended to control spoilage (ICMSF 2005).

## *15.2.2 Microbial Data*

Table 15.1 summarizes useful testing for raw dried grains, flour and flour based mixes. Refer to the text for important details related to specific recommendations.

#### 15.2.2.1 Critical Ingredients

Raw grain is a critical ingredient for the production of flour and dry mixes. Raw grains should be adequately screened for mycotoxins, such as aflatoxins and fumonisins in maize, DON and nivalenol

**Table 15.1** Testing of dried, raw grains and their flour and flour-based mixes for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | High | Visual tests for fungal growth, insect infestation and wet spots. Test grains for appropriate mycotoxins prior to milling<br>• Aflatoxins and fumonisins in maize<br>• Deoxinivalenol and nivalenol in wheat<br>• Ochratoxin A in barley and rye | | | | | | | |
| In-process | Medium | Moisture content in grains should not be higher than: 13% for rice, 11% for wheat, maize and barley and 10% for oats (ICMSF 2005)<br>Test product residues from product contact surfaces for *Salmonella* during normal operation to verify control of the process. Typical guidance levels<br>• *Salmonella* – absent | | | | | | | |
| Processing environment | High | Test environment for *Salmonella* in relevant areas during normal operation to verify control of the process. Typical guidance levels<br>• *Salmonella* – absent | | | | | | | |
| Shelf life | – | Not relevant | | | | | | | |
| End product | Medium | Test end products for appropriate mycotoxins, depending on grain and seasonal concerns<br>Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a possible safety issue, testing is recommended for *Salmonella* | | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Low | Flour and dry mixes | *Salmonella* | ISO 6579 | 10 | 5[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

in wheat, and ochratoxin A in barley and rye, as appropriate, prior to converting to flour. DON presence may be controlled by monitoring crops in the field and by enforcing grain test weight requirements at elevators. This is an example of how Good Agricultural Practices can be used to control mycotoxins rather than testing. Other mycotoxins such as zearalenone, Toxin T-2 and alternariol should also be monitored in grains from certain regions as previously described (ICMSF 2005). The Codex Alimentarius Commission adopted a maximum level of 5 μg/kg for ochratoxin A in raw wheat, barley and rye (Codex Alimentarius 2008). There is no Codex Alimentarius Commission recommendation for other mycotoxins in cereals but different countries have adopted their own limits.

Ingredients in dry mixes such as sugar, salt, baking soda and shortening, are not a serious concern for human health compared to the raw grains and flour. Powdered egg or dry milk ingredients may present a *Salmonella* risk, therefore testing for *Salmonella* may be useful, especially when there is no knowledge of supplier controls. Refer to the appropriate chapters for further guidance.

#### 15.2.2.2 In-Process

In-process testing of grains, flour and dry mixes for mycotoxins is not recommended because the microbial hazard concentrations are not likely to change substantially during processing. However, it is useful to periodically test in-process samples for salmonellae, which should be absent (GMA 2009). As mentioned previously, exposure to water can create a microenvironment that is favorable to growth of salmonellae. Moisture in flour frequently causes lumps that collect on sifter screens, therefore, sifter tailings provide a useful sampling location. Line residues may also provide useful samples in some systems because they represent product produced through an extended period of time.

#### 15.2.2.3 Processing Environment

During storage and transportation of the grains and flours, moisture control is very important because fungal growth and mycotoxin production may take place if the moisture level rises above 12% (ICMSF 2005). Temperature fluctuation may cause condensation, which can lead to wet spots in the grains and the flours and growth of fungi present in harvested grains.

Salmonellae are of concern due to their persistence in dry conditions (Richter et al. 1993). *Salmonella* in the processing environment and equipment may contribute to product contamination. Environmental testing for salmonellae is useful to identify harborage sites (GMA 2009).

#### 15.2.2.4 Shelf Life

Microbiological shelf life testing is not relevant for cereal grains, flours and dry mixes because the low $a_w$ prohibits multiplication.

#### 15.2.2.5 End Product

Mycotoxins are a primary concern in raw grains, therefore routine tests for appropriate toxins are recommended. Rapid screening tests, such as enzyme linked immunosorbent assay (ELISA) and fluorometry for aflatoxins and ochratoxin A, can provide a good indication of the contamination level. However, further analysis of positive samples should be carried out using appropriated methodologies (Scott 1995; Barug et al. 2006).

As long as results from environmental and in-process sampling confirm the absence of *Salmonella*, testing of end products can be considered for only for periodic verification. However, presence of the pathogen in environmental samples should trigger investigative sampling to identify the causes. This investigation may be complemented with sampling of finished product. These products need to be cooked before consumption, therefore case 10 is applicable. Table 15.1 also summarizes recommended testing for other stages of this product category.

## 15.3 Raw, Frozen and Refrigerated Dough Products

Raw dough is an intermediate product for bread, cookie, pasta and cereal production, which involves mixing of flour, leavening agents and other ingredients that may include dairy products, eggs, sweeteners, nuts, chocolate, etc. depending on the final product. Doughs may be prepared and distributed prior to baking in refrigerated or frozen form. These products are typically intended to be cooked by baking or steaming in retail outlets, restaurants and homes. Some dough products are used as an ingredient in other foods, such as ice cream.

### *15.3.1 Significant Organisms*

#### 15.3.1.1 Hazards and Controls

The heat treatments used to fully cook dough products (baking or steaming) reach temperatures that are sufficient to destroy vegetative bacteria. Commercially distributed dough products are typically intended to be cooked. However, ready-to-eat applications, such as cookie dough for ice cream, require special considerations because salmonellae may occasionally contaminate grains and flour. Dough that is incorporated into ready-to-eat products should be prepared using ingredients, including flour, that have been treated to destroy vegetative pathogens. Storage at elevated temperatures has been used commercially to destroy salmonellae in bulk quantities of dry products. However, the heat treatment used may be detrimental to the functional properties of the flour for making traditional baked products, thus this type of treatment may not be appropriate for those products.

#### 15.3.1.2 Spoilage and Controls

Frozen dough products are not subject to microbial spoilage. Refrigerated doughs and other raw pastry products may sour as a result of growth of lactic acid bacteria present in cereal components. Such microorganisms occur in flours used for making doughs and may grow to high numbers on the dough-making equipment. However, the potential for souring depends on the formulation and storage conditions, and the numbers of lactic acid bacteria that can be tolerated in particular products can be determined only by practical tests. Problems are avoided by strict attention to sanitary design and process hygiene.

### *15.3.2 Microbial Data*

Table 15.2 summarizes useful testing for raw frozen and refrigerated dough products. Refer to the text for important details related to specific recommendations.

**Table 15.2** Testing of frozen and refrigerated dough products for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Medium | Test for mycotoxins if confidence in flour or raw grains is low<br>Test sensitive ingredients for *Salmonella* if confidence in supplier is low | | | | | | | |
| In-process | Low–medium | In-process testing depends on the product. See text | | | | | | | |
| Processing environment | High | Test for Salmonella in the processing plant environment. Typical guidance levels<br>• *Salmonella* – absent | | | | | | | |
| Shelf life | – | Not relevant for frozen product. May be relevant for refrigerated product depending on the formulation. See text | | | | | | | |
| End product | | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a possible safety issue, test for *Salmonella* | | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Raw ready-to-cook dough products | *Salmonella* | ISO 6579 | 10 | 5[c] | 0 | 0 | – |
| | Low | Raw ready-to-eat dough products | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 15.3.2.1 Critical Ingredients

Testing of flour for products that are intended to be ready-to-eat is not a reliable control method. Ingredients in raw dough products such as sugar, salt, baking soda and shortening are not a serious concern for human health compared to the raw grains and flour. Powdered egg or dry milk ingredients may present a *Salmonella* risk, therefore testing for *Salmonella* may be useful, especially when there is no knowledge of supplier controls. Refer to the appropriate chapters for further guidance.

Mycotoxins must be controlled at the ingredient level (see Sect. 15.2.1.1).

#### 15.3.2.2 In-Process

In-process testing is of limited use for frozen dough products. For raw dough products, line residues represent a useful sample to verify hygienic control. Microbiological methods have been proposed for lactic acid bacteria in refrigerated dough (Hesseltine et al. 1969); however, the relevant test depends on the product formula and potential for spoilage. Periodic sampling of line residues for *Salmonella* is useful to verify that raw dough products will not become contaminated from the environment.

#### 15.3.2.3 Processing Environment

The processing environment can provide harborage sites for *Salmonella*, which may contaminate in-process materials. Monitoring of the processing environment for *Salmonella* is recommended.

#### 15.3.2.4 Shelf Life

Shelf life testing is not relevant to frozen dough products. It may be considered for refrigerated dough products that are subject to microbial spoilage. Many of these are packaged under pressure generated by carbon dioxide; thus mold is not a problem. However, growth of lactic acid bacteria can lead to excessive gas and spoilage. Specific testing methods should be developed for the product and anticipated distribution conditions encountered.

#### 15.3.2.5 End Product

As long as results from environmental and in-process sampling confirm the absence of *Salmonella*, testing of end products can be considered only for periodic verification. However, presence of the pathogen in environmental samples should trigger investigative sampling to identify the causes. This investigation may be complemented with sampling of finished product. These products need to be cooked before consumption, therefore case 10 is applicable. Table 15.2 also summarizes recommended testing for other stages of this product category.

## 15.4 Dried Cereal Products

Dried cereal products include breakfast cereals, oatmeal, snack foods, rice cakes and infant cereals. Infant cereals are covered in Chap. 25. Dried products are made from grains that are heated during flaking and puffing, or are made from flour that is heated during extrusion after adding water. Dried cereal products are typically ready to eat (RTE) without further cooking, but some may be heated with added milk or hot water. Other ingredients such as sugar, salt, spices, vitamins, flavors, and dried fruits and nuts may be added to produce the final product.

### *15.4.1 Significant Organisms*

#### 15.4.1.1 Hazards and Controls

When good hygienic practices are in place, there are no major hazards. However, *Salmonella* outbreaks have been associated with dried cereal products due to environmental or ingredient contamination. For example, two *Salmonella* Agona outbreaks were associated with breakfast cereal produced at the same manufacturing facility. Investigations revealed that a processing line in a cereal plant was the point of contamination (CDC 1998, 2008).

Mycotoxins must be controlled at the ingredient level (see Sect. 15.2.1.1).

#### 15.4.1.2 Spoilage and Controls

Due to the low water activity, usually there are no microbial spoilage concerns.

### *15.4.2 Microbial Data*

Table 15.3 summarizes useful testing for dried cereal products. Refer to the text for important details related to specific recommendations.

**Table 15.3** Testing of dried cereal products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Test for mycotoxins if confidence in flour or raw grains is low<br>Test nuts, cocoa and other sensitive ingredients not subjected to a subsequent kill step for *Salmonella* if confidence in supplier is low |
| In-process | High | Test appropriate product residues and in-line samples for *Salmonella*. Typical guidance levels<br>• *Salmonella* – absent |
| Processing environment | High | Test for *Salmonella* and Enterobacteriaceae in the processing plant environment. Typical guidance levels<br>• Enterobacteriaceae – $10^2$–$10^3$ CFU/g<br>• *Salmonella* – absent |
| Shelf life | – | Not relevant |
| End product | High | Testing for Enterobacteriaceae is recommended to verify process control |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Dried cereals | Enterobacteriaceae | ISO 21528-2 | 2 | 5 | 2 | 10 | $10^2$ |

| | | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a possible safety issue, testing for *Salmonella* recommended |
|---|---|---|

| | | | | Sampling plan and limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Dried cereals | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 15.4.2.1 Critical Ingredients

Mycotoxins in raw grains survive the processing, therefore tests for mycotoxins are applicable if not controlled by the supplier. Testing other major ingredients such as dried fruits and nuts may be appropriate. Since most dried cereal products are ready-to-eat, nuts, cocoa and other ingredients with a history of *Salmonella* contamination should be tested if not controlled by the supplier.

#### 15.4.2.2 In-Process

Outbreaks associated with dried cereal products demonstrate the utility of periodic in-process testing (e.g., line residues) for *Salmonella*, which should be absent.

#### 15.4.2.3 Processing Environment

The processing environment can provide harborage sites for *Salmonella*, which may contaminate in-process foods. Monitoring of the processing environment for *Salmonella* is recommended.

#### 15.4.2.4 Shelf Life

Microbiological shelf life testing is not relevant for cereal grains, flours and dry mix because the low $a_w$.

#### 15.4.2.5 End Product

Proposed testing for dried cereals is described in Table 15.3. Environmental and in-process monitoring are considered to be more useful than end product testing when properly designed with the intent to identify and correct potential issues.

## 15.5 Baked Dough Products

Breads are made of flour of wheat, maize, barley, oats, rye, soy, millet, or sorghum and are heated (baked) at high temperatures. Frequently yeast fermentation of dough precedes baking. Other ingredients may include water, sugar, salt, milk and eggs. Soda crackers, sour dough bread, panettone, nan, pita (bread in Middle East), pão de queijo (Brazilian cheese rolls) and tortillas are also included in this category. The composition and processing characteristics of these breads, as well as their microbial ecology are described in the previous publication (ICMSF 2005). Temperatures needed to establish acceptable structure and texture of dough products are sufficient to inactivate vegetative cells. In addition, the baking process used by many cultures dehydrates the surface of the baked products, which prevents microbial growth on the surface. Asian cultures sometimes use a steaming process for dough products, which results in a water activity that may support growth of some pathogens on the surface.

### *15.5.1 Significant Organisms*

#### 15.5.1.1 Hazards and Controls

As mentioned in previous sections, mycotoxins may be a concern if not controlled in the grains used to produce flours. A notable exception is lime treated maize that is used for making tortillas. Though salmonellae and *Bacillus cereus* may occasionally be found in dough, they do not cause human illness once the dough is heated to properly develop bread structure.

#### 15.5.1.2 Spoilage and Controls

Mold will grow on baked bread products if stored for sufficient time. The time required for visible mold growth depends on the moisture level of the crust, the initial contamination level on the surface of the bread, preservatives that may be present in the dough and the temperature of storage. Baking destroys mold in the dough but recontamination can occur if the environment between baking and packaging is not controlled. Cooling of baked bread before packaging is recommended to avoid condensation. Maintaining dry and clean conditions in the cooling and packaging environment is critical for the keeping quality of breads.

Rope-causing bacteria (mucoid variants of *Bacillus subtilis* and *Bacillus licheniformis*) that may be present in flour are also of concern for moist breads because they can survive the baking process. Although a method exists that can be used to test for such bacteria, conducting a practical baking test may be more appropriate to determine whether a particular flour is suitable for bread manufacture by observing whether rope develops (ICMSF 1986). Rope spoilage bacteria may also become established in bakery environments as the result of poor cleaning and sanitation.

## *15.5.2 Microbial Data*

Table 15.4 summarizes useful testing for baked dough products. Refer to the text for important details related to specific recommendations.

### 15.5.2.1 Critical Ingredients

The baking process is adequate to inactivate vegetative bacteria, yeasts and molds in the ingredients; therefore, safety concerns (e.g., salmonellae) are minimal unless ingredients are added after baking (e.g., glazes, egg washes, nut toppings). As with other cereal based products, mycotoxins in grains used to produce flour should be controlled by the supplier. If product characteristics support rope formation, flours should be screened for low levels of rope spores or controlled by the supplier.

### 15.5.2.2 In-Process

In-process monitoring for baked products varies considerably, depending on the product and design of the operation. Frequently, product exposure after baking is very limited and in-process sampling may be irrelevant.

### 15.5.2.3 Processing Environment

Control of mold through air filtration and hygiene measures is essential to prevent premature mold spoilage of many baked products that have sufficiently high $a_W$ to permit mold growth after packaging.

**Table 15.4** Testing of baked dough products for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Medium | Test for mycotoxins if confidence in flour or raw grain is low<br>Test nuts, egg wash, dried dairy products and other sensitive ingredients added after baking for *Salmonella* if confidence in supplier is low | | | | | | | |
| In-process | Medium | Appropriate tests depend on the type of product and process involved. Refer to text | | | | | | | |
| Processing environment | High | Test air for mold in cooling and packaging areas for products prone to mold spoilage<br>Hygiene monitoring for equipment cleaning and sanitation procedures is relevant<br>Test for *Salmonella* in the processing plant environment as appropriate (see text). Typical guidance levels<br>• *Salmonella* – absent | | | | | | | |
| Shelf life | Medium | Testing depends on the product, formulation and intended use of the product. Refer to text for general guidance | | | | | | | |
| End product | Low | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a possible safety issue, the following sampling plans are recommended | | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Baked, RTE dough products | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

Monitoring the air through use of settling plates or an air sampler is useful to develop a history of levels that are associated with spoilage. This is especially useful in the cooling and packing area because baked breads need to cool prior to packaging to prevent formation of condensation inside the package.

Because *Salmonella* can persist on flour and in dry environments for extended periods of time, it is prudent to conduct periodic surveillance for salmonellae in the post bake environment. Bakery product manufacturing facilities should be maintained in a dry state, using dry cleaning methods for hygiene. Particular attention when sampling the environment should be given to any area with condensation, standing water and other high moisture conditions that would be favorable to the establishment and growth of *Salmonella*. For example, condensation may form at the entrance of freezer tunnels.

If rope spoilage is a concern, hygiene indicators for equipment cleanliness may be appropriate.

#### 15.5.2.4 Shelf Life

The broad range of products prohibits making general recommendations for this entire category. Spoilage of baked dough products is well documented, and therefore shelf life testing should be performed when the information is beneficial for quality and use-by date coding. For products prone to rope spoilage, shelf life testing using different batches of flour is prudent.

#### 15.5.2.5 End Product

The safety of baked bread products is well documented; therefore routine testing of these products is not recommended (ICMSF 2005). When above testing or process deviations indicate a possible safety issue, Table 15.4 provides recommended testing.

## 15.6 Unfilled Pastas and Noodles

Pastas and noodles are raw dough products made of wheat flour, semolina, buckwheat flour, rice flour or combinations of these. Other ingredients, such as eggs, may be added. Water is added and mixed until gluten is extracted and the dough can be formed into the desired shaped. The dough may be extruded, rolled, or cut into various shapes of pasta and noodles, and is usually dried at temperatures that depend on the product. The fully dried products have long shelf life at ambient temperatures. Fresh, partially dry refrigerated pasta and noodles packaged with a modified atmosphere also are commercially available. Filled pastas such as tortellini and ravioli are described in Chap. 26.

### *15.6.1 Significant Organisms*

#### 15.6.1.1 Hazards and Controls

Mycotoxins are only a concern if the flour is obtained from a supplier with no mycotoxin control program.

Among bacterial hazards, salmonellae from egg ingredients are a concern. They can survive the pasta drying process and remain viable for several months (Rayman et al. 1979). Survival of salmonellae may be a problem if noodles are not properly cooked.

The presence of egg enhances the potential for *Staphylococcus aureus* growth and enterotoxin production in pasta. Enterotoxins would persist in dried pasta and would not be destroyed in boiling water. The hazard of *S. aureus* can be controlled by cleaning product residues from mixers and

extruders and avoiding slow drying times. Pasta manufacturing equipment has narrow and complex shapes, such as mixer hubs and extruder heads, which can be difficult to clean. Daily cleaning is required to prevent residue build up and potential harborage sites. Dry cleaning methods should be used to reduce the potential for growth in inaccessible areas of equipment and the environment.

Flour is used to keep fresh pasta from sticking together or to processing equipment. Excessive build up of flour and dough on processing lines can provide sites for growth of *S. aureus*, *Salmonella* and spoilage bacteria. The extent of growth depends on the water activity of the dough, temperature of production, and other processing and formulation factors. Basic hygiene for equipment is important.

*Clostridium botulinum* may be a concern if fresh, refrigerated pastas are not formulated to prevent growth of this bacterium and are held under abusive temperatures.

#### 15.6.1.2 Spoilage and Controls

Spoilage does not occur in dry pasta and noodles. Fresh pastas may spoil due to growth of yeast, molds and bacteria if kept too long in the refrigerator or when the modified atmosphere packaging is disrupted.

### *15.6.2 Microbial Data*

Table 15.5 summarizes useful testing for unfilled pasta and noodle products. Refer to the text for important details related to specific recommendations.

#### 15.6.2.1 Critical Ingredients

Salmonellae may be present in flour and egg ingredients. Use of pasteurized egg can reduce the potential for *Salmonella* contamination.

#### 15.6.2.2 In-Process

Monitoring in-process samples for *S. aureus*, especially in accumulated residues around mixer hubs and other points of product build up, is useful to determine how long a processing line can operate. The drying process must be monitored to prevent an unacceptable increase in *S. aureus*. Aerobic colony count also may be useful to monitor process control.

#### 15.6.2.3 Processing Environment

In addition to good hygienic practices, monitoring of temperature and moisture is particularly important for the drying area for pasta and noodles. Monitoring environmental samples for salmonellae is useful to identify and correct harborage sites.

#### 15.6.2.4 Shelf Life

Shelf life testing for dry pasta is not relevant, but may be needed for fresh, refrigerated pastas. To address *C. botulinum* and other pathogen concerns in modified atmosphere packaging of fresh pastas, information on time and temperatures to which the products will be exposed and product pH and $a_w$ may be appropriate to verify safety throughout shelf life (ICMSF 2005).

**Table 15.5** Testing of unfilled pasta and noodles for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | High | Test for mycotoxins if confidence in ingredient flour is low<br>Test eggs for *Salmonella* if confidence in the supplier is low (see Chap. 22) | | | | | | | |
| In-process | Medium | Test in-process residues for *S. aureus*, especially at product accumulation points. Typical levels observed<br>• Aerobic colony counts – $<10^6$ CFU/g<br>• *S. aureus* – $<10^3$ CFU/g | | | | | | | |
| Processing environment | Low | Test for *Salmonella* in the processing plant environment. Typical guidance levels<br>• *Salmonella* – absent | | | | | | | |
| Shelf life | – | Not applicable for dry pasta | | | | | | | |
| | High | The shelf life of refrigerated pastas should be established with appropriate tests. Examine $a_w$, pH and atmosphere condition for fresh, refrigerated pasta if determined to be critical for product safety or stability | | | | | | | |
| End product | – | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When process deviations or testing indicate a possible safety issue the following are recommended | | | | | | | |
| | | | | | | Sampling plan and limits/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Low | Pastas and noodles | *S. aureus* | ISO 6888-1 | 8 | 5 | 1 | $10^3$ | $10^4$[c] |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | | | | | *n* | *c* | *m* | *M* |
| | Low | | *Salmonella* | ISO 6579 | 10 | 5[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] *S. aureus* enterotoxin tests may be used in lieu of counts or if counts are exceeded
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 15.6.2.5 End Product

As described above, dry pasta and noodles may contain salmonellae and *S. aureus*. ICMSF (1986) proposed case 10 for testing dry pastas for salmonellae, as these products are to be cooked prior to consumption and case 8 for *S. aureus*. A limit of $M = 10^4$/g was proposed for *S. aureus.* If *S. aureus* is found in pasta in excess of $10^4$ CFU/g, tests for enterotoxins may be considered as they will not be inactivated by boiling. It is important to note that *S. aureus* populations may die off during storage of dry pasta, therefore the recommended sampling plan should be applied near the time of production. Validated testing methods for enterotoxin have become available since earlier guidance (ICMSF 1986) and may be considered for suspect product.

Table 15.5 describes recommended testing for microbiological safety and quality of pasta and noodle products.

## 15.7 Cooked Cereals

This product group includes commercially cooked grains that are distributed and sold in commerce. Some grains are cooked in their original form with only limited drying and threshing treatments. For most Asian countries, boiled or steamed rice, with or without frying, is the principal diet. Wheat is

also consumed after boiling, but is usually mixed with other grains such as rice. Cooked maize is included in this category, but sweet corn is described in Chap. 12.

Rehydration of grains through boiling or steaming increases the water activity to levels that support bacterial growth. Typically these products are consumed just after preparation. However, in some situations, boiled or steamed products may be prepared for later use and consumption. For example, rice may be cooked and frozen with or without other ingredients. Shelf-stable, vacuum packaged rehydrated rice products are a more recent product development.

## *15.7.1 Significant Organisms*

### 15.7.1.1 Hazards and Controls

The potential hazard of mycotoxins was previously discussed. These products are rarely associated with vegetative pathogens because cooking destroys them. However, survival of spore formers is a concern. Numerous *B. cereus* foodborne illness outbreaks have been associated with boiled or re-fried rice (Schiemann 1978; Shinagawa 1990; Granum and Baird-Parker 2000; Haque and Russell 2005). Pumilacidin produced by *Bacillus pumilus* was reported to cause of food poisoning associated with rice in Norway (From et al. 2007). These incidents were the consequence of holding cooked rice for several hours or even overnight at room temperatures, or in large containers in refrigerators with inadequate cooling. These outbreaks could be prevented by consumer education, training of food-handlers and informative labeling of products (e.g., "refrigerate after preparation if stored for later consumption") rather than by setting criteria for the cooked products.

The advent of shelf-stable, vacuum packaged, hydrated rice products provides a potential concern for *B. cereus* and *C. botulinum* unless the products are processed to destroy these spore formers or formulated to prevent growth.

### 15.7.1.2 Spoilage and Controls

Spoilage fungi and bacteria are killed by cooking, but cooked cereals form ideal growth media. Control procedures intended for microbial hazards are also useful for controlling spoilage.

## *15.7.2 Microbial Data*

Table 15.6 summarizes useful testing for cooked rice. Refer to the text for important details related to specific recommendations.

### 15.7.2.1 Critical Ingredients

Mycotoxins derived from raw grains survive the processing procedures applied to these products. Grains should be procured from suppliers that conduct tests for relevant mycotoxins (e.g., aflatoxins, fumonisins, ochratoxin A, DON and zearalenone) in regions where these toxins have frequently occurred.

### 15.7.2.2 In-Process

During cooking, vegetative microorganisms are inactivated and *B. cereus* populations in rice are reduced but may not be totally eliminated (Johnson et al. 1983). Therefore, it may be useful to conduct periodic testing of line residues for *B. cereus* for continuous rice cooking operations to ensure that a

**Table 15.6** Testing of cooked rice for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Test for mycotoxins only if confidence in raw grains is low |
| In-process | High | For continuous rice cooking, test product residues for *B. cereus* during operation to verify control of the process. Typical guidance levels<br>• *B. cereus* – $<10^2$ CFU/g<br>• Aerobic colony count or Enterobacteriaceae may be useful indicators of process control. Typical levels depend on the product and process |
| Processing environment | Medium | Test for *Salmonella* in relevant areas during normal operation to verify control of the process. Typical guidance levels<br>• *Salmonella* – absent |
| Shelf life | Low to high | Not relevant for products consumed directly after cooking<br>For long shelf life products stored at ambient conditions, data to verify safety and stability are essential and may include water activity, pH, atmosphere condition and processing parameters |
| End product | High | Testing product parameters for growth inhibition is essential for shelf-stable products that do not receive a botulinal process (see text) |
| | Low | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a concern or when for rice of unknown history, testing is recommended for *B. cereus* |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Rice | *B. cereus* | ISO 7932 | 8 | 5 | 1 | $10^3$ | $10^4$ |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans

harborage site is not established. The emetic toxin produced by *B. cereus* is heat resistant. Testing for Enterobacteriaceae or total viable counts may be useful indicators of process control. Typical levels will vary depending on the environment, process and product.

#### 15.7.2.3 Processing Environment

The processing environment may provide harborage sites for spore formers and *Salmonella*, which may contaminate exposed food prior to packaging. Monitoring the processing environment may be useful in some situations.

#### 15.7.2.4 Shelf Life

Cereal products are typically consumed shortly after preparation, thus shelf life testing would not be relevant. However, vacuum packed products with long shelf life at ambient temperatures are commercially available. Water activity, pH and the atmosphere inside the package should be carefully reviewed to evaluate the potential for *C. botulinum* growth unless the product is processed to destroy the microorganism.

#### 15.7.2.5 End Product

ICMSF (1986) recommended criteria for *B. cereus* for entrees containing cooked rice or corn flour as a main ingredient. The association of *B. cereus* outbreaks with corn flour based products has not

materialized since that recommendation; however, outbreaks associated with cooked rice continue. Time and temperature monitoring during cooling and storage of rice is appropriate for control when the product will be consumed at a later time. In process or environmental monitoring as described above may be useful for continuous processing situations, with finished product testing only when results suggest a potential loss of time-temperature control, atypical environment or in-process results or when there is no history about the source of the product and level of microbial control. For shelf-stable products formulated to prevent growth of pathogens, relevant tests (e.g., pH, $a_W$, etc. as appropriate) should be conducted to ensure that product equilibrium conditions will continue to inhibit growth.

## 15.8 Topped or Filled Dough Products

A wide variety of topped and filled baked or cooked cereal products were addressed in the previous publication (ICMSF 2005), including cakes, pies, tarts, doughnuts, sweet buns, pizza, lasagna, ravioli, or dumplings, egg rolls, bao zi, empanadas, enchilada and others. Some of them are popular throughout the world and others are local. The fillings and toppings can include a wide variety of raw ingredients from meats, fish, cheese, cream, fats, nuts, vegetables, fruits and their pastes and jams. They may be precooked, but some fillings and toppings are added to dough without cooking and are cooked with the dough. See Chap. 26 for a discussion of these products.

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# Chapter 16
# Nuts, Oilseeds, Dried Legumes and Coffee

## 16.1 Introduction

This chapter categorizes four groups of grains: (1) nuts, including peanuts and tree nuts; (2) oilseeds, such as palm nuts, rapeseed or canola, sesame, sunflower, safflower, cottonseed and cacao seed; (3) dried legumes including beans and bean-based products such as soy flour, soy milk, tofu and sufu; and (4) coffee beans and coffee beverage. This chapter discusses control measures for safety of these products that may be applied from raw materials to finished products, where applicable. This includes microbiological and mycotoxin testing.

These products are minimally processed from their raw state, primarily by drying (in the field or by dryers), although some also are roasted, steamed, blanched, or treated with disinfecting gas such as propylene oxide.

The microbial ecology, processing steps applied to manufacture of these products, typical preparation before consumption, and impact on the microbiota of the final product and control measures for these groups were previously described in detail (ICMSF 2005).

## 16.2 Nuts

Nuts are dry, one seeded fruits, which do not burst open to release seeds at maturity. They are usually enclosed by a rigid outer casing or shell. This section covers the major nuts such as peanuts and tree nuts (almonds, hazelnuts, pistachios and Brazil nuts). While peanuts are not a true nut but rather a legume, it is also covered in this section.

### *16.2.1 Significant Organisms*

#### 16.2.1.1 Hazards and Controls

The major microbiological problem in nuts is the growth of toxigenic fungi, which can infect and proliferate on peanuts and tree nuts in the field and during improper harvesting and storage procedures, resulting in the production of mycotoxins. Aflatoxins are the most relevant hazards associated with nuts. The acute and chronic effects of human exposure to these toxins have been documented (CDC 2004a; ICMSF 2005; Groopman and Kensler 2005)

Aflatoxin is produced by *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus nomius* and related species. Invasion of peanuts mostly occurs before harvest, and depends primarily on plant stress

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_16,

induced by drought or high temperatures (Sanders et al. 1981; Pitt 2006; Pitt and Hocking 2009). Drought stress before harvest is the main factor causing aflatoxin production. The problem can be overcome most effectively by irrigation, but this is not a practical solution in many peanuts growing regions. Applying nontoxigenic strains of *A. flavus* or *A. parasiticus* to soil to compete with aflatoxigenic strains (Dorner and Cole 2002; Cotty 2006; Pitt 2006), or developing peanut genotypes resistant to colonization by *A. flavus* (Asis et al. 2005; Xue et al. 2005; Robens 2006) have been suggested as preventive measures before harvest. *A. flavus* and *A. parasiticus* are capable of growth at about $0.80a_w$ (Pitt and Miscamble 1995); however, the toxin production is limited below $0.85a_w$. The Codex Alimentarius Commission (2004) adopted a code of practice for the prevention and reduction of aflatoxin contamination in peanuts through application of preventive controls in the production, handling, storage and processing of each peanut crop.

For tree nuts, infection by fungi occurs in nuts that have split or are damaged by insects. Measures to reduce aflatoxin formation in nuts include procedures that minimize insect damage, dehulling and drying nuts to a moisture content corresponding to $a_w$ of less than 0.65 as soon as possible after harvesting, and controlling the moisture and temperature during the transportation and storage of the nuts (ICMSF 2005).

For almonds, the production of aflatoxin has been attributed to kernel damage caused by the navel orange worm (Schatzki and Ong 2001), and aflatoxin content in almonds can be related to the extent of insect damage of the nuts.

Brazil nuts are the only crop gathered from forests, thus GAP do not apply. The climatic conditions in the Amazonian environment and gathering activity cannot be controlled, exerting direct or indirect effects on the toxigenic fungi and aflatoxin production.

The Codex Alimentarius Commission (1994, 2005) adopted a code of hygienic practices for tree nuts. The code of practice provides basic hygienic requirements for orchards, farm processing (shelling and hulling), and commercial shelling or in-shell operations, including blanched, diced, ground and similar products. Mycotoxins other than aflatoxin are rarely reported in peanuts and tree nuts. Control of toxins other than aflatoxins is not recommended.

*Salmonella* is an additional hazard for nuts (Danyluk et al. 2007). Although uncommon, outbreaks of salmonellosis have been associated with almonds (CDC 2004b; Isaacs et al. 2005) and peanuts (Kirk et al. 2004). In a follow-up study, 0.87% of 9,274 100-g almond samples were positive for *Salmonella*; positive almonds were found to have ≤10 *Salmonella*/100 g (Danyluk et al. 2007). This study demonstrated no correlation between the presence of *Salmonella* and aerobic colony counts, coliform counts and *Escherichia coli* levels, although Feldsine et al. (2005) suggested that monitoring indicators may be helpful. One study found that *Salmonella* may persist in orchards for years (Uesugi et al. 2007).

The presence of vegetative microorganisms in nuts can result from contamination at multiple points during preharvest, harvest and postharvest, with survival of pathogens to the point of consumption. Vegetative cells can be controlled by a variety of postharvest interventions, including propylene oxide, steam and irradiation (Danyluk et al. 2005; Sanchez-Bel et al. 2005; Du et al. 2007; Brandl et al. 2008). These methods may result in undesirable sensory characteristics and may be insufficient to ensure elimination of pathogens but, may provide some reduction. Primary control measures are based on the selection of reliable suppliers, validation of the effectiveness of inactivation measures and implementation of appropriate GHP designed to prevent postprocessing contamination from the processing line and environment.

Human salmonellosis due to contaminated nuts and peanut butter has been reported (Scheil et al. 1998; CDC 2007, 2009). The peanut roasting process is often managed as a CCP, but for peanut butter the final product is not a CCP. The thermal tolerance of *Salmonella* in peanut butter makes the effectiveness of pasteurizing processes for butters and spreads highly uncertain (Burnett et al. 2000; Shachar and Yaron 2006). Moisture control in equipment and the environment is necessary to reduce the risk of growth of *Salmonella* and other bacterial pathogens in the processing system.

The conditions applied by industry for nut roasting have traditionally been designed to deliver desired quality parameters, which may vary from customer to customer. Nut associated outbreaks and recalls in the early 2000s illustrated the need to validate roasting processes to ensure that the manufacturer is aware of the capability of their operating conditions to effectively eliminate enteric pathogens such as *Salmonella*. When *Salmonella* has been found in roasted nuts or peanut butter, the source has frequently been due to postroasting contamination. Therefore GHP is also essential to prevent recontamination of nuts after roasting.

Mycotoxins are additional hazards in peanut butter and testing for aflatoxin provides assurance of the effectiveness of color sorting and removal of moldy nuts before processing (ICMSF 2005).

#### 16.2.1.2 Spoilage and Controls

Nuts are consumed with or without roasting. Inadequate drying and poor storage conditions can lead to fungal spoilage. Xerophilic fungi capable of growing at low water activity may grow if moisture content and temperature are favorable during drying, transport or storage. Precise quantitative data on the killing effect of a nut roasting process on relevant fungi and bacteria are not available. The control measures outlined to prevent mold growth and mycotoxin formation will also help to control growth of xerophilic spoilage fungi and most bacteria.

### *16.2.2 Microbial Data*

Table 16.1 summarizes useful testing for nuts. Refer to the text for important details related to specific recommendations.

#### 16.2.2.1 Critical Ingredients

Raw materials should be obtained from growers using GHP, even when GAPs are not applicable such as when Brazil nuts are gathered from the forest. Nuts used in nut butters or finished blends without further processing should come from manufacturers using GHP. Testing raw ingredients for bacteria is not recommended for products that are to be roasted by a validated process. All raw ingredients should be adequately segregated from finished product to prevent potential cross contamination.

#### 16.2.2.2 In-Process

After peanuts are shelled, color sorters are used to remove discolored kernels, which are most likely to contain aflatoxin because discoloration is due primarily to mold growth (Pitt and Hocking 2006). Lots may be checked for aflatoxin by chemical and immunochemical methods (Krska and Weleig 2006).

Processes, such as roasting, wet and dry blanching, and gas and steam treatments should be validated to provide adequate lethality for *Salmonella* and other enteric pathogens. When such processes are used, monitoring critical parameters such as time, temperature, etc. is important.

#### 16.2.2.3 Processing Environment

Monitoring GHP designed to prevent postprocessing contamination from the equipment and environment may be useful; Enterobacteriaceae or *E. coli* may be appropriate indicators. Environmental sampling for *Salmonella* is suggested in dry operations (see Chap. 4).

**Table 16.1** Relative importance of testing of nuts for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Good agricultural practices should be used for nut production |
| | Medium | Test for relevant mycotoxins if confidence in the supplier is low |
| | High | Test nuts that have no subsequent kill step for *Salmonella* and indicators if confidence in supplier is low |
| In-process | Low | For raw peanuts and tree nuts, routine microbiological testing is not recommended |
| | High | Monitoring the effectiveness of sorting, as well as temperature and moisture content, is important for mycotoxin control in raw nuts |
| | High | Test for total aflatoxins in peanuts and tree nuts (almonds, hazelnuts, pistachios and Brazil nuts) for further processing: 15 μg/kg |
| Processing environment | High | Monitoring GHP is essential during normal operation to verify control of the process. Internal standards may be useful for indicators, such as Enterobacteriaceae. Test environment for *Salmonella* in relevant areas during normal operation to verify control of the process. Typical guidance levels:<br>• *Salmonella* – absent |
| Shelf life | – | Not applicable |
| End product | High | Test for total aflatoxins<br>• 10 μg/kg for ready-to-eat almonds, hazelnuts, pistachios and Brazil nuts<br>• 15 μg/kg for ready-to-eat peanuts |
| | Medium | Testing for indicators may be useful following internal standards. The diversity of products in this category prevents recommendations for universally applicable criteria. |
| | Low | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a possible safety issue, testing for *Salmonella* is recommended |

| | | | | Sampling plan and limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Ready-to-eat tree nuts, peanuts and nut butter | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 16.2.2.4 Shelf Life

Microbiological shelf life testing is not relevant for dry nuts. If water is added to prepare nut-derived products that have a water activity that supports microbial growth, validating shelf life may be necessary.

#### 16.2.2.5 End Product

Many varieties of tree nuts move in international trade and the bacteriologic quality generally is acceptable (Eglezos et al. 2008). The diversity of products in this category prevents development of recommendations for universally applicable indicator criteria; however, these may be useful when developed using internal or industry specific data. As long as results from environmental and in-process sampling confirm the absence of *Salmonella*, testing of end products can be considered

only for periodic verification. However, presence of the pathogen in environmental samples should trigger investigative sampling to identify the causes. This investigation may be complemented with sampling of finished product. Table 16.1 summarizes recommended testing for other stages of this product category. Case 11 is appropriate for nuts because *Salmonella* will survive but not grow. Testing is advised if the supplier history is unknown.

End product testing for mycotoxins is widely practiced by manufacturers and governments (ICMSF 2005). The Codex Alimentarius Commission (2009b, 2010) adopted a maximum level of 15 μg/kg for total aflatoxins in peanuts and tree nuts (almonds, hazelnuts, pistachios and Brazil nuts) intended for further processing. For ready-to-eat tree nuts, the level of 10 μg/kg was adopted (Codex Alimentarius 2009b, 2010). For ready-to-eat peanuts there is no Codex limit. National and international standards for mycotoxins in nuts have also been established (FAO 2004).

## 16.3 Oilseeds

Seeds are grown mainly for oil production. Oilseeds include palm nuts (*Elaeis guineensis*, *Elaeis olifera* and hybrids), rapeseed or canola (*Brassica rapa*, *Brassica campestris*), sesame (*Sesamum indicum*), sunflowers (*Helianthus annuus*), safflower (*Carthamus tinctorius*), cottonseed (*Gossypium* spp.), cacao seeds (*Theobroma cacao*) and soy (*Glycine max*) (see Sect. 16.4).

Two products can be obtained by pressing oilseeds: oil and meal (cake). Meals are commonly used as animal feed ingredients, and are discussed in details in Chap. 11. For cocoa, the seed is the cocoa bean and the press cake is used for cocoa powder and chocolate (see Chap. 17). Due to the low $a_w$, oil from oilseeds is not relevant for microbiological aspects and will not be discussed in this chapter.

### *16.3.1 Significant Organisms*

#### 16.3.1.1 Hazards and Controls

The major microbiological problem in oilseed is the growth of *A. flavus* and consequent aflatoxin production. High levels of aflatoxin have been found in a variety of oilseeds (ICMSF 2005). *A. flavus* infects cottonseed as a result of insect damage, or through glands in the cotton plant near the flowers which attract insects for pollination (Klich et al. 1984). Cottonseed meal is a common feed for dairy cows and aflatoxins, when present, can be transferred to milk. This is discussed in Chap. 23. Sunflower, rapeseed and other oil seed press cakes are also commonly used for animal feed.

Detoxification of aflatoxin in oilseeds by ammoniation also has been reported; however, neither ammoniation nor any other procedures has been widely used commercially (ICMSF 2005).

#### 16.3.1.2 Spoilage and Controls

Xerophilic fungi capable of growing at low water activity may contaminate oilseed after harvest if there is favorable conditions for these species to grow. Control of fungal growth in oilseeds can be achieved controlling the moisture content.

### *16.3.2 Microbial Data*

Little information is available on relevant tests for oilseeds. Refer to Chaps. 11 and 18 for relevant information.

#### 16.3.2.1 Critical Ingredients

It is prudent to screen for aflatoxins when confidence in the supply is questioned, or when climatic conditions suggest a potential issue. Hazard analysis is useful to determine the need for testing.

#### 16.3.2.2 In-Process

No information is available to recommend appropriate testing.

#### 16.3.2.3 Processing Environment

No information is available to recommend appropriate testing.

#### 16.3.2.4 Shelf Life

Microbiological shelf life testing is not relevant for oilseeds; however, proper time, temperature and relative humidity are important to minimize the potential for fungal growth and subsequent mycotoxin production.

#### 16.3.2.5 End Product

Aflatoxin in oilseeds is distributed in both the oil and meal during pressing but is effectively removed from the oil during refining and alkali treatments. Microbiological testing for oilseeds is not relevant.

## 16.4 Dried Legumes

Dried legumes are the seeds of leguminous plants (family Leguminosae). Dried legumes considered in this chapter include soybeans and other types of beans. Bean-based products, such as soybean flour, soymilk, tofu and sufu are also included. Other leguminous plants are treated under vegetables in Chap. 12, and peanuts are addressed in Sect. 16.2.

Most dried legumes are rich in carbohydrates and low in oils, and are microbiologically similar to cereals. However, soybeans are also high in oil and protein (approximately 20 and 40%, respectively) and resemble oilseeds in their microbiology (ICMSF 2005). Most of the protein is heat stable, which permits high processing temperatures in the manufacture of soy based products such as soymilk, tofu, textured vegetable protein, soy flour and soy protein isolates.

### *16.4.1 Significant Organisms*

#### 16.4.1.1 Hazards and Controls

Storage conditions of less than 65% relative humidity are adequate to control microbial problems associated with dried legumes. Recontamination and growth of bacterial pathogens such as *Salmonella* can occur during further wet processing. The reduced water activity of dry beans and derivatives prevents the growth of most bacteria, but does not inactivate them. Drying generally includes heating, but the internal temperature of the product during drying rarely exceeds 35–49°C

because of evaporation of water, and microbial growth can occur during drying in internal tissues that contain sufficient moisture. In the final dried product the water activity generally is below 0.65, and only some xerophilic fungi and yeasts can multiply. Bacteria on dried legumes are of little consequence when these commodities are consumed after boiling or other heat processing. However, dried legumes prepared into soups and dips (e.g., hummus) may support the growth of pathogens. The appropriate control points and monitoring following mixing and rehydration will depend on the process.

Soybeans are contaminated with mesophilic vegetative microbiota including Enterobacteriaceae, commonly in low numbers, along with low numbers of spores of *Bacillus* and *Clostridium* spp. (ICMSF 2005). While further processing can involve water that could create conditions suitable for microbial growth, the processes also generally include heat that would kill vegetative bacteria such as *Salmonella*. Extraction of soybean oil uses solvents (e.g., hexane) that will eliminate most microorganisms.

Fungal spoilage of soy beans is uncommon and mycotoxin production is rare. If low levels of mycotoxins are present in unprocessed soybeans, extraction during the manufacture of soy protein will eliminate them.

Soybean-based products discussed here are soy flour, soymilk, tofu and sufu. Soy sauce is discussed in Chap. 14. Soy lecithin is another major ingredient, but is not addressed here because microbiological issues are rare.

Soy flour generally is defatted and desolventized without steam treatment to make textured vegetable protein. Soymilk is the liquid filtered from soybean slurry after the beans are soaked in water and blended, and has a pH around 7. The microbiological characteristics of soymilk are influenced by the quality of the soybeans, water, processing environment and the thermal process. During soaking, vegetative bacteria can multiply (ICMSF 2005).

Tofu is a nonfermented soybean product produced by heating soymilk to boiling, precipitating proteins with salt and pressing into cakes. Tofu has a high moisture content and is susceptible to microbial growth. Boiling the soymilk should eliminate vegetative microbiota, but further processing and ingredients may introduce new contaminants. Tofu can be sold and served as fresh tofu, herbed tofu, tofu paste, fried tofu, tofu burgers, sufu and other formulations. The microbial safety and quality of each is influenced by contact with hands, equipment and surfaces, and the added ingredients and processing steps. *Salmonella*, *Bacillus cereus* and *Staphylococcus aureus* are recognized hazards in tofu (ICMSF 2005).

Sufu (furu) is a fermented soybean curd resembling soft creamy cheese. Sufu is treated with starter cultures of mold (*Actinomucor*, *Mucor* and *Rhizopus*) or bacteria (*Micrococcus* and *Bacillus* spp.), salted and ripened in a dressing mixture. Most sufu contains 5–15% NaCl and 0.5–7% ethanol, which will inhibit most vegetative pathogens and molds, but room temperature storage at retail can permit growth of surviving and recontamination microbiota (Han et al. 2001). The pH of the final product varies from 5 to 7.5 and does not change during storage. Greater than 5 log CFU/g of bacterial endospores, can be found in finished sufu; *B. cereus* has been recovered at levels ≥5 log CFU/g, and *Clostridium perfringens* has been found at levels up to 5 log CFU/g (Han et al. 2001).

#### 16.4.1.2 Spoilage and Controls

Fungi capable of growing at low water activity may contaminate dried legumes after harvest from contaminated trucks, conveyors, bags, dust and storage facilities. The most common xerophilic species are *Eurotium* spp., *Aspergillus penicillioides* which causes loss of germination of seeds, and *Aspergillus restrictus* (Pitt and Hocking 2009). The presence of water and favorable temperature and atmosphere stimulate fungal growth. While further processing may include water that could create conditions suitable for microbial growth, the processes generally include heat that would kill vegetative bacteria, and spoilage is reported rarely.

## *16.4.2 Microbial Data*

Table 16.2 summarizes useful testing for dried legumes and bean based products. Refer to the text for important details related to specific recommendations.

### 16.4.2.1 Critical Ingredients

Raw materials should be obtained from growers using GAP. Dried legumes used in finished blends without further processing should come from manufacturers using GHP.

Water is an important ingredient in soymilk and tofu manufacturing, and needs to be of an appropriate quality and not add to the microbial load of the product.

### 16.4.2.2 In-Process

The first step in soybean processing is oil extraction. Further processing of soy protein involves the addition of water, and thus the possibility of microbial contamination and growth. Further processing

**Table 16.2** Relative importance of testing of dried legumes and bean-based products for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low | GAP should be used in production and potable water used for manufacturing | | | | | | | |
| In-process | Low | For dried legumes, routine microbiological testing is not recommended | | | | | | | |
| | High | For bean-based products, test for indicators to verify adequacy of process control and GHP using internal standards | | | | | | | |
| Processing environment | Low | For dried beans, routine environmental monitoring is not recommended | | | | | | | |
| | High | For bean-based products, test for *Salmonella* the processing plant environment. Typical guidance levels<br>• Indicators – consistent with internal standards<br>• *Salmonella* – absent | | | | | | | |
| Shelf life | Low | Not applicable for dry products | | | | | | | |
| | High | For high moisture bean-based products, shelf life should be validated | | | | | | | |
| End product | Medium | Test for indicators for on-going process control and trend analysis<br>Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by above tests. When above testing or process deviations indicate a possible safety issue, testing for *Salmonella* recommended | | | | | | | |
| | | | | | | Sampling plan and limits/25 g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Low | Bean flours, concentrates and isolates | *Salmonella* | ISO 6579 | 10 | 5[c] | 0 | 0 | – |
| | Low | High moisture derivatives of this category | *Salmonella* | ISO 6579 | 12 | 20[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

normally involves additional heating that is lethal to nonspore forming bacteria. Use of indicators to verify adequacy of heat treatment performance may be useful; however, available information is insufficient to specify typical levels encountered.

#### 16.4.2.3 Processing Environment

For facilities that handle dry beans only, environmental monitoring is of limited value. However, monitoring GHP designed to prevent postprocessing contamination from the equipment and environment would be very useful for facilities that manufacture soy products, especially those that will be used in ready-to-eat applications. Enterobacteriaceae and potentially aerobic colony counts may be appropriate indicators, using internally developed standards. Environmental sampling for *Salmonella* is suggested in soy protein operations (see Chap. 4).

#### 16.4.2.4 Shelf Life

Microbiological shelf life testing is not relevant for products that are dry. Once dry products are rehydrated, validating shelf life is recommended.

#### 16.4.2.5 End Product

For dried legumes, the microbiota depends largely on the conditions of growing and harvesting. For monitoring of GMPs, testing for Enterobacteriaceae is suggested. A two-class sampling plan (case 10) for *Salmonella* is recommended for bean-based products that will receive a subsequent heat treatment that will reduce the population (ICMSF 1986). For high moisture derivatives of dried legumes in which multiplication of *Salmonella* may occur, case 12 is recommended (ICMSF 1986). Case 12 may also be relevant for soy protein that is used in ready-to-eat dry mixes, such as instant beverages, depending on the potential for growth under conditions of use.

## 16.5 Coffee

This section categorizes coffee into two distinct groups: coffee beans and coffee beverage. Coffee is consumed as a beverage made by brewing roasted beans, or as instant coffee, which is produced by freeze- or spray-drying extracted coffee.

### *16.5.1 Significant Organisms*

#### 16.5.1.1 Hazards and Controls

The most significant hazard in coffee beans is ochratoxin A (OTA). The fungi that produce OTA are *Aspergillus ochraceus* and related species (*Aspergillus westerdijkiae* and *Aspergillus steynii*), *Aspergillus carbonarius* and a minor number of isolates of *Aspergillus niger* (Taniwaki et al. 2003; Frisvad et al. 2004). The time of invasion of coffee by toxigenic fungi is of great importance for OTA development in coffee.

Coffee cherries contain sufficient moisture to support mold growth and OTA formation on the outer part of the cherries during the initial 3–5 days of drying. Sun drying of coffee cherries, if not done correctly, can potentially lead to OTA formation. Drying is the most favorable time for development of ochratoxigenic species, with the main limitation being the time it takes for the berries to dry beyond a

critical water activity level of about 0.80. No berries should spend more than 4 days to decrease water activity ($a_w$) from 0.97 to 0.80. *A. ochraceus* produced little OTA (0.15 μg/kg) at $a_w$ of 0.80 and temperature of 25°C, but at 0.86 and 0.90 the production was 2,500 and >7,000 μg/kg, respectively (Palacios-Cabrera et al. 2004).

General strategies to reduce or prevent OTA formation in coffee include the implementation of GAP during preharvesting and harvesting periods, and control of moisture and temperature during the postharvesting period and storage. The Codex Alimentarius Commission (2009a) Code of Practice for the Prevention and Reduction of OTA in Coffee gives guidelines to mitigate this hazard in coffee beans.

Coffee roasting removes a very significant percentage of OTA. Depending on the roasting process, destruction ranges from 62 to 98% (Studer-Rhor et al. 1995; Ferraz et al. 2010). Surveys for OTA in retail roasted and soluble coffees all over the world indicate that coffee is not a major source of OTA in the diet, with estimated intakes being within safety limits. The low level of OTA contamination found in roasted and soluble coffee reported in the literature support this conclusion (Taniwaki 2006).

There is no substantial evidence of issues with bacterial pathogens for coffee products.

#### 16.5.1.2 Spoilage and Controls

After harvest coffee goes through three stages of drying: initial, transitional and final. The initial or high moisture phase starts with harvest. The product is in an unstable state, and spoilage can be controlled through competitor microorganisms, restricting oxygen and reducing the drying time which is critical in this state. The transitional phase is the least stable and most difficult to predict, when spoilage can only be controlled by time limitation. Mesophilic and xerophilic spoilage microorganisms have enough water to grow but not their hydrophilic competitors. Turning or stirring of the coffee is essential to promote uniform drying. When harvest coincides with a rainy or high humidity season, measures to optimize drying must be adopted. The final or low moisture phase starts at the end of drying and continues until roasting. The product is in a stable condition and control is necessary to prevent water re-introduction or redistribution in the bulk coffee. At some point during drying, there is no further growth as the product reaches the low moisture phase (Codex Alimentarius 2009a). Pinkas et al. (2010) provides more detail on spoilage during drying.

### *16.5.2 Microbial Data*

Table 16.3 summarizes useful testing for coffee products. Refer to the text for important details related to specific recommendations.

**Table 16.3** Relative importance o19.255 ptf testing coffee for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | There are no critical ingredients for coffee |
| In-process | – | Routine microbiological testing is not recommended |
| Processing environment | – | Routine microbiological testing is not recommended |
| Shelf life | – | Not applicable |
| End product | Low | Consider testing for OTA following international standards (see text) if confidence in the process is low and programs are not in place for coffee beans |

#### 16.5.2.1 Critical Ingredients

Raw materials should be obtained from growers using GAP. Coffee beans to be used in roasted coffee, instant coffee or other finished products should come from manufacturers using GHP.

#### 16.5.2.2 In-Process

Roasting is a heating process in which the raw coffee is submitted to a temperature of 180–250°C for a period of 5–15 min. The conditions of roasting are selected to produce the desired taste, color and other desired sensory characteristics for the finished product. No microbiological testing is recommended.

#### 16.5.2.3 Processing Environment

No microbiological testing is recommended.

#### 16.5.2.4 Shelf Life

Microbiological shelf life testing is not relevant for products that are dry. Refer to previous discussion on storage of coffee beans before further processing.

#### 16.5.2.5 End Product

National and international standards for OTA in coffee have been established (FAO 2004). Microbiological testing of coffee is not recommended.

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# Chapter 17
# Cocoa, Chocolate and Confectionery

## 17.1 Introduction

Raw cocoa beans used for the manufacture of products discussed in this chapter are obtained after complex fermentation processes (Schwan and Wheals 2004; Camu et al. 2008). They are roasted applying one of several processes, either as whole beans, nibs or liquor (ICMSF 2005). To obtain cocoa powder, roasted cocoa nibs or liquor are heated in the presence of water and alkali and pressed to extract the cocoa butter. The press-cake is then broken and milled to obtain the powder. Chocolate is a homogeneous product obtained by mixing cocoa liquor, cocoa mass, cocoa press-cake, and/or cocoa powder with ingredients such as cocoa butter, milk powder and others to obtain a variety of products. Confectionery includes a very large number of products manufactured using very different technologies such as chocolate confectioneries (e.g., bars, blocks, and bonbons) and sugar confectioneries (e.g., boiled sweets, toffees, fudge, fondants, jellies, and pastilles).

Details on the different processing steps applied to manufacture these products as well as their impact on the microbial flora of the final product have been described (ICMSF 2005). Their compositional definitions are included in different Codex Alimentarius Commission standards: 105-1981 for cocoa powders (Codex Alimentarius 2001a), 86-1981 for cocoa butter (Codex Alimentarius 2001b), 87-1981 for chocolate (Codex Alimentarius 2003) and 142-1983 or 147-1985 for several confectionery products (Codex Alimentarius 1983, 1985).

## 17.2 Cocoa Powder, Chocolate and Confectionery

Since the products have similar microbiological hazards, all three product groups are discussed simultaneously, with the differences highlighted when necessary.

### *17.2.1 Significant Organisms*

#### 17.2.1.1 Hazards and Controls

*Salmonella* is the only relevant pathogen of public health significance related to these products as shown by outbreaks that have occurred over the last 30–35 years (ICMSF 2005). Products involved in outbreaks have been shown to be contaminated with levels ranging between 0.005 CFU/g and 23 CFU/g (D'Aoust and Pivnick 1976; Greenwood and Hooper 1983; Hockin et al. 1989; Werber et al. 2005). As of 2011, no specific risk assessment has been performed for these products.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_17,

The only kill step for salmonellae and other members of the Enterobacteriaceae is roasting. This processing step has traditionally been applied to develop the desired sensory qualities and thus only very limited quantitative data on the killing effect have been published, such as by Stobinska et al. (2006). Historically, commercial roasting practices have proven to deliver microbiologically safe products. In addition, modern technologies often combine roasting with a steam treatment able to kill spore formers. For this reason, a reduction of vegetative bacteria in excess of 6 log units is expected.

Cocoa powder manufacture involves an alkalinization step, which involves addition of water, alkali and heat-treatment at 85–115°C. This is frequently considered a Critical Control Point (CCP) and results in destruction of >6 logs of vegetative microorganisms such as *Salmonella*. The predominant microbiota found in cocoa powder are *Bacillus* spore. Some of the spore forming microorganisms may also be destroyed depending on the processing conditions.

In chocolate manufacturing, conching at temperatures ranging between 50° and 80°C is applied to develop the desired sensory characteristics. While a certain reduction of *Salmonella* has been reported (Krapf and Gantenbein-Demarchi 2010), this step is not considered as a controlled bactericidal step and hence not managed as CCP. In the case of confectionery, roasting (for chocolate-based products), and cooking or boiling (for sugar-based products) are bactericidal steps that reduce vegetative microorganisms in excess of 6 log units.

The presence of vegetative microorganisms in cocoa powder, chocolate and confectionery products results from postprocess contamination originating from added ingredients or from the processing equipment or environment. Control measures are therefore based on the selection of reliable suppliers of ingredients and implementation of appropriate GHPs designed to prevent such postprocessing contamination.

The presence of ochratoxin in cocoa beans has been reported (Bonvehi 2004; Amezqueta et al. 2005), and the ecology of ochratoxin A-producing molds and production during cocoa processing have been investigated (Amezquéta et al. 2008; Mounjouenpou et al. 2008; Copetti et al. 2010). However, ochratoxin has not been considered a significant hazard due to its removal during the shelling process (Amezquéta et al. 2005). The need for limits has been discussed and new data may suggest that standards with appropriate limits are relevant.

#### 17.2.1.2 Spoilage and Controls

Spoilage of cocoa and chocolate occurs in very rare cases when uptake of moisture enables the growth of xerophilic molds. In the case of confectionery, and in particular sugar and chocolate candies containing fillings with an intermediate water activity (0.6 or higher) such as marzipan, fudges or syrups, spoilage by xerophilic fungi can occur (Thompson 2010). However, there are no specific control measures other than the application of GHP as described above and control of $a_w$.

### *17.2.2 Microbial Data*

Table 17.1 summarizes useful testing for cocoa powder, chocolate and confectionery products. Refer to the text for important details related to specific recommendations.

#### 17.2.2.1 Critical Ingredients

Ingredients are added to chocolate and confectionery products in dry mixing operations without subsequent heat-treatment. Hazelnuts, almonds, peanuts, and other nuts are typically roasted before

**Table 17.1** Testing of cocoa powder, chocolate and confectionary for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | Test nuts, milk powder, coconut, cocoa, eggs, flour, spices, gelatin and other sensitive ingredients for *Salmonella* if confidence in the supplier is low |
| In-process | Medium | Test intermediate cocoa powder product for *Salmonella*, Enterobacteriaceae and aerobic colony counts (ACC) to demonstrate hygiene control. For products with $a_w$ >0.6 test for osmophilic yeasts and xerophilic molds. Typical levels encountered:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – ≤10 CFU/g<br>• Aerobic colony counts – internal limits<br>• Osmophilic yeasts and xerophilic molds – ≤10–$10^2$ CFU/g |
| | High | Test product residues from product contact surfaces for *Salmonella* and Enterobacteriaceae during operation to verify control of the process. Typical levels encountered:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – ≤10 CFU/g<br>• Aerobic colony counts – internal limits |
| Processing environment | High | Test for *Salmonella* and Enterobacteriaceae in relevant areas during normal operation to verify control of the process. Typical levels encountered:<br>• *Salmonella* – absent<br>• Enterobacteriaceae – ≤$10^2$–$10^3$ CFU/g or sample<br>• Test water in jacketed equipment circuits for residual biocide or ACC |
| Shelf life | Medium | Applies to products that support osmophilic yeast or xerophilic mold growth |
| End product | High | Testing for indicators is essential to verify control of process |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Cocoa powder | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $10^3$ | $10^4$ |
| Cocoa powder; chocolate, confectionary | Enterobacteriaceae | ISO 21528-1 | 2 | 5 | 2 | 10 | $10^2$ |
| Confectionery | Osmophilic yeasts and xerophilic molds | ISO 21527-2 | 2 | 5 | 2 | 10 | $10^2$ |

| | | |
|---|---|---|
| | Low/high | *Salmonella* testing is not recommended when effective GHP and HACCP are confirmed by in-process and environmental tests. Test for *Salmonella* when history is unknown or process deviations indicate a possible safety issue |

| | | | | Sampling plan and limits/25g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Cocoa powder, chocolate, confectionery | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g samples (see Sect. 7.5.2 for compositing)

being added and roasting then is considered a CCP. Nuts and other ingredients such as whey or milk powders, coconut, cocoa powder, egg derivatives, flour, spices and gelatin, are considered high risk for the presence of salmonellae (ICMSF 2005). Due to the absence of a kill step during subsequent processing, the microbiological quality of these ingredients has an important impact on finished

products. This should be reflected in purchasing specifications. Suppliers need to adopt appropriate preventive measures (GHP and HACCP) when manufacturing their ingredients. Consult relevant chapters in ICMSF (2005) and this book for appropriate tests for these ingredients.

#### 17.2.2.2 In-Process Samples

Testing of in-process samples of cocoa powder may be of limited value due to the relatively straightforward processing lines and low exposure of intermediate product. However, in certain cases presscake or powder may be stored for a prolonged period of time for sensory reasons and testing to verify that recontamination has not occurred may be useful.

Chocolate and confectionery processing lines are more complex, involving several different operations such as milling, conching, intermediate storage, tempering, molding, cooling and hardening. A common element of most of these process steps is the use of double-walled equipment containing water, which may be a source of contamination through micro-leaks. Sampling and testing of chocolate masses at intermediate steps such as storage tanks may be performed before they are further processed. Testing for aerobic colony counts or Enterobacteriaceae as well as directly for *Salmonella* could help in detecting issues such as micro-leaks, ingress of water or even growth at interfaces. Analytical results would help in preventing the spread of contamination to the downstream processing lines, which are usually very difficult to clean and sanitize since the use of water should be avoided.

Testing of residues from critical product contact surfaces where the presence or even growth of *Salmonella* or Enterobacteriaceae may occur is very useful to detect contamination originating from the processing environment. Steps such as milling for cocoa powder, conches or cooling tunnels (potential for condensation and thus growth) and intermediate storage of powders (potential for contamination during pneumatic transport) provide useful information. Scrapings of residues are usually the most representative types of samples while, considering the nature of the products, swabs or sponges are much less useful. The results of such samples, where direct contamination of the product is possible should be within the limits applied for finished product.

For specific chocolate or confectionery products that contain fillings with a water activity of >0.6, testing for osmophilic yeasts and molds may be appropriate as they can grow in such products. Sampling points similar to those described above or specific to particular confectionery processing lines can be used.

#### 17.2.2.3 Processing Environment

It is important to implement effective hygiene control measures after roasting to avoid contamination with Enterobacteriaceae and *Salmonella* from the processing environment. The effectiveness of these measures is best demonstrated through sampling and testing of environmental samples. Residues accumulating under or above equipment, in particular those close to exposed product represent the most useful samples and are best collected with scrapers. Enterobacteriaceae are used as a hygiene indicator, which allows timely detection of potential issues such as the presence of water or the ingress of dust from a zone with a lower hygiene level. However, it is important to also include direct testing for *Salmonella* in such samples, particularly in plants that process raw cocoa beans, an important source of the pathogen.

In a closed processing environment, low levels of Enterobacteriaceae should be targeted and *Salmonella* should be absent in all samples analyzed. Enterobacteriaceae levels below $10^2$–$10^3$ CFU/g are usually achievable in this type of dry environment; however, limits should be established in each plant based on historical data. Details on the establishment of environmental sampling programs are provided in ICMSF (2005) and as outlined in Chap. 4.

Considering its impact on chocolate masses, it is also important to monitor the microbiological quality of the water in double-walled systems either by microbiological tests or indirectly through the determination of residual biocides if the water is treated (see Chap. 21, Water).

#### 17.2.2.4 Shelf Life

With the exception of certain products that are sensitive to mold or yeast spoilage due to a higher $a_w$ (>0.6), microbiological shelf life testing is not relevant for these products.

#### 17.2.2.5 End Product

The recommendations proposed in 1986 continue to be appropriate. ICMSF (1986) proposed a 2 class plan ($n=10$, $c=0$, $m=0$) for *Salmonella* in cocoa, chocolate and confectionery products as the sole criterion for these products at the port of entry. Other parameters such as aerobic colony counts or coliforms were not considered relevant to safety or stability.

The recommended sampling plan's performance for *Salmonella* is 1 cell per 180 g (log mean) and 1 cell per 33 g (arithmetic mean)assuming a standard deviation of 0.8. This would enable detection of lots contaminated at levels that have caused outbreaks in the past. Equivalent criteria are included in regulatory requirements of several countries, e.g., Canada, New Zealand.

As long as the results from environmental and in-process sampling confirm the absence of *Salmonella*, testing of end products can be considered as additional verification. However, the presence of *Salmonella* in any environmental or in-process sample should trigger investigative sampling to identify the cause. This investigation may be complemented with a reinforced sampling of finished product. Testing for Enterobacteriaceae or coliforms in environmental samples, intermediate or finished product is a valuable tool to detect deficiencies in preventive measures leading to post-process contamination.

In addition, aerobic colony counts can be a very good indicator for cocoa powders. Levels of $\leq 10^3$ CFU/g are considered normal (Collins-Thompson et al. 1978; Payne et al. 1983) and higher levels would be indicative of lapses in normal GHP. However, in the case of chocolate and confectionery, caution should be exercised when using aerobic colony counts because the level depends on the origin of cocoa beans, roasting conditions and product composition. White chocolate, for example, usually has very low levels while dark chocolate has a much higher level. Baselines established by a manufacturer for individual products are useful references and monitoring of appropriate samples along the processing line will provide useful information indicating a possible issue such as ingress of water. For confectionery products with $a_w$ >0.6 that contain ingredients such as marzipan or syrups, monitoring for osmophilic yeasts and xerophilic molds should be considered.

Table 17.1 lists proposed guidance for indicators and *Salmonella*. Limits m and M for indicators may be tighter and vary depending on the internal historical data of the manufacturer (e.g., different types of products with different ingredients) and the type of process. The use of more lenient limits, in particular for Enterobacteriaceae, would indicate a significant reduction in the effectiveness of control measures.

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# Chapter 18
# Oil- and Fat-Based Foods

## 18.1 Introduction

The microbial ecology of six categories of oil- and fat-based foods was previously discussed by ICMSF (2005): mayonnaise and dressings, mayonnaise-based salads, margarine, reduced-fat spreads, butter and water-continuous spreads. Most oil- and fat-based foods contain some level of moisture and nonfat nutrients. The end products may exist either as fat-continuous water-in-fat systems (e.g., butter and margarine), or as water-continuous oil-in-water systems (e.g., mayonnaise and dressings). Due to their physical structure, fat-continuous products are usually much more stable than water-continuous products. For the latter, safety relates directly to the pH and the types and level of acidulants. The safety of fat-continuous products depends mainly on adequate heat-treatment of ingredients and the stability and structure of the emulsion. A small category of oil- and fat-based products is characterized by extremely low water contents (e.g., butter oil, ghee, vanaspati, cocoa butter substitutes and cooking oils) that contribute to microbial stability.

Industrially produced oil- and fat-based products have a very good safety record and there are no indications that they contribute significantly to foodborne diseases. While the use of microbiological criteria to check end product safety such as at port-of-entry is of very limited value, microbiological testing may be useful to verify process control at particular stages in production. To assure end product safety, raw material quality, hygiene, process control and the application of HACCP in the manufacturing operation are the most important considerations.

## 18.2 Mayonnaise and Dressings

### *18.2.1 Significant Organisms*

#### 18.2.1.1 Hazards and Controls

Epidemiological evidence has not implicated industrially manufactured products; however, homemade and restaurant made mayonnaise and dressings have been implicated in incidents of illness. For these water-continuous products, significant hazards to be controlled include *Salmonella* spp. and *Listeria monocytogenes*. Some strains of these pathogens can be relatively acid tolerant to particular acidulants. Strategies to control presence and growth of significant pathogens include:

- Controlling pathogen specifications of final product via careful selection of ingredients.
- If ingredient control is difficult, inactivation of pathogens by suitable formulation parameters in the end product, such as a combination of a maximum pH (e.g., pH 4.5) and a suitable level

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of acidulant (e.g., 0.2% undissociated acetic acid), with a minimum holding time and temperature.
- Using thermal processing in which ingredient control for spoilage and pathogenic microorganisms, hygienic processing and filling are applied to product that is completely or partially heat processed.

As for all commodities, the adequacy of a chosen product and process design must be validated and proper operational implementation needs to be verified to provide a safe product on an ongoing basis. The product temperature must also be considered as part of validation, especially for chilled products, as the effects of acetic acid or other organic acids tend to increase with temperature.

#### 18.2.1.2 Spoilage and controls

Microbial spoilage is mainly caused by acid-tolerant yeasts and lactobacilli. Spoilage by molds is rare because most molds have a limited tolerance to acetic acid, which is most often used as the acidulant. Spoilage can be controlled by selecting suitable stable formulations, by preventing contamination via raw materials and the process environment, by hygienic packaging, and appropriate storage and distribution (chilled if necessary).

### *18.2.2 Microbial Data*

#### 18.2.2.1 Critical Ingredients

Raw ingredients like egg, milk products, herbs and spices can be contaminated with significant hazards. Such ingredients should be decontaminated, preferably pasteurized, or sourced from suppliers able to provide material of appropriate specification. Refer to relevant chapters for guidance; e.g., Chap. 22 for eggs and Chap. 14 for spices.

#### 18.2.2.2 In-Process

Because of the importance of controlling infectious pathogens, single or combined ingredients constituting intermediate products are best heat treated as part of the manufacturing process. This can be a repeated pasteurization of the egg preparation, cooking of the starch phase or the acetic-acid containing water phase. Verification that processing conditions are met will rely on monitoring operational parameters (e.g., time, temperature), not on microbiological testing.

Heat treatment is not feasible for some products or subcomponents in this category. For such products, reliance on ingredient quality, formulation parameters that inactivate pathogens of concern (e.g., acidification) and process controls can also be effective means of controlling pathogens when properly validated.

Packaging material is usually free of pathogens and acid-resistant spoilage microorganisms, and this can be covered explicitly in specifications used between packaging supplier and food manufacturer. Decontamination and microbiological testing are performed at a low frequency or are not required in manufacture.

#### 18.2.2.3 Processing Environment

Depending on the strategy applied (see Sect. 18.2.1.1), the processing line environment may be considered a potential source for significant hazards or spoilage microorganisms. The layout of the process line and its environment should be easily cleaned, and prevent cross contamination from raw

ingredients to decontaminated intermediate or final product. Improperly or inadequately cleaned manufacturing equipment is a common source of acetic acid-resistant spoilage microorganisms; therefore, hygienic equipment suited for cleaning in place (CIP) is best used for manufacture. Manual cleaning may be required for equipment that is difficult to clean by CIP. The adequacy of cleaning of the processing line and the cleanliness of its environment is best evaluated by visual observations and physical and chemical means, but these can be supported by microbial testing such as swabbing and testing for indicators of process hygiene, e.g., aerobic colony count (ACC). It is relevant to establish how physical or chemical means reflect sound hygiene status by calibration against a suitable hygiene indicator, such as ACC. Using ACC as a supportive measure can help demonstrate ongoing process control or possible loss of control. The values indicating either situation should be established during commissioning of the processing line, as it depends on features of the line equipment, the product manufactured and the production environment. Air quality may also be monitored for yeasts and molds.

In addition to monitoring for sanitation effectiveness, monitoring the plant environment for the presence of pathogens of concern and/or indicators of the presence of these pathogens may be relevant for certain products. Because of the breadth of potential products in this category, specific recommendations are not possible, but guidelines for establishing such a program, if necessary, are presented in Chap. 4.

#### 18.2.2.4 Shelf Life

In many cases mayonnaises and dressings are multiuse products, thus recontamination by spoilage or pathogenic microorganisms may occur after opening. The period before opening is referred to as "closed shelf life" and the period after opening is "open shelf life." For most ambient-stable products, sensory quality limits shelf life. Where needed, the microbiological limit of product shelf life can be established during product development by challenging product with likely spoilage microorganisms and/or select pathogens, as appropriate. These tests do not need to be conducted routinely; however, consider repeating when significant changes are applied to acetic acid level, pH, salt, water content, preservative levels or manufacturing.

Where product stability requires chilling during closed shelf life, microbiological spoilage can be reduced by measuring temperature and correcting deviations during storage and retailing. Frequently, refrigeration is a means for controlling sensory changes in the product rather than for control of microbial growth, as lactobacilli, yeasts and molds may grow slowly under refrigeration in some products.

Labeling instructions for consumers should limit ambient open shelf life or advise refrigeration during open shelf life.

#### 18.2.2.5 End Product

Routine microbiological testing is not recommended since mayonnaises and dressings are inherently safe and stable, provided the product formulation and processing are under control. Microbiological testing may be used to validate the adequacy of product and process design to deliver a safe and stable food product.

Control of chemical product properties, such as pH, acidulant or salt level, is the best way to verify that the product formulation conforms to specifications. For products where the product composition or formulation will not reduce or eliminate the risk posed by infectious agents such as *Salmonella* spp., microbiological testing for ACC or Enterobacteriaceae may be considered to verify process control and hygiene. Where there is no risk of such infectious agents surviving, then testing for only lactobacilli, yeasts and molds may suffice (see Table 18.1). For example, these criteria may be used to verify that the heat treatment has been effective and recontamination during further processing, handling and packing is under control during manufacture of some types of mayonnaise.

**Table 18.1** Relative importance of testing of mayonnaise and dressings for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Raw ingredients like egg, milk products, herbs and spices can be contaminated with significant hazards. Such ingredients should be decontaminated, preferably pasteurized, or sourced from suppliers able to provide material of appropriate specification for the product produced (see text) |
| In-process | Medium | Where applicable, operating parameters for instance for pasteurization may need monitoring; routine microbiological testing is not advised |
| Processing environment | Medium | Verify the efficiency of cleaning of processing lines and cleanliness of the processing environment by chemical and physical means at an appropriate frequency (see text) |
| Shelf life | Low | Testing not applicable; labeling instructions for consumers should limit ambient open shelf life or advise refrigeration during open shelf life |
| End product | Medium | Test for hygiene indicators to verify on-going process control and trend analysis. Consider ACC and Enterobacteriaceae for products where risk for infectious agents surviving cannot be excluded. Consider only lactic acid bacteria, yeasts and molds where this risk can be excluded |

| | | | | | Sampling plan and limits CFU/g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | Mayonnaise and dressings where infectious agents may survive | Aerobic colony count | ISO 4833 | 3 | 5 | 1 | $10^2$ | $10^3$ |
| | | Enterobacteriaceae | ISO21528-2 | 5 | 5 | 2 | 10 | $10^2$ |
| | Mayonnaise and dressings where infectious agents do not survive | Lactic acid bacteria | ISO15214 | 5 | 5 | 2 | 10 | $10^2$ |
| | | Yeasts and molds | ISO 21527-2 | 5 | 5 | 2 | 10 | $10^2$ |
| Low to high | Routine pathogen testing is not recommended. For egg containing products in which rapid die off of vegetative pathogens *cannot* be ensured, test for *Salmonella* when utility or hygiene indicator results signal loss of control | | | | | | | |

| | | | | Sampling plan and limits CFU/25g[b] | | | |
|---|---|---|---|---|---|---|---|
| | | | | *n* | *c* | *m* | *M* |
| Mayonnaise and dressings | *Salmonella* | ISO 6579 | 11 | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

The frequency of verification testing can be reduced as confidence in process control builds up over time. Regulators may use the same criteria to determine whether a lot for which they have no pertinent history regarding hygiene and safety, has been manufactured hygienically.

## 18.3 Mayonnaise-Based Salads

Mayonnaise-based salads or dressed salads are cold mixed, nonheat treated mixtures of mayonnaise or dressing with a variety of foods (e.g., chicken, meat, egg, seafood, potato, vegetables, herbs or fruits) and may contain a number of components (e.g., starch, sugar, spices, organic acids, flavors and

colors). The considerations for Combination Foods (see Chap. 26) apply to this product category. Because of the diverse products that may be included in this category, specific recommendations for criteria are not possible as they depend on the specific ingredients used. However, considerations for testing of mayonnaise-based salads are summarized below.

## *18.3.1 Significant Organisms*

### 18.3.1.1 Hazards and Controls

A wide variety of microorganisms can be introduced in the finished product from ingredients, processes and environments used to produce mayonnaise-based salads. It is important that the selection of ingredients, the formulation of the final product (e.g., pH, acidulant, salt, preservative) and the hygiene measures applied during manufacturing minimize the number of hazards to consider, and are suitable to control those hazards. Mayonnaise-based salads and dressed salads are generally more vulnerable to spoilage and survival of pathogens than properly formulated and processed mayonnaises and dressings, due to higher equilibrium pH values. Therefore, product and processing designs require careful adherence to good practices and chilled distribution and storage of the final product.

There is no concrete epidemiological evidence that industrially produced mayonnaise-based salads present a significant foodborne disease burden. Products prepared in food service operations have led to incidents with *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7, which may survive at low temperature and are relatively acid-tolerant. *Staphylococcus aureus* may also be considered a significant hazard, having caused incidents in high-pH or low-acid formulations.

### 18.3.1.2 Spoilage and Controls

Microbial spoilage may be caused by acid-tolerant yeasts and lactobacilli. Chilling can be applied to avoid spoilage of sensitive product formulations. It is important to ensure that the addition of water-containing ingredients or the presence of larger pieces of food do not cause changes to the projected product criteria (i.e., pH; acidulant, salt and preservative level). A nonhomogeneous product mixture can increase the vulnerability of the end product.

## *18.3.2 Microbial Data*

Table 18.2 summarizes useful testing for mayonnaise-based salads. Refer to the text for important details related to specific recommendations.

### 18.3.2.1 Critical Ingredients

Ingredient selection should assure that the introduction of spoilage microorganisms (i.e., acid-tolerant yeasts and lactobacilli) is minimized and pathogens are absent from ingredients that do not receive any further decontamination treatment. High-risk ingredients such as meat and chicken should be cooked (see Chaps. 8 and 9, respectively), and ingredients such as herbs and vegetables should be well cleaned and/or decontaminated to assure consumer safety (see Chap. 12). Ingredients may be selected to conform to particular specifications. Water (see Chap. 21) used should be of potable quality and free of pathogens and acid-tolerant microorganisms.

**Table 18.2** Testing of mayonnaise-based salads for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium to high | Consult relevant chapters for microbiological testing recommendations for specific ingredients |
| In-process | Low | Routine microbiological testing is not recommended |
| Processing environment | Medium | Sample processing equipment to verify the efficiency of cleaning before start-up by chemical and physical means or by testing aerobic colony count. Cleanliness of the processing line environment should also be verified at appropriate frequency (see text) |
| Shelf life | Medium | Routine shelf life testing is not recommended. Testing may be useful to validate shelflife of new retail products or when new packaging systems are installed. Labeling instructions for consumers should advise on suitable refrigeration during open shelf life |
| End product | Medium | Consider tests for hygiene indicator microorganisms to verify on-going process control and trend analysis depending on ingredients (see text). Routine pathogen testing is not recommended. When indicators suggest an issue, pathogen testing relevant to the product and ingredients may be considered (see text) |

#### 18.3.2.2 In-Process

Storage conditions for ingredients and intermediate products should minimize microbial growth. Time and temperature should be monitored for verification of good storage practices. Where appropriate, key intermediate product criteria may be checked by physical and chemical means.

Packaging materials are generally free of pathogens and spoilage microorganisms, though molds may occur. Decontamination may be appropriate for sensitive product formulations or specifications could be used between the packaging supplier and food manufacturer. This should be determined during product development. Microbial testing is normally not necessary during production.

#### 18.3.2.3 Processing Environment

Inadequately cleaned equipment can be a source of spoilage microorganisms and pathogens; therefore, hygienically designed equipment is important. When this is not possible, frequent and complete dismantling of equipment for cleaning should be considered. The adequacy of the cleaning process is best evaluated by physical and chemical means, potentially with supporting microbial testing.

The environment of the processing line may be a source of pathogens or spoilage microorganisms. The lay-out of the process line should support ease of cleaning and minimize the potential for cross-contamination. Cleaning efficacy is best evaluated by physical or chemical means, supported by microbiological testing.

#### 18.3.2.4 Shelf Life

The refrigerated shelf life of a typical mayonnaise-based salad may vary from a few days to up to 8 weeks depending on the level of spoilage microorganisms, pH, acidulant preservatives and ingredients used. Temperature should be monitored in the chill chain to assure that the required chill temperature is achieved at all times.

Routine microbial testing is not necessary and would not be useful. However, selected microbiological testing may be applied during product development to validate that the product and process design will deliver a safe and stable food product for its intended shelf life. Validation tests include shelf life tests and pathogen challenge tests. While none of the validation tests need to be conducted

routinely during operation, they may have to be repeated when significant changes to the formulation, manufacturing process or scale of operation are applied.

#### 18.3.2.5 End Product

Routine end product testing is not recommended because the safety of the end product is best assured by monitoring physical and chemical parameters in the product and the manufacturing environment, as detailed above. Limited microbiological testing may be used to verify process control during manufacture using internally developed standards. Specific criteria depend on the ingredients and processing used for the product. It is important to remember that certain ingredients used in mayonnaise-based salads may naturally have very high microbial levels of indicators. For example, fresh sliced onion ACC may range from $10^3$ to $10^6$ CFU/g or more (ICMSF 2005).

The frequency of any microbiological testing can be increasingly reduced the longer the production process is found to be well under control. When significant changes are introduced, testing may increase temporarily. Table 18.2 summarizes testing for mayonnaise-based salads.

## 18.4 Margarine

### *18.4.1 Significant Organisms*

#### 18.4.1.1 Hazards and Controls

Margarines are inherently stable water-in-oil emulsions containing at least 80% fat and up to 20% water. Other ingredients may include emulsifiers, acidulants, salt, milk or milk products, vitamins, preservatives, herbs and spices. Margarines are stabilized by a very special physical principle. The aqueous phase, in which the microorganisms may occur, is dispersed as very small water droplets in a fat-continuous matrix such that these droplets restrict microbial growth by limiting space and access to nutrients. Control of significant microorganisms primarily depends on stability of the emulsion, but also on the microbial quality of the ingredients, product criteria and hygiene during production and packaging.

There is no epidemiological evidence of stable margarine formulations causing illness. The adequacy of the product and process design to control pathogens such as *Salmonella* should be validated. Blends of margarines with butter need to consider the impact of blending on the stability of the final product and need to control the potentially hazardous microorganisms that are significant for butter.

#### 18.4.1.2 Spoilage and Controls

Microbial spoilage of margarines is mainly by molds, which may be able to grow through the fat matrix, are not affected by preservatives and take advantage of moisture condensate present on the product surface. Other significant microorganisms are lipolytic yeasts and bacteria that can destabilize the emulsion and contribute to product spoilage.

### *18.4.2 Microbial Data*

#### 18.4.2.1 Critical Ingredients

Ingredient selection and sourcing should ensure that the introduction of spoilage microorganisms is minimized and pathogens are absent from ingredients that do not receive any further decontamination treatment. Critical ingredients (e.g., water, herbs, spices, dairy products), especially those added to the

fat-phase, are best pasteurized before use. Ingredients can be sourced from suppliers able to meet appropriate specifications. Specifications used in the trade for ingredients used in margarine products include: ACC $<10^3$ CFU/g, Enterobacteriaceae <10 CFU/g, yeasts $<10^2$ CFU/g, molds <10 CFU/g, *Salmonella* absent/25 g ($n=5$); *L. monocytogenes* absent/g ($n=5$). Typical trade specifications for dry milk ingredients used in margarine may vary with region but include: ACC $<10^4$ CFU/g, Enterobacteriaceae $<10^2$ CFU/g, coliforms <10 CFU/g, yeasts and molds $<10^2$ CFU/g, and absence of infectious pathogens.

#### 18.4.2.2 In-Process

Storage conditions for ingredients and stock solutions, water- and fat-phase mixes, and other intermediate products should minimize growth of spoilage microorganisms and avoid recontamination from the environment. Selection of good quality ingredients in combination with monitoring time and temperature will generally suffice to verify process control. It is advisable to check key parameters (e.g., pH, salt levels, acidulant or preservative) of stock solutions and intermediate products by physical and chemical means. Stock solutions of ingredients or the aqueous phase containing water soluble ingredients are commonly pasteurized before mixing with the fat-phase to give a pre-emulsion. The process parameters for pasteurization need to be monitored and deviations acted upon to assure process control.

For more sensitive formulations or where manufacturing hygiene makes recontamination likely, the microbiological status should be verified at selected stages during production. For example, this could include regular monitoring of the microbial content of water used to make the aqueous phase, especially when pasteurization is not practiced. The pre-emulsion is a key stage for microbial testing for process control verification because there is no subsequent heat-treatment to control microorganisms if contamination occurs. Should intermediate product be kept at elevated temperature (>40°C), growth of thermophilic microorganisms can be monitored.

Packaging materials will be free of pathogens and spoilage bacteria. Molds may occur. For sensitive product formulations, decontamination may be appropriate or specifications could be used between packaging supplier and food manufacturer. Where necessary, air quality at packaging needs to be carefully controlled. The relevance of these aspects should be determined at the product development stage. Microbial testing should not be necessary during operation.

#### 18.4.2.3 Processing Environment

Margarine manufacture requires equipment that can be easily cleaned and sanitized, preferably by CIP. The adequacy of the cleaning of process equipment is best evaluated by physical and chemical means, supported by microbial testing. While the processing equipment is wet-cleaned and sanitized, the working environment should be maintained as dry as possible during production as limiting water use helps to control *Listeria.*

The processing line environment may be a source of significant hazards or spoilage microorganisms. The lay-out of the process line environment should be easily cleaned and prevent cross-contamination from raw ingredients to decontaminated intermediate or final product. Recycled cardboard can be a source of mold spores.

#### 18.4.2.4 Shelf Life

While most margarines are stable during closed shelf life and can be stored and distributed at ambient temperature, they benefit from chilled storage during open shelf life. For sensitive formulations, refrigeration may be needed directly after manufacture and the temperature in the chill chain needs

to be monitored and deviations acted upon. Routine microbial testing is not necessary. It is essential to assure that storage is in a dry environment and that condensation is avoided.

Selected microbiological testing may be applied during product development to validate that the chosen product and process design will deliver a safe and stable food product. Tests to consider in this regard are shelf life tests and pathogen challenge tests. While none of these tests need to be conducted routinely during operation, they may have to be repeated when significant changes to the formulation, manufacturing process or scale of operation are applied.

#### 18.4.2.5 End Product

Considering full-scale manufacture, it is not advised to use microbiological criteria for assessing end product safety and stability on a routine basis. The safety of the end product is best assured by monitoring physical and chemical parameters in intermediate products and the manufacturing environment, the processing line and in samples of the end product. At the start of manufacturing, measurement of the emulsion characteristics is advised, e.g., by measuring the volume weighted geometric mean diameter and the geometric standard deviation of the droplet size distribution (Alderliesten 1990, 1991) or by microscopy.

Microbiological testing may be used to verify process control with the possibility to gradually decrease the frequency the longer the production process is found to be well under control. When significant changes are introduced, such testing may be temporarily intensified. Examples of end product microbiological limits that are used in the trade are noted in Table 18.3. Notably, adherence

**Table 18.3** Testing of margarine and reduced-fat spreads for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Medium | Critical ingredients (e.g., water, herbs/spices, dairy products, etc.) are best pasteurized. Ingredients may be selected to conform to particular specifications (see text) | | | | | | | |
| In-process | Low – margarine | Routine microbiological testing is not advised. Emulsion characteristics and process criteria (i.e., pH, preservative and/or organic acid level) should be monitored for verification of process control. For sensitive formulations, the microbiological status of the pre-emulsion and the water used for the aqueous phase can be tested | | | | | | | |
| | Medium – reduced-fat spreads | In addition to testing for margarine, process criteria should be monitored when in-line pasteurization of the complete emulsion is applied. Mold contamination of packaging may need to be considered for particularly sensitive products | | | | | | | |
| Processing environment | Medium | Efficiency of cleaning can be verified before process start-up, by chemical and physical means or by testing ACC; cleanliness of the process environment can be verified at appropriate frequency, by testing ACC | | | | | | | |
| Shelf life | Low | Testing not applicable; labeling instructions for consumers should limit ambient open shelf life or advise refrigeration during open shelf life | | | | | | | |
| End product | Medium | Test for indicators for on-going process control and trend analysis, e.g., using the microbiological criteria for hygiene indicators listed below | | | | | | | |
| | | | | | | Sampling plan and limits CFU/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Margarine and reduced fat spreads | Aerobic colony count | ISO 4833 | 3 | 5 | 1 | $10^2$ | $10^3$ |
| | | | Enterobacteriaceae | ISO 21528-2 | 5 | 5 | 2 | 10 | $10^2$ |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans

to good practices should allow much lower levels to be routinely achieved than those quoted. End product microbiological limits noted in the trade for verification of process and hygiene control are, for example: aerobic colony count $<10^4$ CFU/g, Enterobacteriaceae $<10^2$ CFU/g, yeasts $<10^3$ CFU/g, molds $<10^2$ CFU/g, spore formers $<10^4$ CFU/g, *S. aureus* $<10^3$ CFU/g, *Salmonella* spp. absent/25 g; *L. monocytogenes* absent/g.

## 18.5 Reduced-Fat Spreads

While margarine products contain over 80% fat, reduced-fat spreads may contain between 20 and 80% fat. There is a wide variation in reduced-fat spreads, relating to the fat level, the use of dairy ingredients etc.

### *18.5.1 Significant Organisms*

#### 18.5.1.1 Hazards and controls

As long as these spreads are true water-in-oil emulsions, the same basis for product and process safety applies as for margarines, although reduced-fat spreads are generally more vulnerable to microbiological problems. Notably, the lower the fat content and the courser the water droplet dispersion, the more vulnerable spreads will be and the more likely they will support growth of pathogens, if present. The presence of dairy ingredients in reduced-fat spreads may add to their vulnerability and needs to be considered when establishing safe product and process design. Below 20% fat, spreads are oil-in-water emulsions and likely to support pathogen growth (see Sect. 18.6).

Control of significant microorganisms for reduced-fat spreads relies on a combination of factors, such as the stability of the emulsion, microbial quality of ingredients, product criteria, and hygiene during production and packaging. Additionally, preservatives such as sorbic acid and benzoic acid may be used. Although pH levels are best <4.5, such low levels may cause precipitation of dairy proteins when present, and slightly higher pH levels need to be chosen. It is advised to validate the adequacy of the product and process design to control pathogens such as *Salmonella* spp. and *L. monocytogenes*.

#### 18.5.1.2 Spoilage and Controls

Microbial spoilage is mainly by molds, as described in Sect. 18.4.1.2. Other significant microorganisms are yeasts and bacteria not effectively controlled by the product formulation/emulsion and possibly able to destabilize the emulsion.

### *18.5.2 Microbial Data*

#### 18.5.2.1 Critical Ingredients

The vulnerability of the product formulation and emulsion strongly determines the need for considering critical ingredients. Careful selection and sourcing should ensure spoilage microorganisms and pathogens are not introduced, especially when ingredients are used without a decontamination

treatment such as pasteurization. Critical ingredients (e.g., water, thickeners, dairy products) are best pasteurized before use. Specifications used in the trade for ingredients such as starches and gums are: aerobic colony count $<10^4$ CFU/g, Enterobacteriaceae $<10^2$ CFU/g, yeasts and molds <500 CFU/g, and absence of infectious pathogens.

#### 18.5.2.2 In-Process

Stock-solutions of ingredients or the aqueous phase containing various water-soluble ingredients are commonly pasteurized before being mixed with the fat-phase containing the fat-soluble ingredients to give a pre-emulsion. Vulnerable formulations may require in-line pasteurization of the complete emulsion and process control parameters (time/temperature) need monitoring for process control verification. Key product criteria (e.g., pH, acidulant, preservative) of stock-solutions and intermediate products require monitoring by physical and chemical means.

For vulnerable formulations in particular or where manufacturing hygiene makes recontamination likely, the microbiological status is best verified at selected stages during production. This can, for instance, relate to regularly monitoring the microbial content of water used to make the aqueous phase.

Packaging materials will generally be free of pathogens and spoilage microorganisms, though molds may occur. For sensitive product formulations, decontamination may be appropriate or appropriate specifications can be agreed on between packaging supplier and food manufacturer. Where necessary, air quality at packaging needs to be carefully controlled. The relevance of these aspects should be determined at the product development stage. Microbial testing should not be necessary during operation.

#### 18.5.2.3 Processing Environment

The considerations and requirements for the processing environment for reduced-fat spreads is as described for margarines, in Sect. 18.4.2.3.

#### 18.5.2.4 Shelf Life

Depending on the formulation and emulsion characteristics, reduced-fat spreads may be stable during closed shelf life and can be stored and distributed at ambient temperature. However, most formulations/emulsions require chilled storage during open shelf life. Vulnerable products will require refrigeration from manufacture onward and in this case the temperature in the chill chain needs to be monitored and deviations acted upon. Routine microbial testing is not necessary. It is essential to assure that storage is in a dry environment and that condensation is avoided.

#### 18.5.2.5 End Product

It is not advised to routinely use microbiological criteria for the end product. Selected microbiological testing including use of suitable microbiological criteria may be applied during product development to validate the product and process design for safety and stability. Tests to consider here are shelf life tests and pathogen challenge tests. While none of these need to be conducted routinely during operation, they may have to be repeated when significant changes to the formulation, manufacturing process or scale of operation are applied.

End product safety during full scale manufacture is best verified by monitoring physical and chemical parameters in intermediate products, processing equipment and process environment, as

well as in samples of end product. Microbiological testing may be used to verify ongoing process control. However, the frequency can be increasingly reduced the longer the production process is found to be well under control. When significant changes are introduced, such testing may be temporarily intensified. Examples of end product microbiological limits that are used in the trade are noted in Table 18.3. Adherence to good practices should allow much lower levels to be routinely achieved than those quoted. Specifications vary by country; e.g., the US includes coliforms at 10 CFU/g and Australia may allow an ACC of $M = 1.5 \times 10^5$ CFU/g.

## 18.6 Butter

### *18.6.1 Significant Organisms*

#### 18.6.1.1 Hazards and Controls

Significant hazards for butter are *L. monocytogenes* and *S. aureus*, based on epidemiology of outbreaks involving butter. Other hazards may include *Salmonella* and *E. coli* O157:H7, although there is less epidemiological evidence linking these to butter-associated foodborne illness.

The principle methods for controlling pathogens in butter are quality of ingredients, pasteurization of some raw materials (e.g., milk or cream), hygiene during production and packaging, the size and distribution of the water droplets in the fat matrix (as for margarine) and presence of salt. Preservatives are often not permitted for use in butter. Refrigeration is necessary during open shelf life.

#### 18.6.1.2 Spoilage and Controls

Microbial spoilage of butter is caused mainly by yeasts and molds, and sometimes bacteria. These may be introduced through poor hygiene before or during packaging, or during use. Refrigeration is an important feature of closed and open shelf life, for prevention of spoilage, as is the use of clean packaging materials and prevention of condensates forming on the product surface.

### *18.6.2 Microbial Data*

#### 18.6.2.1 Critical Ingredients

Ingredient selection should ensure that significant pathogens are absent in raw materials and that introduction of spoilage microorganisms is minimized. The steps involved in butter manufacture are not designed to reduce or eliminate microbiological contamination. Cream is a critical ingredient and is typically pasteurized to remove infectious agents and other vegetative microflora, but will still contain bacterial spores and some heat-resistant vegetative spoilage microorganisms. Some butter-making processes use commercially available starter cultures. The starter cultures should not become a source of contamination. Therefore, the number of subcultures should be limited. Ingredients such as salt, coloring agents, and neutralizers are generally free of microbial contamination because of the way they are manufactured; chemicals should be of food-grade quality. When water is used in butter manufacture after pasteurization (e.g., for washing), the water should be of potable quality. Ingredients sourced from suppliers should meet appropriate specifications, including, for cream: ACC $<10^3$ CFU/g, Enterobacteriaceae $<10^2$ CFU/g, and absence of infectious pathogens.

#### 18.6.2.2 In-Process

Verification of process control can generally be carried out by selection of good quality ingredients and monitoring of time and temperature of intermediate products. Key parameters of stock solutions (e.g., levels of salt or preservatives, if these are used and allowed by regulations) should be checked by physical and chemical means. Moisture content, salt distribution and the water droplet size/distribution are important for microbiological stability, and pH is an important parameter of sour cream butter. The verification program should incorporate measurements of these factors and include trend analysis. To limit mold contamination, a laminar flow cabinet (or other means of controlling air quality) at the packaging stage may be necessary. Product (mold) spoilage is limited further by storage at refrigeration temperature. Microbial testing should not be necessary during operation. Water use during production should be very limited in order to control the environmental risk of *L. monocytogenes*.

#### 18.6.2.3 Processing Environment

Use of hygienic equipment is important for cleaning and sanitation, otherwise equipment should be dismantled for cleaning. Samples from the start-up of the process as well as from the end of the run should be analyzed. Efficacy of cleaning and sanitation is best evaluated by physical or chemical means, with microbiological testing providing a supporting role. Cardboard packing material may be an important source of mold spores, especially when recycled cardboard is used. Condensation on the product surface should be avoided.

#### 18.6.2.4 Shelf Life

Butter should be kept free from moisture during distribution. Refrigerated shelf life of butter varies between 3 and 9 months, depending on the level of salt or other preservatives present, if permitted by regulation. Temperature of storage should be monitored.

#### 18.6.2.5 End Product

Microbiological testing may be carried out during product development to validate that the product and process design will deliver safe and stable butter. Tests that may be applied for these purposes include shelf life tests and challenge tests. These tests are not carried out during routine manufacturing but should be repeated when there are significant changes in the formulation or manufacturing process. If challenge test data are not available or if there is information to suggest that the formulation/structure of the product will not prevent growth of microorganisms such as *L. monocytogenes* or *S. aureus*, then microbiological criteria for these microorganisms in end product would be appropriate. In this situation, case 5, where $n=5$, $c=2$, $m=10$ and $M=10$ can be applied for *S. aureus* and a two class plan where $n=5$, $c=0$ and $m=0$ can be applied for *L. monocytogenes*.

For end products, microbiological testing is not considered a primary means of routinely assessing product safety and stability. Assessment of safety is best carried out through monitoring of chemical and physical parameters in intermediate products, the environment, processing line and in samples of end product. Microbiological testing can provide a supporting role here, to verify process control, and can be reduced on the basis of results demonstrating the process is well under control. If significant changes are introduced or if there is a failure in process control leading to manufacture of substandard product, then testing can be intensified temporarily, to verify that the process returns to being in control (Table 18.4).

**Table 18.4** Testing butter for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Medium | Critical ingredients (e.g., cream, water) are best pasteurized or selected to conform to particular specifications (see text) | | | | | | |
| In-process | Medium | Routine microbiological testing is not advised. Emulsion characteristics and process criteria (i.e., pH, salt level) should be monitored for verification of process control. For sensitive formulations, the microbiological status of water used for washing may be tested as an additional verification measure. Mold contamination of packaging may need to be considered for particularly sensitive products | | | | | | |
| Processing environment | Medium | Efficiency of cleaning can be verified before process start-up, for instance by chemical and physical means or by testing aerobic colony count; cleanliness of the process environment can be verified at appropriate frequency, for instance by testing aerobic colony count | | | | | | |
| Shelf life | Low | Testing not applicable; labeling instructions for consumers should limit ambient open shelf life or advise refrigeration during closed and open shelf life | | | | | | |
| End product | Medium | Test for indicators for on-going process control and trend analysis, for instance using the microbiological criteria for hygiene indicators listed below | | | | | | |
| | | | | | Sampling plan and limits CFU/g[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Butter | Aerobic colony count | ISO 4833 | 3 | 5 | 1 | $10^2$ | $10^3$ |
| | | | Enterobacteriaceae | ISO 21528-2 | 5 | 5 | 2 | 10 | $10^2$ |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans

## 18.7 Water-Continuous Spreads

The principles outlined above for reduced-fat spreads also hold for water-continuous spreads. These products are more vulnerable to spoilage caused by molds, yeast and bacteria and they should undergo challenge tests with relevant microorganism to validate the product and process design. Emphasis should be placed on physical and chemical measurements and if necessary, these can be supported with microbiological tests as recommended for reduced-fat spreads. A key consideration for these products is more limited open shelf life and products will need to be stored and transported under refrigeration.

## 18.8 Miscellaneous

Included in this group are butter oil, ghee, vanaspati, cocoa butter substitutes, and cooking oils (soy, olive, canola, cottonseed, sunflower and other oils). Due to the extremely low water content (<0.5%) of these products, they do not allow microbial growth. When stored under moist conditions, mold spoilage may occur on the product surface. Also survival of infectious pathogens, in principle, is possible. However, microbiological testing of these products should not be necessary.

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# Chapter 19
# Sugar, Syrups and Honey

## 19.1 Introduction

ICMSF (2005) previously discussed the microbial ecology of sugar, syrups and hone in *Microorganisms in Foods 6: Microbial Ecology of Food Commodities*. These products are rarely associated with food safety issues because of the naturally low water activity. When used as an ingredient, spoilage may be a concern in certain products. This is discussed in relevant chapters in this book, as well as in ICMSF (2005).

## 19.2 Cane and Beet Sugar

Sugar is obtained from sugar cane (*Saccharum officinalis*) or sugar beets (*Beta vulgaris*). It is sold in both crystalline and liquid form. Sucrose is the most widely distributed sugar in nature. Other sugars, such as dextrose (glucose), fructose, lactose, mannitol, sorbitol and xylitol, also play an important economic role. Specifications for sugars are given in the Codex Alimentarius Commission Standard 212-1999 (Codex Alimentarius 2001b).

### *19.2.1 Significant Organisms*

#### 19.2.1.1 Hazards and Controls

Dry refined sugar is a safe product and is not associated with foodborne outbreaks. Processing destroys the vegetative microorganisms present in the raw material. *Clostridium botulinum* has been detected in raw sugar and molasses but not in refined sugar (Nakano et al. 1992).

#### 19.2.1.2 Spoilage and Controls

The prevalence of the microbial spoilage microbiota in cane sugar depends on climatic conditions, sugar content, pH of the exudates and damage to cane caused by insects, frost and other causes. Xerophilic molds are the microorganisms of major concern, because growth to high levels will lead to lost yield of sucrose due to formation of acids, dextrans and slime. Losses in sucrose content may be substantial unless the time between harvest and crushing is minimized. Dextran is a polysaccharide that causes processing problems because it increases the viscosity of the process liquid, resulting in slower processing. It can also damage equipment and necessitate an increased frequency

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_19,

of cleaning. The refining of raw sugar also has an impact on the microbiological quality of the final product (ICMSF 2005).

Many microbial species involved in spoilage are encountered in beet sugar and originate from the soil adhering to the beets. Processing sugar beets at temperatures above 70°C, ideally at 75°C, prevents growth of spore forming thermophilic bacteria. In molasses, osmophilic yeasts are the microorganisms of major concern, which can cause spoilage during storage but their growth depends on the water activity ($a_w$). The $a_w$ of sugar varies from 0.575 to 0.825, but spoilage does not occur at $a_w$ levels lower than 0.65. During growth in molasses, the fructose component of the invert sugars is metabolized and water and acid are produced. The increase of $a_w$ and decrease of pH favors growth of osmophilic yeasts and the hydrolysis of sucrose into invert sugar. A few osmophilic species of yeasts produce invertases causing inversion of sugar. Under favorable conditions growth of yeasts can continue during bulk storage and transport and populations may reach $10^7$–$10^8$ CFU/g, affecting the sensory characteristics of the final product. However, after reaching the maximum, the number of viable cells may decline significantly. The sequence of operations during cane sugar processing affects the microbiota.

Control of $a_w$ to <0.65 ensures that spoilage microorganisms will not grow in these products. There are no specific control measures other than application of GHP. No microbiological testing is recommended for dry sugar or molasses, unless they are used as an ingredient for specific products and processes.

## *19.2.2 Microbial Data*

### 19.2.2.1 Critical Ingredients

There are no critical ingredients in the production of sugar.

### 19.2.2.2 In-Process

For verification of adherence to GHP during processing and handling, testing for hygiene indicators may be performed.

### 19.2.2.3 Processing Environment

Processing environment data include environment samples. The purpose of this testing is to check that the environment is clean and under control.

### 19.2.2.4 Shelf Life

Microbial growth is not relevant because dry sugar is shelf-stable.

### 19.2.2.5 End Product

Microbiological criteria for cane and beet sugar are not recommended for most applications (table sugar, sugar applied as a coating on baked goods). However, thermophilic spores in sugars are of concern to manufacturers of certain canned products and soft drinks (ICMSF 2005). There is a long history for the application by industry of the criteria specified in Chap. 24.

For sugar that is to be used as an ingredient in foods that do not receive a subsequent microbial reduction step (e.g., heating) testing may be needed to ensure the final product (e.g., chocolate, infant

formula) will meet established criteria. In such applications, the stringency of the sampling plan for sugar should reflect the relative risk associated with the food. For example, the sampling plan for sugar added to powdered infant formula would be more stringent than the plan for sugar added to chocolate. The sampling plans should be specified in the purchase specifications as agreed between buyer and supplier. In addition, more stringent requirements for verification of GHP may be required to address specific concerns when sugar is used in a more sensitive food.

## 19.3 Syrups

Glucose syrup is a purified, concentrated, aqueous solution of nutritive saccharides obtained from starch or inulin. Glucose syrup has a dextrose equivalent content of not less than 20% m/m (expressed as D-glucose on a dry basis) and a total solids content of not less than 70% m/m. An increasingly important sweetener is high-fructose corn syrup, made by enzymatic conversion of glucose syrup to fructose. Specifications for glucose syrup are given in the Codex Alimentarius Commission Standard 212-1999 (Codex Alimentarius 2001b).

### *19.3.1 Significant Organisms*

#### 19.3.1.1 Hazards and Controls

Syrups and liquid sugar products have not been linked to outbreaks of foodborne disease. There were some reports on the presence of *Clostridium botulinum* in maize syrup (ICMSF 2005) but growth cannot occur due to the low $a_w$.

#### 19.3.1.2 Spoilage and Controls

Depending on the sugar content, syrups have $a_w$ values ranging between 0.70 and 0.85. Gradients of $a_w$ may be present that can permit growth of osmophilic yeasts in regions of higher $a_w$ and cause spoilage.

Control of $a_w$ to <0.65 ensures that spoilage microorganisms will not grow in the product. Other controls include preventing recontamination through the application of GHP, preventing condensation and other causes of increased $a_w$ in storage tanks and using air filters and ultraviolet lamps in storage tanks. No microbiological testing is recommended for syrups, unless they are used as ingredient in foods that may be more prone to spoilage (e.g., shelf-stable beverages).

### *19.3.2 Microbial Data*

#### 19.3.2.1 Critical Ingredients

There are no critical ingredients in the production of syrups.

#### 19.3.2.2 In-Process

Liquid sugar is refined sugar concentrated after the decolorizing step or made by dissolving refined sugar in water. The usual sugar content is 66–76° Brix. For both liquid sugar and syrups, recontamination with osmophilic yeasts may occur during storage and transport.

For verification of adherence to GHP during processing and handling, testing for hygiene indicators may be performed. Thermophilic spoilage bacteria, xerophilic molds and spore formers should be tested in syrups when these microorganisms are important for canned and bottled foods, see Chap. 24.

#### 19.3.2.3 Processing Environment

Sampling the processing environment generally is not performed in facilities producing syrups.

#### 19.3.2.4 Shelf Life

Microbial growth is not relevant for shelf life, because liquid sugar and syrups are shelf-stable at $a_w$ <0.65.

#### 19.3.2.5 End Product

In general, microbiological criteria for liquid sugar and syrups are not recommended. The thermophilic spores in sugar solutions are of concern for manufacturers of certain canned products and soft drinks (ICMSF 2005). See Chap. 24.

## 19.4 Honey

Honey is the natural substance produced by honey bees predominantly from the nectar of flowering plants, secretions of plants or excretions of plant sucking insects. The material collected by bees is transformed in the honey comb, where it ripens and matures. Honey should not contain any additives unless they are declared on the label. Its composition varies greatly according to the type of plant from which the nectar and other substances are derived. The sugar content (fructose and glucose) should not be less that 60g/100g and sucrose content should not be more than 5g/100g. Specifications for honey are given in the Codex Alimentarius Commission Standard 12-1981 (Codex Alimentarius 2001a).

### *19.4.1 Significant Organisms*

#### 19.4.1.1 Hazards and Controls

Four factors contribute to the microbiological safety and stability of honey. They are the low $a_w$, low pH, hydrogen peroxide and other less well defined antimicrobial substances (TGA 1998, Taormina et al. 2001).

*C. botulinum* spores have been isolated from 7 to 16% of honey samples from a variety of sources (ICMSF 2005). No practical procedures can prevent contamination of honey in the hive by spores of *C. botulinum*. The spores survive processing and storage for long periods in honey. An increased incidence seems to be linked to growth and sporulation in dead bees and pupae in hives (Nakano et al. 1994).

Honey is the only food that has been recognized as a risk factor for infant botulism. Infant botulism due to consuming honey has been reported from many countries (CDC 1984, Fenicia et al. 1993,

Centorbi et al. 1999, Jung and Ottosson 2001, Thomasse et al. 2005, van der Vorst et al. 2006). Infant botulism occurs at less than 12 months with 95% of cases occurring in the first 6 months of age. The World Health Organization and the US Centers for Disease Control recommend that honey should not be fed to infants under the age of 6 months and 12 months, respectively (WHO 2002, CDC 2008). Honey added as an ingredient in commercially manufactured formula for infants up to 1 year of age must be thermally processed to destroy botulinal spores.

There have been no reports that using honey as an ingredient in other foods has resulted in such foods being implicated in botulism. Testing honey for *C. botulinum* is not recommended as a control measure.

#### 19.4.1.2 Spoilage and Controls

The microorganisms of interest in honey processing are those adapted to the characteristics of honey (i.e., high sugar content, low acidity and the presence of naturally occurring antimicrobials). The microbial content is generally low, with counts $<10^2$ CFU/g, exceptionally up to $10^3$ or $10^4$ CFU/g. The microflora of commercial importance are the osmophilic yeasts, which may cause fermentation if the $a_W$ is abnormally high (Snowdown and Cliver 1996, ICMSF 2005).

There are no specific control measures other than application of GHP and ensuring $a_W$ or moisture content are within acceptable limits (Codex Alimentarius 2001a).

### *19.4.2 Microbial Data*

#### 19.4.2.1 Critical Ingredients

There are no critical ingredients in the production of honey.

#### 19.4.2.2 In-Process

As extracted from the comb, honey has a water content of about 17%, corresponding to an $a_W$ of about 0.60. The minimum $a_W$ for growth of osmophilic yeasts is 0.65. The heating given to honey after extraction to control crystallization provides a microbial reduction step despite the increased heat resistance provided by the reduced $a_W$. This heating step is not sporicidal. There are no other specific control measures other than application of GHP.

#### 19.4.2.3 Processing Environment

Sampling the processing environment is not performed in facilities used for extracting and processing honey.

#### 19.4.2.4 Shelf Life

The low $a_W$ (<0.65) prevents growth of osmophilic yeasts. Honey is shelf-stable.

#### 19.4.2.5 End Product

Microbiological criteria are not recommended for honey.

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# Chapter 20
# Nonalcoholic Beverages

## 20.1 Introduction

The nonalcoholic beverages covered in this chapter include soft drinks, fruit juices, concentrates, vegetable juices, coconut milk, coconut water and tea-based beverages. The reader is referred to *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005) for more information on microbial ecology and control of nonalcoholic beverages. This chapter discusses various control measures for safety and spoilage of these products that may be applied from raw materials to finished products, where applicable. This may include microbiological testing.

## 20.2 Soft Drinks

Soft drinks include carbonated and noncarbonated products. Besides the typical ingredients included in soft drinks, they may also contain fruit juices, pulp or peel extracts. Carbonated soft drinks account for about 50% of the soft drink market and are nonalcoholic beverages that are made by absorbing carbon dioxide (carbonation) and are typically not pasteurized. Noncarbonated drinks are predominantly fruit based, do not contain carbon dioxide, and usually undergo a heat treatment or are chemically preserved to control spoilage microbiota (Fujikawa 1997; Ashurst 2005; ICMSF 2005). Sports drinks, also known as electrolyte drinks, are also covered in this section. These typically contain carbohydrates and the main electrolytes, sodium and potassium, although many are enriched with vitamins and other ingredients (Shirreffs 2003; FSANZ 2010; SIPA 2010).

### *20.2.1 Significant Organisms*

#### 20.2.1.1 Hazards and Controls

This product category has no significant microbiological hazards because of the nature of the product and processing methods used for production. Although the initial microbiota of the various ingredients used in their manufacture could include a small number of pathogens or adventitious contaminants, product formulation and good hygienic practices (GHP) control significant hazards. In addition, most noncarbonated soft drinks undergo pasteurization, which not only inactivates enzymes, but also destroys any relevant pathogens. Carbonated soft drinks, which are not heat treated, are usually manufactured from ingredients without significant microbial hazards and the final product is preserved.

Testing for pathogens or their indicators is not recommended for soft drinks.

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#### 20.2.1.2 Spoilage and Controls

Microbiological spoilage associated with soft drinks can be a serious economic problem but is rarely a public health issue. Most spoilage is associated with use of poor quality raw materials, such as the fruit from which many soft drinks are made. Bacteria and yeasts may be controlled by formulation, pasteurization or use of adequate levels of permissible preservatives (ICMSF 2005). Carbonated cola beverages are typically robust and rarely encounter microbial spoilage (DiGiacomo and Gallagher 2001); however, noncarbonated products may be susceptible to spoilage, mainly due to heat resistant fungi, preservative-resistant yeasts and thermoacidophilic spore forming bacteria that may survive these preservation techniques. Yeasts account for most of the spoilage in the soft drink industry due to their high acid tolerance, ability to grow anaerobically and the presence of fermentable sugars in these products. The types of yeast found include *Zygossaccharomyces, Brettanomyces, Saccharomyces, Candida, Torulopsis, Pichia, Hansenula* and *Rhodotorula*. The highly preservative-resistant *Zygosaccharomyces* are the most significant spoilage yeasts, with *Z. bailii* documented as the most prevalent spoilage yeast in soft drinks (Pitt and Hocking 2009). This species can grow even in the presence of the maximum permitted levels of preservatives. Spoilage by this yeast results in pronounced off-odors, off-taste, visible sediment, increased package pressure and package failure due to production of carbon dioxide. *Brettanomyces* spp. are sensitive to benzoic and sorbic acids but are highly resistant to carbonation. These yeasts have been implicated in the spoilage of both low and nonpreserved diet beverages, flavored carbonated water, and sugar sweetened products. *B. naardenensis* is most commonly associated with spoilage of soft drinks.

Most bacteria will not grow in the high acid environment of this product category and vegetative cells are rapidly inactivated. However, a few are aciduric and able to grow at low pH, most notably *Gluconobacter* and *Acetobacter*. Both of these genera are strict aerobes and are of concern in noncarbonated beverages. These microorganisms are restricted by gas-impermeable packaging and minimal head space (Stratford et al. 2000; DiGiacomo and Gallagher 2001; Wareing and Davenport 2005).

Mold spores can survive in carbonated beverages but cannot grow due to the lack of oxygen and the preservation effect of carbon dioxide. However, when carbonation is lost due to loss of package integrity, molds can cause spoilage. Common fungi found in the soft drink environment are *Aspergillus, Penicillium, Rhizopus* and *Fusarium* (Pitt and Hocking 2009). In pasteurized, noncarbonated beverages, heat resistant molds may also be a problem similar to those found in fruit juices, which are discussed in Sect. 20.3.1.2.

Synthetic ingredients in soft drinks, such as artificial flavors and colors, and soft drinks containing natural sweeteners and flavor oils usually lack nitrogen sources suitable to support yeast growth, and rarely spoil. However, soft drinks that contain fruit juices, tea or other sources of nitrogenous compounds are particularly susceptible to microbial spoilage (ICMSF 2005).

Applying GHP is essential for spoilage control in sensitive products. In particular, use of hygienically designed equipment, proper cleaning and sanitation of equipment, and strict attention to factory hygiene are essential. Proper stabilization of these products, as described in ICMSF (2005), is also recommended.

### *20.2.2 Microbial Data*

Table 20.1 summarizes useful testing for soft drinks. Refer to the text for important details related to specific recommendations.

**Table 20.1** Testing of soft drinks for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Test sugar and syrups for spoilage microorganisms when confidence in the supplier is low (see text) |
| | Low | Test water for indicators if water quality is in question |
| In-process | – | None |
| Processing environment | Medium | For microbiologically sensitive products, test sanitation rinse water for yeasts and other applicable microorganisms to verify the effectiveness of sanitation (see text) |
| Shelf life | – | Not applicable |
| End product | – | Not applicable |

#### 20.2.2.1 Critical Ingredients

Water is the major ingredient in soft drink manufacturing, and must be of an appropriate quality and not add to the microbial load of the product. Although *Cryptosporidium parvum* is an important hazard in water, a number of successful treatments are available, i.e., ion exchange or reverse osmosis, filtration (through sand or carbon) or decontamination by adequate amounts of chlorine, UV treatment or ozone (ICMSF 2005). *E. coli* or thermotolerant coliforms are useful for verification of microbial quality, and testing for these microorganisms may be appropriate when the suitability of the water supply is in question (see Chap. 21).

The microbiological quality of dry sugar and sugar syrups incorporated in soft drinks is important for sensitive products and should be assessed through ingredient specifications or testing. In the US, bottler's standards used for many years by the beverage manufacturing industry include the following (Smittle and Erickson 2001):

- Dry, granulated sugar – aerobic colony count <200 CFU/10 g; yeast <10 CFU/10 g; molds <10 CFU/10 g
- Liquid sugar or sugar syrup in 10 g of dry sugar equivalent (DSE) – aerobic colony count <100 CFU; yeast <10 CFU; molds <10 CFU

#### 20.2.2.2 In-Process

Since control of the processing conditions is essential for proper stabilization of these products, the following conditions should be monitored as appropriate (ICMSF 2005):

- Temperature of pasteurization treatment, if applicable (or equivalent nonthermal method)
- Storage temperature of raw materials subject to microbial spoilage
- Closure integrity of bottles, cans, glass jars or other packaging materials
- Proper cleaning and decontamination of packaging material, especially when recycled or reused as for return bottles

#### 20.2.2.3 Processing Environment

The most significant source of spoilage yeast and bacteria is the bottling plant environment and equipment. The majority of microbial contamination occurs from the blender and all equipment downstream through the filler. Sanitation is thus an important factor in the successful production of

soft drinks that are sensitive to microbial spoilage, and sampling is focused on verifying the effectiveness of the sanitation program. Collecting sanitation rinse-water samples, particularly at the fillers, is useful since they represent product flow during manufacture. Typical levels of yeast encountered are <15 CFU/100 mL for sensitive products and <100 CFU/100 mL for colas (DiGiacomo and Gallagher 2001). Sanitation rinse samples from other areas such as blending pumps, tanks, carbonators, etc. may also be sampled, particularly when there are quality concerns or new products are being introduced. Swab samples can be taken if rinse water sampling is not practical. The standard method used in the soft drinks industry for microbiological testing is the membrane filtration method, because it is useful for detecting low levels of yeast, bacteria and molds. Detection methods should include appropriate media for total yeast counts. When spoilage issues occur, enumeration of preservative-resistant yeasts, such as *Zygosaccharomyces bailii* and *Brettanomyces* spp. may be appropriate (Pitt and Hocking 2009).

An example of a 3-class sampling plan for yeast in sanitation rinse waters for a 120 valve filler was discussed by DiGiacomo and Gallagher (2001), i.e. $n=30$, $c=3$, $m=15$ CFU/100 mL, and $M=50$ CFU/100 mL for microbiologically sensitive products. This was based on randomly sampling 25% of the valves on the filler. Similar programs could be established for specific applications, depending on the sensitivity of products to spoilage, history of spoilage issues and other factors. Because of the variation of products and processes that can be used, universal standards are not recommended.

#### 20.2.2.4 Shelf-Life

Considering the nature of the product and processing methods used for production, microbiological shelf life testing is not considered appropriate for these products.

#### 20.2.2.5 End Product

No routine testing is recommended as GHP, processing methods and monitoring hygiene of the processing environment control significant health hazards and spoilage concerns.

## 20.3 Fruit Juice and Related Products

Typical products included in this section are fruit juices, concentrated fruit juice, fruit nectars and cordials, and fruit purees. Fruit juices are the unfermented liquids obtained from the edible part of sound, appropriately mature fruits, and concentrated fruit juice is juice from which water has been physically removed. Fruit nectars and cordials are the unfermented pulpy-liquid drinks prepared from one or more fruits to which sweeteners and other ingredients may be added. Fruit purees are unfermented products obtained by appropriately processing the edible part of the whole or peeled fruit without removing the juice. Fruit juices and related products may or may not undergo thermal treatment. Stabilizing nonthermal treatments include hydrostatic pressure as described in ICMSF (2005).

### *20.3.1 Significant Organisms*

#### 20.3.1.1 Hazards and Controls

Any microorganism present on or below the fruit surface may potentially contaminate fruit juices and concentrates. ICMSF (2005) lists a number of outbreaks that have occurred due to the consumption of contaminated fruit juices.

The growth of filamentous fungi in fresh fruit and their juices may lead to the formation of mycotoxins such as patulin and ochratoxin A. Patulin is mainly found in apple and pear juice and is produced by *Penicillium, Aspergillus* and *Byssochlamys*, of which *P. expansum* is the most commonly encountered species (ICMSF 2005). Ochratoxin A may be found in grape juice and is produced by *Aspergillus carbonarius* or *A. niger* and related species (Varga and Kozakiewicz 2006).

Control of mycotoxins in fruit juice is achievable. Use of raw material of appropriate quality minimizes the presence of mycotoxins in the processed product. Good Agricultural Practices (GAP) both pre- and postharvest, are necessary to keep the contamination level on fruits as low as possible. At the factory, physical removal of spoiled and visually damaged fruit from the product stream, an initial water treatment step and refrigeration of stored fruit at ≤8°C are essential (ICMSF 2005). A Codex Alimentarius Commission Code of Practice provides guidelines to avoid patulin in apple juice and related product (Codex Alimentarius 2003a).

Fresh juice became a recognized source of serious food poisoning outbreaks and fatalities in the 1990s. Unpasteurized juice has been implicated in outbreaks associated with *Salmonella* and other pathogens such as *E. coli* O157:H7 and *Cryptosporidium parvum* (ICMSF 2005). Use of dropped ("windfalls") or damaged fruits must be avoided and the control measures recommended for raw fruits discussed in Chap. 13 should be followed. A minimal standard of a cumulative 5-log reduction of the hazard of concern is required by the FDA (2004) for fruit juice. ICMSF (2005) describes useful processing strategies to achieve this reduction. For unpasteurized fruit juices with low acid concentrations such as tomato, melon and orange, refrigeration is necessary as an additional barrier to prevent growth of a number of bacterial pathogens.

Microbiological testing for pathogens in fruit juices is not recommended, although testing for indicator microorganisms may be useful during processing.

#### 20.3.1.2 Spoilage and Controls

Microbiological spoilage is frequently associated with use of poor quality raw materials, such as the fruit from which fruit juices are made. Naturally occurring bacteria and fungi on fruits are generally controlled by pasteurization or use of adequate levels of preservatives. However, heat resistant fungi, preservative resistant yeasts and the acid dependent, thermotolerant bacterium *Alicyclobacillus* may survive these preservation techniques (ICMSF 2005). Because of the wide variety of products and processes that may be used in this product category, it is not possible to recommend criteria for specific microorganisms in raw materials. However, the quality and wholesomeness of fruit bases from which products are to be made is important to control spoilage. Using GAP and GHP to keep contamination of fresh fruit as low as possible prior to processing is essential to minimize the risk of spoilage by *Alicyclobacillus* because conventional pasteurization temperatures are unlikely to substantially reduce existing levels of *Alicyclobacillus* spores. The use of excessively long process times is also impractical because these may damage sensory characteristics of the product. Refrigeration of fruit juices after pasteurization can also be useful to control spoilage.

For most fruits, pasteurization at temperatures of about 70–75°C is effective to inactivate most enzymes, yeasts and the conidia of common contaminant fungi. However, fungi producing ascospores are capable of surviving such processes, causing spoilage. *Byssochlamys fulva* and *B. nivea* have been reported to cause spoilage in strawberries in cans or bottles, blended juices containing passion fruit, and fruit gel baby foods. *Paecilomyces* may also be present in such products, as the anamorph of *Byssochlamys* (i.e., asexual spores (conidia)), as it too has mesophilic, thermotolerant and thermophilic characteristics (Houbraken et al. 2006). For example, *P. varioti* has caused spoilage in fruit juices as the anamorph of *B. spectabilis* (Houbraken et al. 2008) Other heat resistant fungi isolated from different fruit juices are *Neosartorya fischeri, Talaromyces trachyspermus, T. macrosporus, T. bacillisporus* and *Eupenicillium* (Hocking and Pitt 1984). Raw materials that should be screened

**Table 20.2** Testing of fruit juice and related products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Test water for indicators if the water quality is in question |
| In-process | High | Test unpasteurized fruit juice samples for generic *E. coli* prior to filling |
| Processing environment | Medium | The most important microorganisms in this product are preservative-resistant yeasts such as *Zygosaccharomyces bailii* and *Brettanomyces* spp. Test sanitation rinse water for yeasts and other applicable microorganisms to verify the effectiveness of sanitation (see text) |
| Shelf life | | Not relevant (see text) |
| End product | | Not relevant for shelf-stable product. For refrigerated product, no testing is recommended when filler sampling is conducted as above |

routinely for heat resistant molds are grapes, passion fruit, pineapple and mango juices and pulps, strawberries and other berries, and any raw material that may come in contact with soil directly or as a result of rain splash (Pitt and Hocking 2009).

## *20.3.2 Microbial Data*

Table 20.2 summarizes useful testing for fruit juices and related products. Refer to the text for important details related to specific recommendations.

### 20.3.2.1 Critical Ingredients

Water is an important ingredient in fruit juice manufacturing, and needs to be of an appropriate quality (see Chap. 21).

### 20.3.2.2 In-Process

Process control measures discussed for soft drinks are also appropriate for fruit juice and related products (see Sect. 20.2.2.2). Where thermal treatment such as pasteurization is used to control *E. coli* O157:H7, monitoring of the time and temperature is essential. A variety of combinations have been proposed by FDA (2004).

Microbiological testing for generic *E. coli* as indicator of enteric pathogens is recommended for unpasteurized fruit juices because of the food poisoning incidents that have been associated with such products.

### 20.3.2.3 Processing Environment

Environmental contamination with yeast and molds is an important factor to control for fruit juices, as previously discussed for soft drinks. Inadequate factory hygiene has also been linked to fruit juice outbreaks (ICMSF 2005). Therefore, scrupulous attention to cleaning of the lines, fillers and cooling meter (if used) downstream from the pasteurizer is essential to prevent recontamination of the product. This should include thermal as well as chemical sanitation. Such processes are essential in products

without preservatives, as any fermentative yeast contamination will lead to spoilage. The sampling plan for testing for yeasts and molds described for soft drinks in Sect. 20.2.2.3 is applicable.

#### 20.3.2.4 Shelf Life

The shelf life of unpasteurized fruit juices is short due to enzymatic activity and the presence of high numbers of microorganisms. These juices are typically obtained from freshly pressed fruit and are usually packed and delivered to retailers within 24 h. These juices must be kept refrigerated as they have a very limited life of only a few days (British Soft Drinks Association 2010).

The shelf life of pasteurized juices is longer than that for unpasteurized juices because of the varying degrees of treatments they receive. Typically, long life, hot filled juices usually keep for 6–9 months and do not require refrigeration in unopened packages, whereas short life, pasteurized products have a shelf life of 2–6 weeks and typically require refrigeration (British Soft Drinks Association 2010). In both cases, routine microbiological testing is not recommended, provided that careful attention is paid to implementation and regular monitoring of GHP and processing parameters as discussed previously.

#### 20.3.2.5 End Product

Because of the extensive heat treatment received, no product testing is recommended for canned or hot filled fruit juices, purees and nectars. A possible exception is sampling for the presence of mycotoxins, where applicable. A marker used by industry to assess the quality of apples used to manufacture juice is patulin. A level in excess of 50 μg/kg (in single strength juice) may indicate use of a high percentage of unwholesome apples to manufacture the product (Pitt and Hocking 2009).

End product testing of pasteurized products can be used for verification. Several traditional methods such as aerobic colony counts, yeast and mold counts or direct microscopic examination may be considered (ICMSF 2005). In the case of *Alicyclobacillus*, a method using K agar and a heat shock treatment to ensure germination of spores prior to plating has been found to be most effective (Orr and Beuchat 2000; Walls and Chuyate 2000). For routine juice assays, sample dilution is generally unnecessary. However, sample dilution is recommended for concentrated fruit juices, purees and nectars.

The US FDA (2004) permits unpasteurized citrus juice processors to use multiple methods to decontaminate surfaces of fruit to achieve part of the 5-D pathogen reduction requirement if they use undamaged, tree-picked fruit to prepare the juice. The 5-D pathogen reduction must start after initial culling and cleaning and must take place in a single facility. End product testing for generic *E. coli* and *E. coli* Biotype I is a requirement. Both types of *E. coli* must be absent in the juice (<1 CFU/20 mL). One 20 mL sample/3785 L (1,000 gal) of juice produced must be analyzed. Where <3,785 L/week are produced, one sample must be analyzed per week. When two out of seven consecutive samples are positive for *E. coli*, the process is considered inadequate.

## 20.4 Tea-Based Beverages

Ready-to-drink tea-based beverages range from relatively unformulated still products produced from direct leaf extraction, which may be slightly sweetened and flavored with lemon or other fruits; to carbonated soft drinks made from instant tea solids and lemon juice, which may have a lower pH and be preserved with weak acids. Because of their diversity, these products have a wide range of microbiological susceptibilities. This section addresses commercially prepared and distributed liquid tea beverages and not tea that is prepared directly before service.

## *20.4.1 Significant Organisms*

### 20.4.1.1 Hazards and Controls

Tea-based beverages (including herbal teas) are extremely diverse and do not allow for a simple summary of significant hazards and controls that would be appropriate for all products. However, the mycotoxins fumonisin $B_1$ and fumonisin $B_2$ have been found in certain herbal teas and medicinal plants consumed regularly in Turkey (Omurtag and Yazicioglu 2004).

Tea crops should be grown under GAP and tea production should be conducted under GHP. For simple tea-based beverages, pasteurization and avoidance of postprocess recontamination will prevent significant safety concerns. Microbiological testing is therefore not recommended. However, addition of fruit juices may require use of controls discussed under fruit juices above, and addition of protein sources, such as milk and soy protein, requires validation of the control of *Clostridium botulinum*, as described in Chap. 24.

### 20.4.1.2 Spoilage and Controls

Aerobic colony counts as high as $1.9 \times 10^8$ CFU/g of raw tea have been reported in certain herbal teas (Wilson et al. 2004), and processed dry tea leaves of the tea plant (*Camellia sinensis*) are prone to microbial contamination during post-processing handling and storage. Tea should be produced under GHP to minimize the potential for spoilage issues. Gamma irradiation of the teas may be of use in countries, where it is approved. An irradiation dose of 5 kGy has been reported to be effective (Mishra et al. 2006).

## *20.4.2 Microbial Data*

### 20.4.2.1 Critical Ingredients

Typical ingredients are tea, fruit juices, sweeteners and protein sources that could be added to the teas, as well as the water from which the teas are made. Refer to appropriate chapters for specific ingredients that are used.

### 20.4.2.2 In-Process

Microbiological testing of in-process samples is not recommended.

### 20.4.2.3 Processing Environment

Tea-based beverages are frequently processed on the same lines used for soft drinks manufacturing. Therefore, the testing recommendations described in Sect. 20.2.2.3 are applicable. Airborne contamination by yeast and molds is an important factor to control. Major vectors such as dust and insects may contribute to microbes into the factory environment. Factory hygiene is therefore a major factor in the control of product stability.

### 20.4.2.4 Shelf Life

Tea-based beverages are generally shelf-stable, therefore microbiological shelf life testing is not recommended.

#### 20.4.2.5 End Product

Microbiological testing of shelf-stable tea-based beverages is not recommended.

## 20.5 Coconut Milk, Coconut Cream and Coconut Water

Coconut milk, coconut cream and coconut water are products derived from the separated endosperm (kernel) of the coconut palm (*Cocus nucifera* L.). Coconut milk is the dilute emulsion of comminuted coconut endosperm in water. The Codex Alimentarius Commission (2003b) standard for aqueous coconut products describes standards for different types of coconut products (light, regular, creams and concentrated creams) and prescribes that these products are generally treated by heat pasteurization, commercial sterilization or ultrahigh temperature (UHT) processes to generate shelf-stable products. Coconut water is the albumen of the coconut. It is a white milky liquid that will change into flesh as the fruit matures. This product should be pasteurized or thermally processed.

### *20.5.1 Significant Organisms*

#### 20.5.1.1 Hazards and Controls

Little information is available on coconut milk, coconut cream or coconut water as vehicles for foodborne illnesses; however, there is a history of *Salmonella* issues with coconut. Fresh-frozen coconut milk was also implicated in a *Vibrio cholerae* O1 outbreak (CDC 1991). Processes are typically used to make them shelf-stable will control these hazards (see Chap. 24).

#### 20.5.1.2 Spoilage and Controls

Little information is available on spoilage of coconut milk, coconut cream and coconut water and considering that most of the products are shelf-stable through heat treatment, it is unlikely that spoilage will result within a reasonable shelf life expectation. The high water activity, neutral pH, and available protein in these products would make them prone to spoilage if heat processing were not used. Manufacture of these products under GHP conditions is a necessity to minimize contamination prior to heat treatment.

### *20.5.2 Microbial Data*

#### 20.5.2.1 Critical Ingredients

There are no critical ingredients, other than the raw material.

#### 20.5.2.2 In-Process

Monitoring of the time and temperature of the process is essential. In-process sampling is not recommended for microbiological testing.

#### 20.5.2.3 Processing Environment

Testing of the processing environment is not relevant for shelf-stable products.

#### 20.5.2.4 Shelf Life

Long shelf life is expected because of the thermal process used to make them shelf-stable. No microbiological testing for shelf life is recommended.

#### 20.5.2.5 End Product

Microbiological testing is not relevant for shelf-stable products (see Chap. 24).

## 20.6 Vegetable Juices

Vegetable juices may be low-acid, pasteurized chilled products that receive a mild heat treatment and contain no additives or preservatives. These attributes may render them susceptible to contamination with certain pathogens and if temperature-abused, could result in growth of these pathogens, some of which may produce toxins. Vegetable juices can also be heat treated shelf-stable products. This chapter considers only chilled vegetable juices. See Chap. 24 for recommendations related to shelf-stable products.

### *20.6.1 Significant Organisms*

#### 20.6.1.1 Hazards and Controls

Chapter 12 provides information on significant hazards associated with vegetables and vegetable products. Contamination of fresh vegetables could significantly affect the safety of vegetable juices subsequently produced. Four cases of botulism linked to refrigerated carrot juice occurred in the United States and two cases occurred in Canada in 2006. The implicated products were pasteurized, but not heated to a temperature that would eliminate spores of proteolytic (the most heat resistant type) *C. botulinum*. Subsequent testing of the product revealed the presence of botulinum toxin in the juice. Because proteolytic *C. botulinum* spores are known to grow and produce toxin only under severe temperature abuse conditions, an important control measure is to keep the product refrigerated below 4°C (Guinebretiere et al. 2001; FDA 2007). Acidification to a pH <4.6 may also be considered as a control measure to prevent *C. botulinum* growth under conditions of abuse.

#### 20.6.1.2 Spoilage and Controls

Many microbiological problems arise because of poor quality of raw materials, such as the vegetables from which juices are made. Applying preharvest and postharvest GAP would be useful to minimize the initial contamination of the vegetables. Manufacture of these products under GHP is a necessity to prevent further contamination of the product prior to heat treatment. Even though the bacteria and fungi normal present on vegetables are destroyed by pasteurization, some spore formers such as *Bacillus* and *Clostridium* spp., may still survive in the product. Postpasteurization recontamination should be avoided and refrigeration to below 4°C after processing is essential to prevent outgrowth (Guinebretiere et al. 2001).

## *20.6.2 Microbial Data*

### 20.6.2.1 Critical Ingredients

Water is an important ingredient in fruit juice manufacturing, and must be of an appropriate quality (see Chap. 21).

### 20.6.2.2 In-Process

Process control monitoring discussed for carbonated beverages, fruit juices and related products is also appropriate for vegetable juices (see Sect. 20.2.2.2). The thermal treatment applied to the product requires monitoring of the time, temperature and other controls. Validated control measures for all *C. botulinum* spores should be incorporated into HACCP plans to ensure that *C. botulinum* growth and toxin production will not occur should the juice be kept unrefrigerated in distribution or by consumers. This could be achieved by a number of validated treatment methods such as acidification of the juice to a pH of ≤4.6, thermal treatment of the juice or addition of preservatives (FDA 2007). No in-process microbiological sampling is recommended; however, monitoring of pH levels as part of a HACCP plan is a strong recommendation if this is a control measure.

### 20.6.2.3 Processing Environment

As for soft drinks, fruit juices and tea, equipment sanitation is important, particularly in postprocessing equipment, such as fillers. Collection of sanitation rinse water samples as described above is recommended for appropriate testing (see Sect. 20.2.2.3). Because vegetable juices may have a neutral pH, tests for aerobic colony counts may be useful instead of, or in addition to yeast and mold counts. Control measures should also include testing the performance of container closures (plastic caps, foil seals) in minimizing any risk of post process contamination of the juice by *C. botulinum* spores.

### 20.6.2.4 Shelf Life

No microbiological testing is recommended.

### 20.6.2.5 End Product

End product sampling and inspection of pasteurized products, does not deliver reliable control, but samples incubated at elevated temperatures and then tested microbiologically or examined for gas production may be useful for trend analysis. Microbiological enumeration may be used for verification purposes, where HACCP programs are in place. Several traditional methods, e.g., aerobic colony counts, yeast and mold counts or direct microscopic examination may be considered (ICMSF 2005). Criteria would depend on the product and processing conditions, therefore no specific recommendations can be made.

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# Chapter 21
# Water

## 21.1 Introduction

Water is an essential part of human nutrition, both directly as drinking water or indirectly as a constituent of food. Water is not only essential for life; it remains one of the most important vectors of illness.

One of the major objectives of the physicochemical treatments applied to raw water is to eliminate pathogens and to obtain safe drinking and processing water. Production of water of appropriate quality is becoming increasingly difficult due to the growing demand as well as to the increasing environmental pollution.

Irrigation water is discussed in Chap. 12.

## 21.2 Drinking Water

The WHO has established Guidelines for Drinking Water Quality defining parameters and values governing its quality in 1979. Since then, the parameters and associated limits are subject to constant updates, which are posted on the WHO website (2009). The quality of drinking water is also defined in numerous national or international regulations and guidelines.

Since the first publications on the subject (Gale 1996), several risk assessments related to the safety of drinking water have been performed, either in general terms or focused on specific microbial pathogens or parasites (Gale 2003; Hoornstra and Hartog 2003; Percival et al. 2004; WHO 2008; Mena and Gerba 2009). Several water guidelines are moving through the risk management approach. As a consequence, less emphasis will be placed on assessing end-of-treatment contaminant concentration. Rather, the major focus will be placed on process performance at major control points.

### *21.2.1 Significant Organisms*

#### 21.2.1.1 Hazards and Controls

The microbial population of the raw water used to make drinking water depends on its origin, which can be surface water from rivers, lakes or reservoirs, or ground water from springs, wells or boreholes. For untreated surface waters, the presence of potentially pathogenic bacteria (e.g., *Campylobacter jejuni*, enterohemorrhagic *E. coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*,

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_21,

*Yersinia enterocolitica*), viruses (e.g., hepatitis A, norovirus), parasites (e.g., *Entamoeba histolitica, Giardia intestinalis, Cyclospora cayatenensis, Cryptosporidium parvum*) or helminths is likely. The type of pathogens, their incidence and levels will vary depending on the type of surface water, the region as well as environmental and weather conditions. Details are provided in ICMSF (2005) and WHO (2009).

Ground water is usually of a much better initial microbiological quality and sometimes may comply with the definition of drinking water without any further treatment. In other cases the water source may become contaminated with the previously mentioned pathogens through environmental conditions or during collection.

The microbial population can be reduced by primary treatment of the raw water, usually applied as combined steps. Pretreatments will depend on the origin of the water and include impoundments, coagulation, flocculation and clarification, as well as different types of filtration. While such pretreatments may reduce the microbial load, it is necessary to perform subsequent disinfection to inactivate any remaining pathogens. Disinfectants such as chlorine and chloramine, chloride dioxide, bromine, bromine, ozone or UV are normally used.

Drinking water may be recontaminated with pathogens during distribution. Numerous outbreaks related to enteric pathogens, viruses or parasites have been reported. Examples of recontamination outbreaks include Rooney et al. (2004), Schuster et al. (2005), Karanis et al. (2007), September et al. (2007), La Rosa et al. (2008) and Reynolds et al. (2008). Recontamination can be controlled or minimized by maintaining residual levels of biocides to avoid after-growth in the distribution systems or by ensuring the integrity of the distribution system to avoid ingress of pathogens.

#### 21.2.1.2 Spoilage and Controls

Spoilage is mainly due to sensory deterioration of water, usually caused by the growth of microorganisms such as *Streptomyces* spp., molds, and Gram-negative bacteria. Such spoilage has been previously described (Zaitlin and Watson 2006; Boleda et al. 2007; Krishnani et al. 2008) but is generally not a major issue.

### *21.2.2 Microbial Data*

Table 21.1 summarizes useful testing for drinking water. Refer to the text for important details related to specific recommendations.

#### 21.2.2.1 Critical Ingredients

Not relevant for drinking water.

#### 21.2.2.2 In-Process

Sampling and testing of drinking water at different points in the distribution system, including intermediate storage, allows for the detection of recontamination before the product reaches the consumer. Monitoring of residual biocidal activity in the water (depending on the type of disinfectant used) provides rapid information on the residual levels. This can be complemented by microbiological analyses of hygiene indicators or of pathogens for verification.

Considering the nature of the drinking water, the difference between in-process and end product samples is minimal.

**Table 21.1** Testing of drinking water for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | – | Not relevant for drinking water | | | | | | | |
| In-process | High | Test water for residual biocidal agents (where appropriate and depending of biocides used). Typical levels range between 0.2 and 0.5 ppm or according to local regulations | | | | | | | |
| | Medium | Test drinking water in distribution system for *E. coli* or other appropriate indicators for verification (often regulated). Testing for specific pathogens is usually done only for investigation. See End Product for guidance levels<br>Investigative sampling may be conducted to determine the root cause of off-flavors or off-odors | | | | | | | |
| Processing environment | Low | Not relevant for drinking water | | | | | | | |
| Shelf life | Low | Not relevant for drinking water | | | | | | | |
| End product | High | Testing for indicators is essential to verify control of the process after treatments and during distribution | | | | | | | |
| | | | | | | Sampling plan and limits/100 mL[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Drinking water | *E. coli* (or other hygiene indicators if used) | ISO 9308-1 | NA | 1 | 0 | 0 | – |
| | Low | Testing for pathogens is not recommended to verify control process and is only applied in investigation in case of positive hygiene indicators results. For this reason, no specific sampling plan is provided | | | | | | | |

*NA* not applicable
[a] Alternative methods may be used when validated against ISO methods.
[b] Refer to Appendix A for performance of these sampling plans

#### 21.2.2.3 Processing Environment

Not relevant for drinking water.

#### 21.2.2.4 Shelf Life

Not relevant for drinking water.

#### 21.2.2.5 End Product

It is the responsibility of water authorities or suppliers (in the case of private sources) to ensure the microbiological safety of potable water supplies. Regular monitoring of the water for *E. coli*, as an indicator of fecal recontamination, is performed by authorities or private companies. Other indicators such as enterococci, total viable counts or total or fecal coliforms are used in addition, depending on local or supra-national legislation, and testing for pathogens such as *Salmonella* or parasites is performed when issues are detected.

In a number of standards, microbiological requirements are expressed as mean values or 90$^{th}$ percentiles – such standards are representative of trends of individual samples taken over a defined period of time.

Analysis of residual disinfectants, where applicable, is much more useful than the testing of end products for pathogens and is therefore recommended.

## 21.3 Process or Product Water

Water plays a major role in food production and is used either as an ingredient or during processing. Three situations can be distinguished during processing:

1. Direct contact in operations such as washing, conveying and blanching of vegetables and fruits; scalding, cleaning and chilling of poultry or slaughtered animals; storage of fish and meat in ice; washing to remove certain components during manufacture of cheese or butter; and cutting of products or lubricating of conveyor belts.
2. Indirect contact from inadequately drained equipment after cleaning;
3. Accidental contact with water that is normally not intended to contact with food. Examples are cooling water for retorted containers, water circulating in closed heat-exchange systems, aerosols and condensation.

Process or product water must be of potable quality and thus is purchased as drinking water from authorities or private companies or processed directly by food manufacturers as described in the previous section. However, for certain applications, for example cleaning of vegetables or fruits that will be submitted to a heat-treatment, water with higher counts may be suitable and will not affect the wholesomeness of the end product. In such cases, it is appropriate to consider the use of recycled water, providing substantial savings of drinking water. Conversely, water fulfilling specific physical–chemical requirements is needed as an ingredient for specific products, requiring electrodialysis, ion exchange, filtration, or reverse osmosis, which may have an impact on the microbiological quality of the water if not managed in an appropriate manner.

### *21.3.1 Significant Organisms*

#### 21.3.1.1 Hazards and Controls

Hazards and controls for process and product water are the same as for drinking water (see Sect. 21.2.1.1)

#### 21.3.1.2 Spoilage and Controls

Spoilage and controls for process and product water are the same as for drinking water (see Sect. 21.2.1.2).

### *21.3.2 Microbial Data*

Table 21.2 summarizes useful testing for processing and product water. Refer to the text for important details related to specific recommendations.

**Table 21.2** Testing of processing and product water for microbiological safety and quality

| Relative importance | | Useful testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Critical ingredients | Low | Incoming purchased water can be considered an in-process sample of the distribution system | | | | | | | |
| In-process | High | Test water for residual biocidal agents, where appropriate and depending on the biocides used | | | | | | | |
| | Medium | Test drinking water in the distribution system for coliforms or other appropriate indicators for verification using end product criteria<br>For water used to wash or transport vegetables, fruits, etc. that will be further processed (including a kill step), higher levels of indicator organisms or even the sporadic presence of pathogens may be accepted<br>Analysis of water used in processing plants for an extended number of microbiological parameters may be required by regulators for verification, with a minimum number of samples per year<br>For off-flavors or off-odors, investigative sampling to determine the root cause is useful | | | | | | | |
| Processing environment | – | Not relevant for process or product water | | | | | | | |
| Shelf life | – | Not relevant for process or product water | | | | | | | |
| End product | Medium | Testing for indicators is essential to verify control of process after treatments (if applied) and during distribution in or closed systems | | | | | | | |
| | | | | | | Sampling plan and limits/100 mL[b] | | | |
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | | Process water | Coliforms | ISO 9308-1 | NA | 1 | 0 | 0 | – |
| | Low | Testing for pathogens is not recommended to verify control process and is only applied in investigation in case of positive results in tests for hygiene indicators. For this reason no specific sampling plan is provided | | | | | | | |

*NA* not applicable
[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans

#### 21.3.2.1 Critical Ingredients

Water is the only ingredient for process and product water (see Sect. 21.3.2.2).

#### 21.3.2.2 In-Process

Monitoring of purchased drinking water for residual biocides is relevant at the point of entry in the factory and at different points in the distribution system, including the most remote one. This allows rapid detection of issues and implementation of corrective actions, such as additional biocidal treatments, when needed. Usually, microbiological testing is performed only periodically as a verification. For process or product water, the most frequently used hygiene indicator is the coliform group. However, *E. coli*, fecal coliforms or enterococci may be used depending on the situation, type of products manufactured or distribution system.

However in the absence of biocides, for specially treated water or water in individual closed circuits, an increased frequency testing is recommended.

#### 21.3.2.3 Processing Environment

Sampling of the processing environment is not relevant for processing and product water.

#### 21.3.2.4 Shelf Life

Shelf life testing is not relevant for processing and product water.

#### 21.3.2.5 End Product

Considerations for in-process samples are applicable to end product for process or product water, but samples are taken at the point of use (e.g., used as an ingredient for reconstitution or rehydration of dry ingredients).

## 21.4 Packaged Waters

Two types of bottled water are considered, namely spring or mineral water and other bottled water. Spring and mineral (natural) waters are drawn from underground sources such as boreholes or springs, and must fulfill compositional requirements as defined by the national or international bodies. In Europe labeling as "natural" allows only limited treatments, such as the separation of iron, manganese and sulfur compounds but no bactericidal treatment before bottling (EC 2009).

Bottled water can originate from springs and wells or drinking water from the distribution system. Such water can be submitted to different types of treatments before bottling such as carbonation, distillation, ionization, etc. Bactericidal treatments such as filtration, UV-treatment or ozonation are also permitted.

A thorough review of the different categories of water, including regulations has been published by Dege (2005).

### *21.4.1 Significant Organisms*

#### 21.4.1.1 Hazards and Controls

Pathogens such as *Salmonella* spp., *Campylobacter* spp. or viruses are occasionally found in bottled water surveys. Although sporadic cases of human illness have been reported such as one attributed to *Salmonella* (Palmer-Suárez et al. 2007) or *Pseudomonas aeruginosa* (Eckmanns et al. 2008), these products are rarely associated with outbreaks (ICMSF 2005) and, in several cases no definitive link has been demonstrated.

The absence of pathogens is ensured by the application of GHP from the source to bottling of natural waters and appropriate treatments and prevention of recontamination before bottling. Although, the role of *Pseudomonas aeruginosa* as cause of waterborne diseases remains unclear, it is considered a relevant indicator organism by certain Public Health Authorities but as a pathogen by others.

#### 21.4.1.2 Spoilage and Controls

The control measures for pathogens are also effective in preventing spoilage, and only rare cases of visible growth of molds or *Streptomyces* spp., leading to visual or sensory deviations have been described.

## *21.4.2 Microbial Data*

### 21.4.2.1 Critical Ingredients

For natural mineral water, the water pumped from the source is the only ingredient and microbiological requirements are regulated. For bottled water, the water itself can be considered a critical ingredient; however, biocidal treatments such as ozonation or UV-treatment are usually applied to the manufacture bottled water.

### 21.4.2.2 In-Process

Sampling and testing for general hygiene indicators such as heterotrophic counts or specific indicators such as *E. coli* or coliforms, is normally performed on a regular basis. The choice of the sampling points depends on the design of the processing line and the presence of elements such as intermediate storage tanks, the distance of filling from the catchment, etc. It is of particular importance to assess the absence of recontamination from biofilms building up on product contact surfaces in equipment such as pumps, pipes and storage or balance tanks. Testing for *Salmonella* spp. or *P. aeruginosa* may be performed as well for surveillance, but at a much lower frequency than for indicators.

### 21.4.2.3 Processing Environment

Sampling of the processing environment is not relevant for packaged waters.

### 21.4.2.4 Shelf Life

Shelf life testing is not relevant for packaged waters.

### 21.4.2.5 End Product

The Code of Hygienic Practice for Natural Mineral Water (Codex Alimentarius 1985) provided a two step approach in terms of testing; a first examination on one sample of 250mL, followed by a second examination of four samples depending on the extent of the initial deviation. A revision of the Code of Hygienic Practice for Natural Mineral Water was initiated in 2010 to align this Code with the General Principles of Food Hygiene (Codex Alimentarium1969) and to eliminate discrepancies with the Codex Alimentarius Commission Standard for Natural Mineral Waters (Codex Alimentarius 1981). The criteria summarized in Table 21.3 reflect the proposed criteria (Codex Alimentarius 2010), which specify the absence of several indicator organisms, including *P. aeruginosa*, to demonstrate strict control over a potential recontamination with pathogens. For natural spring or mineral waters, testing for heterotrophic counts is only useful at source, during processing and within 12h of filling since during subsequent storage and distribution the natural microbiota will develop.

In terms of microbiological requirements, the General Standard and Recommended Code of Hygienic Practice for Bottled/Packaged Drinking Water (Other than Natural Mineral Water) (Codex Alimentarius 2001a, 2001b) refer to the application of the WHO Guidelines for Drinking Water. National or supranational regulations are aligned with the WHO Guidelines or have adopted more stringent criteria or additional parameters. For more details refer to Table 21.1.

**Table 21.3** Testing of natural mineral water for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | Testing for heterotrophic plate counts (22°C and 37°C) and hygiene indicators provide valuable information on the hygiene status of the catchment. Levels of 100 CFU/mL (22°C) and 20 CFU/mL (37°C) are used as limits |
| In-process | High | Depending on the layout and complexity of the line testing for heterotrophic plate counts is performed to assess the hygiene status of the lines and in particular to detect the build-up of biofilms. Target levels are as above |
| Processing environment | – | Not relevant |
| Shelf life | – | Not relevant |
| End product | High | Testing for indicators is essential to verify control of process from the source to the filling |

| Product | Microorganism | Analytical method[a] | Case | Sampling plan and limits/250 mL[b] | | | |
|---|---|---|---|---|---|---|---|
| | | | | *n* | *c* | *m* | M |
| Natural mineral water | *E. coli* | ISO 9308-1 | NA[c] | 5[d] | 0 | 0 | – |
| | Coliforms | ISO 9308-1 | NA | 5[d] | 0 | 0 | – |
| | Enterococci | ISO 7899-2 | NA | 5[d] | 0 | 0 | – |
| | *P. aeruginosa* | ISO 16266 | NA | 5[d] | 0 | 0 | – |
| | Spore forming, sulfite reducing anaerobes | ISO 6461-2 | NA | 5[d] | 0 | 0 | – |
| | | | | Sampling plan and limits/mL[b] | | | |
| | Heterotrophic plate count/Aerobic colony counts[e] | ISO 4833 | NA | 5 | 0 | $10^2$ | – |

[a] lternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] NA = Not applicable due to use of Codex criteria proposed in 2010 (Codex Alimentarius 2010)
[d] Individual 250 mL analytical units
[e] At the source, during production or within 12 h after bottling

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# Chapter 22
# Eggs and Egg Products

## 22.1 Introduction

Eggs and egg products represent a large commodity group and are consumed as eggs or as ingredients in many further processed products. This chapter includes appropriate testing related to the safety and quality of avian eggs and egg products, primarily from the domestic chicken. However, the discussion is equally applicable to eggs from other species such as ducks. Eggs are largely marketed as shell eggs and pasteurized egg products (liquid, frozen or dried; whole, whites or yolk) and fully cooked egg products (refrigerated or frozen). Hens eggs and egg products are associated with foodborne disease outbreaks, some of which involve a significant number of cases (Ayres et al. 2009; EFSA 2007; Lynch et al. 2006). Duck eggs have also been associated with outbreaks of foodborne disease (HPSC 2010). *Salmonella* is the most common etiological agent involved in foodborne diseases from eggs in the USA (Ayres et al. 2009) and Europe (Food Safety Authority 2007). In contrast, *Campylobacter* which is a common cause of poultry related foodborne disease is infrequently linked to egg products (Ayres et al. 2009; EFSA 2007; Lynch et al. 2006). FAO/WHO (2002) conducted a risk assessment on *Salmonella* in eggs.

Egg products are typically used in foods that are cooked or handled in such a way that *Salmonella* spp. are destroyed. However, contaminated egg ingredients entering a facility present a potential hazard for contaminating other food products. Egg products are frequently used as a substitute for traditional shell eggs, both in the home and food service operations. Products such as meringue pie, mousses, eggnog, or dry diet mixes, when insufficiently cooked, remain potential hazards from salmonellae that might have survived or were re-introduced after pasteurization.

Refer to *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (ICMSF 2005) for detailed information on the microbial ecology and control of eggs and egg products. From primary production to the point of consumption, control measures should be used to achieve the appropriate level of public health protection. Good agricultural and hygienic manufacturing practices should be implemented during primary production, shell egg processing and egg product processing. Guidance is provided in international standards on hygienic practice for eggs and egg products, hygienic principles and HACCP, and transport of foods in bulk and semi-packed food (Codex Alimentarius 2007, 2003 and 2001, respectively).

## 22.2 Primary Production

Eggs become contaminated with *Salmonella* by two primary means, trans-ovarian or trans-shell infection. Prevention of *Salmonella* in laying flocks requires application of testing and control measures from hatchery supply flocks through to the laying flocks themselves. Important control measures include

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_22,

appropriate farm measures, such as rearing of flock controls, farm hygiene, elimination of contaminated flocks, vaccination, competitive exclusion techniques and disinfection of facilities between flocks (Codex Alimentarius 2007). A number of control programs have included microbiological testing of the laying environment (fluff, dust) to identify infected flocks, but no international agreement has been achieved on the efficacy of this approach and the actions that should be taken once a positive flock has been identified. National and regional programs have been implemented for detection and elimination of *Salmonella* Enteritidis (SE), which is the primary concern for transovarian infection of eggs. Positive flocks are either eradicated or all eggs produced are diverted to further processing and pasteurization in regions where egg associated SE illness is an issue. The need for such a practice for local and domestic use must be carefully assessed, because shell eggs are a valuable source of protein in some regions.

## 22.3 Shell Eggs

### *22.3.1 Significant Organisms*

#### 22.3.1.1 Hazards and Controls

*Salmonella* is the primary pathogen of concern, especially SE, and control of both trans-ovarian and trans-shell contamination is needed. Control consists of on-farm practices, chilling of eggs following collection and during transport, removing cracked eggs from shell egg commerce, avoiding free water on eggs and condensation due to changes in temperature, and washing of eggs with a biocide where this is allowed. Washing is an important step for removal of debris containing organisms which allows for proper egg sanitizing and inspection for cracked eggs. When washing, it is important for the wash water temperature to be higher than the internal egg temperature to minimize opportunities for ingress of microorganisms into the pore structure which would protect them from biocides and facilitate reaching internal contents of the egg. Wash water pH is typically above 10 which also aides in the egg cleaning step prior to sanitizing. Cooling shell eggs to 7°C or below is a requirement in some countries. However, in many countries refrigeration is not readily available and eggs are distributed at ambient temperature. Cooling eggs to 7°C or below prevents the growth of salmonellae but also prolongs their survival. Further, disruption of the cold chain increases the risk of condensation, which facilitates egg penetration by salmonellae. This has led to a recommendation for a quantitative assessment on the benefits and adverse consequences of egg cooling (EFSA 2009). Storage on-farm before collection and prior to distribution or further processing should be under suitable relative humidity, i.e., 70–85% RH.

*Campylobacter jejuni* does not readily penetrate the egg shell and trans-ovarian transfer does not appear to occur. Further, *Campylobacter* does not survive well on the egg surface; therefore testing for the organism is of little significance for the safety of eggs.

Whole shell eggs may be in-shell pasteurized to control *Salmonella* and enhance safety. This practice, however, may alter the functional properties of eggs used for such purposes as whipping egg whites for cakes and meringues unless pasteurization temperatures are well controlled.

*Staphylococcus aureus* enterotoxin has been found on occasion in shell eggs, primarily associated with incubator rejects; i.e., infertile eggs that have been held in incubators. Because these eggs are held at high temperatures, there is a risk associated with *S. aureus* enterotoxin production inside the egg. Incubator rejects should not be used as table eggs or for breaking stock. The FDA, USDA and European Union prohibit the commercial use of any egg that has been subject to incubation. Because refrigeration of eggs is not required in many countries, *S. aureus* enterotoxin production may be a risk for eggs of lower quality (cracks and checked eggs) in addition to incubator rejects. The low

prevalence of *S. aureus* enterotoxin in shell eggs does not warrant testing; however, cracked eggs should not be used as shell eggs in commerce. Their use is discouraged in further processing of egg products because *S. aureus* enterotoxin is heat stable.

#### 22.3.1.2 Spoilage and Controls

A major cause of spoilage during and immediately after removal of shell eggs from storage is fluorescent pseudomonads. Besides pseudomonads, a limited number of other bacteria are capable of acting as primary invaders of shell eggs. Examples include strains of the genera *Alcaligenes*, *Proteus*, *Flavobacterium* and *Citrobacter*. Control measures for spoilage are based on controlling egg shell penetration and growth.

The practice of oiling eggs using food grade oil under hygienic conditions after washing, which removes the protective cuticle, can be used to maintain quality and slow microbial penetration into the egg. In countries where refrigeration is not common and seasonal fluctuations in egg production require storage of shell eggs over several months to assure a consistent supply in the market, a requirement for shell oiling may be considered.

### *22.3.2 Microbial Data*

Table 22.1 summarizes useful testing for shell egg products. Refer to the text for important details related to specific recommendations.

#### 22.3.2.1 Critical Ingredients

Eggs marketed as shell eggs should be from SE-negative flocks (Sheenan and van Oort 2006). Testing for SE as described in primary production is essential for SE control. To maintain an SE-negative flock, feed must be produced in a way that controls *Salmonella*. *Salmonella* control methods may include heat treatments, use of biocides or other methods. Testing may be useful for verification if there is limited history with the feed supplier. Refer to Chap. 11, for additional information.

**Table 22.1** Testing of shell eggs for microbiological safety and quality

| | Relative importance | Useful testing |
|---|---|---|
| Primary production | Medium | Monitoring of layer flocks for SE and other salmonellae using procedures adopted by national or regional authorities |
| Critical ingredients | Low | There are no ingredients in shell eggs; however, consideration should be given to the source of feed (see text) |
| In-process | Medium | Periodic or continual monitoring of biocide levels and relevant physical parameters such as temperature and pH of egg wash water (see text)<br>May test for indicator organisms if wash water is recycled<br>Monitor temperature during chilling and storage of fresh eggs |
| Processing environment | Low | Indicators may be useful to verify sanitation and general hygienic conditions (see text) |
| Shelf life | Low | Not relevant |
| End product | Low | Periodic testing on a plant level or national surveys to monitor trends and provide information for verification of adequacy of control programs over time |

#### 22.3.2.2 In-Process

Egg washing is a practice that is not allowed in all countries. For example, hens egg washing is prohibited in the EU. However, where washing is permitted, businesses should monitor the level of biocide used in egg wash water to assure that it remains at an effective level. Biocides used must comply with local regulations and may include chlorine, calcium hypochlorite, quaternary ammonium compounds, iodine and others (ICMSF 2005). Regulations usually require registration and specific use instructions for egg washes. These instructions should guide use limits and appropriate methods to test for concentrations of the biocide. Wash water temperatures should be monitored to ensure that the temperature of the wash water is 5.5C° above the egg temperature (Board 1980). Recommendations for the temperature of wash water vary, and can be 11C° above egg temperature or even higher (EFSA 2005). Wash water pH above 10 should also be considered as an important part of the egg shell cleaning process.

Enterobacteriaceae may be a useful indicator for process control for egg wash water, especially if water is recycled and if antimicrobial treatments are not allowed. With increasing focus on water reuse for sustainability reasons, a variety of practices may continue to evolve. Typical levels of indicator organisms will vary depending on the process used.

Candling, or observing for cracks in shell eggs, is an important monitoring procedure. Cracks in eggs can allow entry of pathogens and spoilage organisms into the shell eggs. Cracked eggs should be removed from shell egg distribution channels.

#### 22.3.2.3 Processing Environment

Total colony count or Enterobacteriaceae may be useful to verify sanitation and general hygienic conditions. Levels encountered may vary by sample site and should be compared to internally developed guidelines.

#### 22.3.2.4 Shelf Life

Shelf life testing of shell eggs is not usually conducted.

#### 22.3.2.5 End Product

Routine microbiological testing of shell eggs for salmonellae is not recommended due to the low frequency and levels of contamination. However, testing may be useful for national surveys to monitor trends and provide information for verification of the adequacy of control programs over time.

## 22.4 Liquid and Frozen Eggs

Shell eggs may be separated from their shells to produce liquid egg products. Eggs are received, washed, rinsed, sanitized, then candled to identify and remove eggs with imperfections before breakage. The liquid egg may be homogenized as whole egg or separated into white and yolk. Whole or separated eggs are filtered to remove shell particles and chilled before pasteurization. The times and temperatures for pasteurization vary depending on the product. Post-pasteurization, all liquid egg products should be chilled, filled into containers or tankers, and shipped refrigerated

or frozen. After chilling, liquid eggs might also be stored in a refrigerated state and used to produce fully cooked egg products. Salt, sugar, or acidulants may be added to liquid eggs destined for further processing.

## *22.4.1 Significant Organisms*

### 22.4.1.1 Hazards and Controls

Eggs used for the production of liquid egg may include eggs from *Salmonella*-positive flocks; however, adequate pasteurization will inactivate salmonellae, including SE, the most important pathogen in liquid egg. However, the heat treatment of liquid egg products is limited by the heat coagulation of egg proteins and *Salmonella* spp. are occasionally isolated. For example, *Salmonella* detection in 100 g samples of liquid whole eggs and liquid egg whites was 0.3% and 0.6% from 1995 to 2008 (USDA/FSIS 2009). *Listeria monocytogenes* may also demonstrate similar survival and it may grow in pasteurized whole liquid eggs during refrigerated storage. USDA baseline survey results from 2001 to 2003 found *L. monocytogenes* levels to be below 2% incidence in whole egg and yolk at levels typically below 1 cell/g. *L. monocytogenes* was not found in liquid egg whites. Current epidemiological data do not suggest that liquid egg products are a significant cause of foodborne listeriosis.

Proper facility design to separate raw product areas from areas packaging pasteurized liquid eggs is very important for controlling cross contamination. Unless eggs are already clean, they should be washed immediately before the breaking operation. This should be done in a separate room from the breaking operation to prevent cross-contamination. Pasteurization temperatures and times for liquid egg required by various countries vary substantially, with process criteria ranging from 4 to >6D reductions of salmonellae. Ingredients added to liquid egg before pasteurization may also alter time/temperature requirements. The process must be validated for such products.

Liquid egg products should be quickly chilled to below 7°C after breaking and pasteurization. Alternatively, freezing can be applied. Strict procedures to prevent cross contamination should be used in the pasteurization room, including procedures for connecting pipes for carrying pasteurized and chilled liquid egg to storage tanks for holding prior to packaging.

### 22.4.1.2 Spoilage and Controls

The contaminating microorganisms at time of breaking are primarily those on the shell and within the occasional egg. Pasteurization destroys microorganisms such as *Pseudomonas*, *Acinetobacter* and *Enterobacter* spp., which grow in raw albumen and whole eggs. Spoilage organisms that may survive the process include mesophilic microorganisms like micrococci, staphylococci, *Bacillus* spp., enterococci and catalase-negative rods that are capable of growth if the product is temperature abused. Some of these bacteria (i.e., *Micrococcus*, lactic acid bacteria and some *Bacillus* species) may potentially grow under refrigerated storage to spoil product. Good hygienic practices post-pasteurization and during packaging are essential to control spoilage of refrigerated liquid egg products. Freezing for prolonged shelf life reduces spoilage concerns. Aseptic packing systems and those based on this concept are the best means for control along with good hygienic practices.

## *22.4.2 Microbial Data*

Table 22.2 summarizes useful testing for liquid and frozen egg products. Refer to the text for important details related to specific recommendations.

**Table 22.2** Testing of pasteurized liquid, frozen, dried and cooked egg products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | May be relevant for ingredients used in cooked egg products (see text) |
| In-process | High | Monitoring the pasteurization parameters is essential |
| | Medium | Testing of in-line samples can be used to verify hygiene and effectiveness of processing. Typical levels encountered post-pasteurization:<br>• Aerobic colony count $<5\times10^2$ CFU/g<br>• Enterobacteriaceae <10 CFU/g |
| Processing environment | High | Environmental monitoring for salmonellae is relevant where processed product is exposed before packaging. This is especially appropriate for dried product. Typical guidance levels:<br>• *Salmonella* – absent |
| | High | Collect sponge samples from large areas during production where cooked product is exposed before packaging. Typical levels encountered:<br>• *Listeria* species – absent |
| | Medium | Testing for indicator microorganisms is useful for liquid and cooked product to verify sanitation and hygienic conditions. See text for typical levels |
| Shelf life | Low | Shelf life testing is not relevant for frozen or dry egg products |
| | High | Shelf life for refrigerated liquid and cooked egg products should be evaluated using anticipated storage and distribution conditions (see text) |
| End product | Medium | Test for indicators for verification of control |

| Product | Microorganism | Analytical method[a] | Case | Sampling plan and limits/g[b] n | c | m | M |
|---|---|---|---|---|---|---|---|
| Pasteurized liquid, frozen, dry or cooked egg | Aerobic colony count[c] | ISO 4833 | 2 | 5 | 2 | $10^3$ | $10^4$ |
| | Enterobacteriaceae | ISO 21528-2 | 5 | 5 | 2 | 10 | $10^2$ |

Tests for pathogens when data indicate potential for contamination or when production conditions and history are not known

| | Product | Microorganism | Analytical method[a] | Case | Sampling plan and limits/25 g[b] n | c | m | M |
|---|---|---|---|---|---|---|---|---|
| High | Pasteurized liquid, frozen, dry or cooked egg products | *Salmonella* | ISO 6579 | 10[f] | 5[d] | 0 | 0 | – |
| | | | | 12[f] | 20[d] | 0 | 0 | – |
| High | Cooked egg products: Supports growth | *L. monocytogenes* | ISO 11290-1 | NA[e] | 5[c] | 0 | 0 | – |

| | | | | | Sampling plan and limits/g[b] n | c | m | M |
|---|---|---|---|---|---|---|---|---|
| Medium | No growth | *L. monocytogenes* | ISO 11290-2 | NA | 5 | 0 | $10^2$ | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Aerobic colony count not recommended for egg albumin
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[e] NA = Not applicable due to use of Codex criteria
[f] Case 10 for products to be cooked, case 12 for RTE applications with potential for abuse

#### 22.4.2.1 Critical Ingredients

Many different ingredients may be added to liquid egg products before pasteurization. Ingredients can be separated into six main categories:

1. Texturizing agents such as gums and starches
2. Acidifying agents such as citric acid and phosphates
3. Flavors such as butter flavor
4. Nutritional fortification agents such as vitamins and minerals
5. Preservatives such as salt or sugar
6. Whipping aides for whites such as tri-ethyl citrate

Microbial risks associated with *Salmonella* survival during pasteurization should be determined since addition of ingredients has the potential to increase the level of *Salmonella* or affect pasteurization efficacy.

#### 22.4.2.2 In-Process

Time and temperature monitoring of the pasteurization process is critical. Pasteurizers designed with regeneration sections (i.e., hot pasteurized liquid is used to warm cold, raw egg on the other side of the metal plate) should be maintained such that the pressure is higher on the pasteurized liquid side compared to the unpasteurized liquid side of the system. Temperature control before and after pasteurization is also important. In-process samples may be useful to confirm that the control measures are effective. Such samples may include representative samples from in-line filters and product prior to filling operations. In broken eggs before pasteurization, typical aerobic colony counts may range from $10^2$ to $10^5$ CFU/g, with counts above $10^6$ CFU/g indicative of hygiene or egg quality issues (Stadelman and Cotterill 1995). While the sampling frequency needs to be adapted to the situation in the factory, the samples should be selected to verify the system is in control and the end product criteria will be met. Use of process control and trend analysis is recommended to fulfill the same microbiological requirements as the finished product for *Salmonella* and indicators such as Enterobacteriaceae. Testing for α-amylase activity may be useful to verify pasteurization where eggs are processed at temperatures higher than 64°C for 2.5 min. For time/temperatures below these limits, amylase is not denatured therefore this test is of no value.

#### 22.4.2.3 Processing Environment

Processing equipment includes breaking utensils, pipes, pumps, heat exchangers, filters, pails, churns and holding tanks. Verification of equipment hygiene would apply. Environmental monitoring for salmonellae is useful in post-pasteurization areas to identify potential harborage sites, which could lead to post process contamination.

#### 22.4.2.4 Shelf Life

Shelf life should be established using appropriate spoilage microorganism tests that consider the distribution and storage conditions, as well as a reasonable assumption of the potential for abuse.

#### 22.4.2.5 End Product

Application of effective GHP and HACCP is essential to control salmonellae and spoilage microorganisms and to and prevent recontamination. If the conditions of manufacture are not known or if the

reliable application of GHP and HACCP is in question, testing for indicators (e.g., Enterobacteriaceae) and salmonellae is appropriate. Recommendations are made in Table 22.2.

The ICMSF (1986) proposed aerobic colony count, coliform and *Salmonella* criteria for liquid and frozen egg products. At the point of manufacture, aerobic colony count may provide information to verify the adequacy of the pasteurization process, as well as the general quality of the product produced. The aerobic colony count is not recommended for egg albumen intended for drying because growth of Group D streptococci may occur during de-sugaring. These bacteria are more heat resistant than microorganisms of concern such as *Salmonella* and will initiate growth at the pH of egg albumen. In this book, Enterobacteriaceae replaces coliform testing because it represents a broader group of organisms which should be inactivated during pasteurization.

Because pasteurized egg products are used in institutional settings (e.g., hospitals, long term care), more stringent sampling plans should be considered for products targeted for that market.

In Europe, egg products and ready-to-eat (RTE) foods containing raw egg are subject to a food safety criterion for *Salmonella* of $n=5$, $c=0$, $m=$absence in 25 g, and a process hygiene criterion for Enterobacteriaceae of $n=5$, $c=2$, $m=10$/g and $M=10^2$/g (EC 2005). The number of sampling units of the sampling plan may be reduced if the food business operator can demonstrate by historical documentation that effective HACCP-based procedures are in place. The USDA/FSIS (2009) standard method for pasteurized egg products examines 100 g of egg products for the presence of *Salmonella* ($n=4$, $c=0$, $m=$absence in 25 g).

## 22.5 Dried Eggs

Three methods are widely used for drying liquid egg products: spray-drying, pan or drum drying (drying on a heated surface), or freeze drying. Glucose removal before drying improves the stability of dried eggs. Pasteurized or unpasteurized liquid egg may be used as a starting material; if unpasteurized, hot storage following drying is employed to kill salmonellae. However, this control measure is feasible only for certain dried egg products due to decreased quality and functionality attributes.

### *22.5.1 Significant Organisms*

#### 22.5.1.1 Hazards and Controls

Salmonellae may occasionally be present in the final dried packaged product. Proper design should be utilized to separate high risk and low risk areas of the processing plant whenever possible. Control measures include proper equipment (impervious materials without cracks, crevices and dead-end pockets); sanitation of equipment and proper process hygiene; avoiding recontamination during processing and packaging; maintaining dry product, production and storage environments. Hot storage (e.g., 55°C for 7 days) may reduce levels of salmonellae, with reductions influenced by moisture levels, temperature and time of holding. Dried egg products may be used in other products that may not be subject to a process that is lethal to *Salmonella*. Therefore control of salmonellae is important when dried eggs are used as an ingredient in such products. The Grocery Manufacturer's Association has provided guidance on control of salmonellae in dry environments (GMA 2009).

#### 22.5.1.2 Spoilage and Controls

Spoilage bacteria may survive, but will die slowly over time in dried product. Maintaining dry conditions during processing and storage is essential.

### *22.5.2 Microbial Data*

Table 22.2 summarizes useful testing for dried egg products. Refer to the text for important details related to specific recommendations.

#### 22.5.2.1 Critical Ingredients

There are no critical ingredients in dried eggs.

#### 22.5.2.2 In-Process

Monitoring time and temperature is essential for products that are pasteurized using heat after packaging. In-process samples play an important role to confirm that control measures are effective, particularly between drying and filling. Typical samples are the first dried product manufactured, and samples where residues or lumps occur. The sampling frequency needs to be adapted to the conditions of the factory. The samples should be selected to verify the system is in control and the end product criteria will be met. Use of process control and trend analysis is recommended.

#### 22.5.2.3 Processing Environment

Verification of equipment hygiene is important for dried egg processing. Controls should also be established to minimize condensation and moisture in the processing environment and inside storage/shipping vessels. The major cause of *Salmonella* or Enterobacteriaceae in finished product is recontamination from the processing environment. Thus, environmental samples play a key role in verifying effectiveness of the preventive measures. Testing for *Salmonella* and Enterobacteriaceae can be used to indicate the effectiveness of GHP.

#### 22.5.2.4 Shelf Life

Dried eggs are shelf-stable; therefore shelf life testing is not relevant.

#### 22.5.2.5 End Product

End product testing recommendations for dried egg are similar to those for liquid and frozen egg products (see Table 22.2). The aerobic colony count is not recommended for dried egg albumen because growth of Group D streptococci may occur during de-sugaring. These bacteria are more heat resistant than organisms of concern such as *Salmonella* and will initiate growth at the pH of egg albumen. Routine sampling for *Salmonella* is recommended for the manufacturer because of the history of outbreaks with egg products. Enterobacteriaceae is a useful indicator of process control.

Because pasteurized egg products are used in institutional settings (e.g., hospitals, long term care), more stringent sampling plans should be considered for products targeted for that market.

## 22.6 Cooked Egg Products

In 2011, the vast majority of egg products are sold in liquid or dried forms but the market for fully cooked eggs such as omelets, egg patties, French toast, scrambled eggs and hard cooked eggs is growing. These items are perishable and must be kept refrigerated or frozen.

## 22.6.1 Significant Organisms

### 22.6.1.1 Hazards and Controls

*Salmonella* and *L. monocytogenes* are the principle hazards to consider for cooked egg products. Baseline levels for *Listeria* in raw liquid egg were established by the United States Department of Agriculture (USDA) in 2001–2003 with only 2% of all whole egg and yolk samples containing *L. monocytogenes*. Levels were typically in the range of <1 CFU/g and all results were below 4 log MPN/g (Victor Cook, personal communication). *L. monocytogenes* was not found in any liquid egg white sample taken. *Listeria* cannot grow while egg products are maintained in a frozen state.

*Salmonella* is the principle hazard of concern especially in those countries where refrigeration of shell eggs is not required prior to breaking or processing. Baseline levels for *Salmonella* in raw liquid egg were established by the USDA in 2001–2003. *Salmonella* was found in over 70% of all raw liquid egg samples taken at levels ranging from not detected to 5 log MPN/g (Victor Cook, personal communication). *Salmonella* grows well in liquid whole egg and yolk but cannot multiply if products are maintained below about 7°C.

*Salmonella* and *L. monocytogenes* are controlled using validated cooking procedures managed through the HACCP plan. Recontamination is managed through the application of general principles of food hygiene (Codex Alimentarius 2003). *L. monocytogenes* recontamination is also managed through effective application of Codex Alimentarius Commission procedures designed for *Listeria* control to include verification by environmental monitoring (Codex Alimentarius 2007).

Little information exists related to potential spore former incidence such as *Clostridium* species in cooked egg products, but where a hazard is identified procedures used to control spore former growth in cooked meat will also apply to cooked eggs.

### 22.6.1.2 Spoilage and Controls

Cooked egg product spoilage depends on numerous factors such as storage temperature, numbers and types of microorganisms, ingredients used as part of formulation and type of finished product packaging. Under aerobic packaging, spoilage is caused by pseudomonads, *Serratia* species, yeasts, molds and other microorganisms found in egg processing plants. Yeasts and molds are also capable of spoiling hard cooked eggs packed in high acid brines. Low pH of brine packed eggs will slow growth of spoilage microorganisms; however, pH is normally buffered by the egg over time which allows for spoilage bacteria to grow. Control is best achieved by implementing procedures related to sanitation, personal hygiene and other prerequisite programs to prevent recontamination of spoilage microorganisms after cooking. Controlling sanitation practices during cooling, peeling and packaging is necessary for hard cooked eggs.

## 22.6.2 Microbial Data

Table 22.2 summarizes useful testing for cooked egg products. Refer to the text for important details related to specific recommendations.

### 22.6.2.1 Critical Ingredients

The nonegg ingredients in cooked egg products are rarely a source of significant pathogen or spoilage microbiota unless ingredients are added to cooked egg products after cooking or other lethality step.

Some ingredients (e.g., nisin, benzoate, sorbate, citric acid, acetic acid) can reduce the rate of spoilage and growth of *L. monocytogenes* or other Gram-positive microorganisms.

#### 22.6.2.2 In-Process

In-process samples are recommended for validation of time/temperature conditions during establishment of cooking CCPs and to verify controls after modifications are made to established cooking systems. In-process samples are also useful when investigating problems. Routine sampling for *Salmonella* is not recommended since the risk associated with this pathogen is best controlled through GHP and HACCP.

#### 22.6.2.3 Processing Environment

Environmental testing focuses on control of *L. monocytogenes* since it is a significant concern for products that have a long refrigerated shelf life and support its growth. Control of *Listeria* will also effectively control spoilage microorganisms and *Salmonella*.

Of highest concern are products with longer than 10 days refrigerated shelf life that (1) support *L. monocytogenes* growth during normal storage/distribution, (2) do not have validated growth inhibitors, (3) do not have a listericidal treatment after final packaging and (4) are intended for consumers who are susceptible to listeriosis. The frequency and extent of sampling should reflect history of public health issues seen in the industry and specific to the location of production.

Sampling of contact surfaces, indirect contact surfaces and environmental areas (e.g., floors, drains), and post cooking before the final package is recommended (Codex Alimentarius 2007). Sponge samples from large areas should be collected during production. The benefit of environmental sampling for products given a validated listericidal treatment after final packaging is questionable.

Some processors use indicator microorganism testing as a means to monitor changes in general microbiota after significant control of *Listeria* is achieved and when environmental *Listeria* monitoring yields very few positives for all areas sampled. However, use of indicator organisms should be tied directly to control of *Listeria* for such a program to be meaningful.

Spoilage microorganism control and monitoring in the processing environment is best accomplished using an approach similar to that for cooked meat processing. Swab or sponge samples can be collected before the start of operations to verify the effectiveness of cleaning and sanitizing. Analysis for aerobic colony count is a common method. Typical aerobic colony counts on thoroughly cleaned and sanitized food contact surfaces are $<10^2$ CFU/cm$^2$. Higher numbers will be encountered during production.

#### 22.6.2.4 Shelf Life

Finished product shelf life can be validated by holding the product at a controlled temperature and performing sensory evaluation, in conjunction with microbiological analysis at selected intervals, including packages before, on and after the expected expiration date. For cooked egg products, unacceptable sensory characteristics are typically found before microbial spoilage is observed. Therefore, sensory analysis is primarily recommended for establishing shelf life for cooked egg products. It is recommended that shelf life validation be performed to mimic expected storage conditions as well as labeled storage requirements. Subsequent shelf life verification can be performed at a frequency that reflects confidence that the product will consistently meet the stated expiration date on the package.

Validation that growth of *L. monocytogenes* will not occur within the expiration date on the package may be of interest in some regions (Scott et al. 2005).

#### 22.6.2.5 End Product

It is recommended to test for indicator microorganisms (e.g., aerobic colony count, Enterobacteriaceae) for ongoing control and trend analysis. Typical aerobic colony counts are $<10^3$ CFU/g for cooked egg products and Enterobacteriaceae counts are usually <10 CFU/g.

Processors should apply validated HACCP plans to eliminate *Salmonella* and *L. monocytogenes* and apply effective GHP to prevent recontamination from microorganisms in the processing environment. If the reliable application of GHP and HACCP is in question, sampling for *Salmonella* and *L. monocytogenes* may be appropriate. When evidence indicates a potential for contamination with *L. monocytogenes* (e.g., positive food contact surface) sampling the food should be considered.

The *Salmonella* sampling plan in Table 22.2 is for foods in which *Salmonella* will not grow under normal conditions of distribution and storage (i.e., case 11). Sampling plans for *L. monocytogenes* are for ready-to-eat foods produced following the general principles of food hygiene for control of *L. monocytogenes* and with an appropriate environmental monitoring program (Codex 2007). For products that do not support growth of *L. monocytogenes*, sampling plans presented will provide 95% confidence that a lot of food containing a geometric mean concentration of 93 CFU/g with a standard deviation of 0.25 log CFU/g would be detected and rejected based on any of five samples exceeding $10^2$ CFU/g. Such a lot may have 55% of the samples below $10^2$ CFU/g and up to 45% of samples above $10^2$ CFU/g but only 0.002% of all the samples from this lot could be above$10^3$ CFU/g.

The typical actions to take when end product pathogen testing criteria are not met would be to (1) prevent the affected lot from being released for human consumption, (2) recall the product if it has been released for human consumption, (3) determine and correct the root cause of the failure, and (4) verify the effectiveness of the corrective action(s) going forward. For process hygiene criteria (EC 2005), these set an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with European food law.

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# Chapter 23
# Milk and Dairy Products

## 23.1 Introduction

This chapter groups a wide range of products manufactured with milk obtained from cows. They are manufactured using a wide variety of technologies and processing conditions and encompass commodities such as fluid milk, milk powders and traditional products such as cheese and other fermented milks. References on milk obtained from other animals such as sheep, goats, buffaloes, camels or horses can be found in ICMSF (2005), which also discusses different processing technologies and their impact on the microorganisms in finished products.

The Codex Alimentarius Commission (2009) established the code of hygienic practice for milk and milk products, and definitions of several products are established such as those for evaporated milk (Codex Alimentarius 1971a), sweetened condensed milk (Codex Alimentarius 1971b), whey cheese (Codex Alimentarius 1971c), cream and prepared cream (Codex Alimentarius 1976), cheese (Codex Alimentarius 1978), fresh cheese (Codex Alimentarius 2001) and milk and cream powder (Codex Alimentarius 1999a). A detailed listing of all definitions used for dairy products can be found in the *General Standard for the Use of Dairy Terms* (Codex Alimentarius 1999b). Other products such as fluid milk or cream are normally differentiated based on local regulations. Ice cream and ice milk are formulated milk products intended for consumption in the frozen or partially frozen state.

## 23.2 Raw Milk for Direct Consumption

Raw milk contains numerous microorganisms that originate from the animal itself. Levels and composition of the initial microbiota are influenced by factors such as the health status of animals including udder disease, fecal contamination of the udder, antimicrobial systems in the milk, and inhibitory substances or veterinary drugs used to treat diseased animals.

Additional secondary contamination originates from the environment (bedding, milking machines, air etc.) as well as from persons handling the milk. Details of these different factors can be found in ICMSF (2005).

### *23.2.1 Significant Organisms*

Significant zoonotic agents such as *Brucella* spp. and *Mycobacterium bovis* have been eradicated from animal stocks and no longer play an important role. *Salmonella* spp., verotoxigenic and

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enterohemorrhagic *E. coli* (EHEC), *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus* spp., *Yersinia* spp. and *Coxiella burnetii* are the most frequent pathogens and several publications have been published on the subject (Jayarao et al. 2006; Oliver et al. 2005, 2009; LeJeune and Rajala-Schultz 2009).

Numerous other microorganisms such as lactic acid bacteria, micrococci, *Bacillus* spp., Enterobacteriaceae, *Pseudomonas* spp., *Mycobacterium avium* subsp. *paratuberculosis,* etc. are also part of the initial biota of raw milk. Composition and levels found depend on the health status of the herds and the hygiene conditions under which the milk is collected (Chambers 2005; Hantsis-Zacharov and Halpern 2007; Eltholth et al. 2009; Aly et al. 2010). Details on both pathogens and commensals can be found in ICMSF (2005).

#### 23.2.1.1 Hazards and Controls

Pathogens are likely to be present in raw milk but low levels can be maintained if appropriate hygiene programs are implemented to control the initial contamination. Such programs include:

- Mastitis control programs
- Farm management and environmental provisions including feed
- Milking machine and milking procedure hygiene programs
- On farm cooling programs

The effect of handling of raw milk on the microbiota is described in detail by ICMSF (2005), Verdier-Metz et al. (2009), Rysanek et al. (2009) and Sraïri et al. (2009).

Although reduction is possible, pathogens or spoilage microorganisms in raw milk cannot be completely eliminated and growth can take place readily. For this reason shelf life of raw milk, even when refrigerated, is limited. In many countries sale of raw milk for direct consumption is restricted or completely prohibited because of the potential risk to public health. Where sale of raw milk is allowed, it is usually sold directly at the farm or through local or regional organizations. Commercialization of such raw milk is subjected to specific requirements and must originate from certified herds. Certification includes strict rules on animal keeping, regular surveillance of their health status, frequent and extended microbiological testing of the milk, and provisions for labeling including an expiration date for the product.

Mycotoxins, especially aflatoxins B and G, which can be ingested by ruminants through contaminated feed and excreted in the milk as aflatoxin $M_1$, are relevant hazards in parts of the world (Elgerbi et al. 2004; Coffey et al. 2009; Prandini et al. 2009). Details on control measures are provided in Chap. 11.

#### 23.2.1.2 Spoilage and Controls

Spoilage can be caused by a wide range of microorganisms present in the raw milk and many undesirable sensory and physical changes in raw milk have been described. For details consult ICMSF (2005) and Ledenbach and Marshall (2009).

Control of spoilage is achieved through refrigeration of the raw milk and short storage periods before further processing.

### *23.2.2 Microbial Data*

Table 23.1 summarizes useful testing of raw milk intended for raw consumption. Refer to the text for important details related to specific recommendations.

**Table 23.1** Testing of raw milk intended for raw consumption for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | No additional ingredients than the milk itself. Milk should be obtained from healthy herds |
| In-process | High | Regular examination of animal health to exclude chronically diseased animals from production and prevent contamination of raw milk through diseased animals (e.g., mastitis). Typical guidance levels for on farm examination could be:<br>• Somatic cell count per animal $<3\times10^5$ – $5\times10^5$/mL and no detection of mastitis agents<br>• *Salmonella* absent in animals and serologically negative for *Coxiella burnetii*<br>• Other agents may be used depending on the relevance of pathogens for a specific region |
| Processing environment | Low | Testing of processing environment is of limited use and not recommended other than potentially monitoring the hygienic status of equipment |
| Shelf life | Low | Due to the very short shelf life of raw milk, testing for shelf life is not useful |
| End product | High | Testing for indicators can be used to verify hygiene control measures during milking and handling (trend analyses). Testing for aerobic colony counts is frequently performed to determine payment, usually without a specific sampling plan (e.g., one sample/supplier on a daily or periodic basis) |

| | | | | Sampling plan and limits/mL[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Raw milk | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $2\times10^4$ | $5\times10^4$ |
| | Enterobacteriaceae | ISO 21528 | 6 | 5 | 1 | 10 | $10^2$ |
| | *S. aureus* | ISO 6888 | 7 | 5 | 2 | 10 | $10^2$ |

These limits are appropriate for milk manufactured under the highest hygiene conditions found in developed and certain developing countries. Significantly higher levels may be observed in regions with unfavorable hygiene and temperature conditions in the supply chain. Under such conditions, limits should be adjusted as the situation improves

| | | |
|---|---|---|
| | Low | Testing for *Salmonella* and other pathogens in raw milk that will be submitted to a kill step is not recommended |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans

#### 23.2.2.1 Critical Ingredients

The raw milk itself is the only ingredient. Monitoring and maintenance of an appropriate health status in the herds is appropriate.

#### 23.2.2.2 In-Process

No routine microbiological testing is recommended. Milk should be examined to monitor heard health status. See Table 23.1 for guidance.

#### 23.2.2.3 Processing Environment

The hygienic status of equipment may be monitored prior to start up using rapid tests such as ATP. No routine microbiological testing is recommended.

#### 23.2.2.4 Shelf Life

Microbiological shelf life testing is not relevant for these products because shelf life is short.

#### 23.2.2.5 End Product

End product testing is usually performed to determine milk quality and payment scheme. High aerobic mesophilic counts indicate poor hygiene during milking and subsequent handling, and thus are usually penalized with reduced payments to the supplier.

For raw milk for direct consumption, strict requirements and control measures are established by authorities, as for example in Germany (Anonymous 2007). Regular testing for pathogens against established microbial criteria may also be included to demonstrate control over microorganisms of public health concern. Specific criteria are normally established by national or local authorities, as raw milk is only traded locally or regionally. These criteria may vary according to the epidemiological situation. For this reason no specific criteria are made in Table 23.1.

## 23.3 Processed Fluid Milk

Processed fluid milk is produced using heat treatments to reduce the initial microbiota of the raw milk. It may contain added ingredients such as flavors and vitamins, and can also be made from reconstituted dried milk. Different types of heat treatments exist, ranging from mild treatments such as thermization, to intermediate treatments such as pasteurization, to more severe treatments such as sterilization or UHT treatment (ICMSF 2005; Goff and Griffiths 2006). The severity of the treatment is normally related to the intended shelf life and storage conditions of the fluid milk, ranging from short shelf life under refrigeration to prolonged shelf life at ambient temperature.

### *23.3.1 Significant Organisms*

#### 23.3.1.1 Hazards and Controls

Low levels of vegetative and spore forming pathogens are likely to be present in raw milk and the levels and occurrence depend on several factors outlined in Sect. 23.2.

Thermization at temperatures ranging between 57 and 68°C for up to 30 s reduces vegetative microorganisms about 3–4 log cycles. However, it does not provide full control over pathogens and is usually applied only to extend shelf life of raw milk for a limited period of time before being further processed.

Pasteurization is applied to destroy vegetative pathogens and to extend the shelf life of the products during refrigerated distribution and storage. It may include treatments at low temperature for a long time (LTLT, 62–65°C for 30–32 min) or high temperature for a short time (HTST, ≥71°C for ≥15 s). Conditions are frequently regulated and may therefore vary from country to country. For example, in the United States, the HTST temperature used in practice is close to 80°C.

Sterilization and UHT treatments are performed as batch processes in closed containers or continuously with subsequent aseptic packaging. Conditions vary between 120°C for 10–30 min for sterilization and ≥135°C for a few seconds for the UHT. Such processes produce products that are commercially sterile and thus have a prolonged shelf life at ambient temperature. Other technologies such as microfiltration are not considered in this book.

Sporadic outbreaks due to the presence of pathogens such as *Salmonella* or *L. monocytogenes* in flavored and unflavored pasteurized milk have generally been shown to be due to postprocess contamination (ICMSF 2005; CDC 2008). Pasteurized milk was included in *L. monocytogenes* risk assessments on ready-to-eat foods and despite of the ability to grow in the product, the risk per serving was considered to be low (FAO/WHO 2004a, b).

#### 23.3.1.2 Spoilage and Controls

The pasteurization conditions described in Sect. 23.3.1.1 also eliminate vegetative bacteria and reduce spore forming psychrophilic spoilage microorganisms for refrigerated products. Processes discussed in the same section for shelf-stable products also eliminate, mesophilic or thermophilic spore forming spoilage microorganisms. As previously mentioned, post processing contamination can lead to spoilage concerns, thus strict control of hygiene in addition to pasteurization is essential for control.

### *23.3.2 Microbial Data*

Table 23.2 summarizes useful testing of processed fluid milk products for microbiological safety and quality. Refer to the text for important details related to specific recommendations.

#### 23.3.2.1 Critical Ingredients

Raw milk is the major ingredient used to manufacture fluid milk. However, in several countries the use of milk powder in reconstitution processes is common. Other ingredients, such as cocoa powder, sugar, fruit concentrates, thickeners and flavors may be added to produce pasteurized or sterilized flavored products. The relevant microorganisms for these ingredients are described in appropriate chapters in ICMSF (2005) and in this book. Addition of such ingredients does not affect the safety of products and testing for vegetative microorganisms (pathogens or indicators) is normally of limited use. Testing is normally performed only to ensure that ingredients are manufactured according to GHP, thus only as a periodic verification and not for lot acceptance.

The presence of spore formers in ingredients used for sterilized or UHT products is a relevant consideration. Certain ingredients, such as milk powder, cocoa powder or thickeners, can be the source of highly heat resistant spores and thus selection of these ingredients are considered critical to guarantee commercial sterility of the products. The presence of high spore counts may lead to spoilage problems, which can be overcome by the adjustment of processing conditions or through establishment of microbial specifications to ensure that maximal levels of spores are not exceeded. Typical specifications include limits of 10–$10^2$ CFU/g for mesophilic or thermophilic spores, depending on the processing conditions.

#### 23.3.2.2 In-Process

For this type of product, neither samples of intermediate products nor residues at critical steps are taken on a routine basis. However, investigative sampling is important for issues such as increased spoilage rates. Thorough investigation for weaknesses in the processing line or of the cleaning procedures may include microbiological sampling and testing in points such as balance or storage tanks, seals, pumps, valve clusters, plate heat exchangers or filling heads.

**Table 23.2** Testing of processed fluid milk products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low | Testing for vegetative pathogens or indicators is only useful to verify that ingredients have been manufactured applying GHP |
| | Medium | For sterilized or UHT products, testing for mesophilic and or thermophilic spore formers is useful for critical ingredients and in particular if the heat treatments applied are at the lower end of the scale. Typical industry standards are$10–10^2$ CFU/g |
| In-process | Low | Routine in-process testing is not recommended. It is important for trouble-shooting to identify potential sources of contamination. Such investigative sampling should include critical steps of the processing line such as the plate heat exchangers, fillers, and intermediate storage tanks |
| Processing environment | Low | Routine testing of the environment for vegetative and spore forming pathogens or spoilage microorganisms is not recommended. It can, however, be useful for trouble-shooting to identify potential sources of contamination (e.g., filter units, areas of the filling chamber or the fillers themselves) |
| Shelf life | Medium | For refrigerated products with extended shelf life (>17 days), shelf life testing may be useful to identify potential issues (see text) |
| End product | Low to high | Low for pasteurized products, high for sterilized or UHT products, for which testing and trend analyses to assess the performance of the line and detection of major deviations is recommended |

| | | | | Sampling plan and limits/mL[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Pasteurized milk[c] | Enterobacteriaceae | ISO 21528 | 5 | 5 | 2 | <1 | 5 |
| Sterilized or UHT products | Presence/absence tests for spoilage microorganisms | Incubate at 30 and 55°C (if suitable) for 10–14 and 5–7 days, respectively | Fixed numbers of samples up to 100% of batches depending on product type (see text) | Destructive and nondestructive methods | | | |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] EC (2005)

#### 23.3.2.3 Processing Environment

Testing of the processing environment is not recommended on a routine basis.

#### 23.3.2.4 Shelf Life

Microbiological shelf life testing is not relevant for commercially sterile, shelf-stable products. However, testing may be relevant for refrigerated products depending on the intended shelf life and distribution use patterns in a specific market. For example, shelf life testing on HTST milks is widely used in the US, where the shelf life of milks is typically >17 days and can range from 21 to 30 days. The risk of spoilage and potential pathogen growth may increase in these long shelf life products due to low levels of competing microorganisms. The Mosely Keeping Quality test is one method that has

been used to evaluate shelf life (Wehr and Frank 2004) while other more rapid tests may also be considered (Richter and Vedamuthu 2001) when long refrigerated shelf life is practiced.

#### 23.3.2.5 End Product

End product testing for pathogens is normally not performed for refrigerated pasteurized products due to their short shelf life. Testing for vegetative indicators such as mesophilic aerobes, Gram negatives or Enterobacteriaceae can serve as verification of the effectiveness of pasteurization conditions or control over recontamination at the end of manufacturing. See country or regional specific standards, for example EC (2005).

For sterilized or UHT products manufactured on well-performing lines, sampling and testing is of limited use for individual lots. However, incubation of product units taken at random and, for UHT products, when events such as start up or machine stoppage, changes of packaging rolls, etc. is frequently done to determine the performance of processing lines over prolonged periods of time. Such sampling and testing may detect major issues that could lead to high spoilage rates. Incubation is usually performed at 30°C to verify commercial sterility during distribution. Other temperatures may be relevant if, for example, the product will be distributed in tropical regions. Incubation of a limited number of samples at 55°C for short periods of time (5–7 days) is frequently done for monitoring or to meet local regulatory requirements, and will detect insufficient sterilization more rapidly than lower incubation temperatures.

Sampling regimes for incubation vary from limited numbers of units to 100% of the production for sensitive products such as liquid infant formulae. Testing after incubation is usually done by combining destructive methods such as pH determination, ATP measurement or classical microbiological tests using nondestructive methods such as vacuum tests or determination of changes in viscosity. It is important that the evaluation of such incubation results is performed using statistical tools such as cumulative trend analyses to assess the overall performance of the lines over time.

## 23.4 Cream

Cream is the fat rich fraction of the milk, which is usually obtained by skimming in centrifuges and separators. Classification depends on regulatory requirements and is usually based on the fat content: half-cream (12%) to double cream (48 and 53%). Product categories are similar to those described in Sect. 23.3.

### *23.4.1 Significant Organisms*

#### 23.4.1.1 Hazards and Controls

The composition of the microbiota of unprocessed cream is very similar to that of raw milk but the skimming processes applied may lead to a concentration of microorganisms in the fat phase. Therefore it is also likely that low levels of pathogens are present in unprocessed cream.

Because of the higher fat content and its protective effect on microorganisms, heat treatments applied are usually more severe than those for fluid milk (i.e., a few degrees higher or longer times).

#### 23.4.1.2 Spoilage and Controls

The quality of raw cream depends on the quality of the milk used for manufacture, but the microbiota is basically the same.

### *23.4.2 Microbial Data*

Since the microbiology and processes to manufacture cream products are similar to those of fluid milk, refer to Sect. 23.3.2 for further details.

## 23.5 Concentrated Milk

Concentrated milk is processed from either raw milk or after reconstitution of milk powders. They can be subdivided into three main groups: (1) condensed and evaporated milk, (2) sweetened condensed milk and (3) retentates obtained by reverse osmosis, micro-filtration or ultra filtration. These products have a reduced water content and their microbial stability is achieved through sterilization or combinations of milder heat-treatments with additional hurdles such as a low pH or the addition of sugar to lower the water activity to about 0.83–0.85.

### *23.5.1 Significant Organisms*

#### 23.5.1.1 Hazards and Controls

The same comments as for pasteurized and sterilized milks in Sect. 23.3.1 holds for concentrated milk and the primary concern is to control postprocess contamination. For sweetened condensed milk with a water activity of about 0.85, the only pathogen that may be able to grow is *S. aureus*. However under the anaerobic conditions in unopened packaging units both growth and enterotoxin formation are inhibited.

#### 23.5.1.2 Spoilage and Controls

Concentrated and evaporated milks are a favorable media for microbial growth and spoilage problems are usually the same as those observed for pasteurized or sterilized/UHT milk. For sweetened condensed milk, only osmotolerant micrococci or xerophilic fungi are able to grow and to cause spoilage.

Control is achieved in both cases through the application of GHP to avoid postheat treatment contamination.

### *23.5.2 Microbial Data*

Table 23.3 summarizes useful testing for concentrated milk products. Refer to the text for important details related to specific recommendations.

#### 23.5.2.1 Critical Ingredients

If not manufactured with dry dairy products in reconstitution processes, evaporated milk is usually manufactured from fresh milk without the addition of ingredients. For sweetened condensed milk, a critical ingredient is seeding lactose added after the heat-treatment to control appropriate crystallization of the sugar. Requirements on the level of osmophilic yeasts are normally included in raw

**Table 23.3** Testing of concentrated milk products for microbiological safety and quality

| Relative importance | | Useful testing | | | |
|---|---|---|---|---|---|
| Critical ingredients | Low | Testing for hygiene indicators such as Enterobacteriaceae is only useful to verify that ingredients have been manufactured under GHP<br>Testing for spore formers may be useful for sterilized evaporated milk and in such cases limits of 10–$10^2$ CFU/g are usual industry standards | | | |
| In-process | Low to high | Routine in-process testing for evaporated milk is not recommended, but can be useful for trouble-shooting to identify potential sources<br>For sweetened condensed milk, testing of samples for osmophilic yeasts and xerophilic molds or micrococci is useful and absence per manufactured unit (after incubation) should be the objective | | | |
| Processing environment | Low | Routine testing of the environment for vegetative and spore forming pathogens or spoilage microorganisms is not recommended, but can be useful for trouble-shooting to identify potential sources of contamination | | | |
| Shelf life | Low to medium | Not applicable, except for sweetened condensed milk packed under modified atmosphere (which inhibits mold growth) for detection of xerophilic mold spoilage after prolonged shelf life | | | |
| End product | High | For sterilized evaporated products and sweetened condensed milk after incubation of finished products (predefined number of units or percentage of production), testing and trend analyses to assess the performance of the line and the detection of major deviations is useful<br>For sweetened condensed milk, testing is done for xerophilic fungi or *S. aureus* if able to grow at the water activity of the product | | | |
| | | Product | Microorganism | Analytical method | Sampling plan and limits |
| | | Sterilized evaporated milks | Presence/absence tests for spoilage microorganisms | Incubation at 30 and 55°C (if suitable)<br>Destructive and nondestructive methods | Fixed number or percentage of samples/batch (see text) |
| | | Sweetened condensed milk | Presence/absence of molds and *S. aureus* | Incubation at 25 and 37°C respectively | Fixed number or percentage of samples/batch (see text) |

material specifications. If ingredients such as cocoa powder, flavors or fruit concentrates are added to concentrated milks then the same type of approach as described under Sect. 23.3.2.1 is recommended.

#### 23.5.2.2 In-Process

For evaporated milk no routine in-process sampling is recommended. For sweetened condensed milk which is not a sterile product despite the heat treatment, sampling of intermediate product at critical steps such as the seeding and crystallization tanks or fillers, is useful to provide information on possible hygiene problems. Samples are usually incubated for a few days at 25 and 37°C and examined for the presence of yeasts and molds or micrococci.

#### 23.5.2.3 Processing Environment

Testing is not recommended for the processing environment for concentrated milk.

#### 23.5.2.4 Shelf Life

Shelf life testing is typically not relevant for condensed milk products. The exception is testing for xerophilic molds in sweetened condensed milk packed under modified atmosphere, which may develop only after prolonged periods of time (usually weeks and months) after production. These results are only useful for monitoring purpose and trend analyses.

#### 23.5.2.5 End Product

Concentrated and evaporated milks are usually handled in a manner similar to sterilized and UHT products (see Sect. 23.3.2.5). For sweetened condensed milk, samples are usually incubated for about 3 days at 37°C and about 5 days at 25°C, respectively and then tested for the presence of fungi or micrococci and in particular of *S. aureus* (see Table 23.3).

## 23.6 Dried Dairy Products

Many milk products, including whole milk, skimmed milk, whey, buttermilk, cheese and cream, may be dried using appropriate technologies such as spray or roller drying. Dried milk products may be consumed directly after reconstitution, but more commonly they are used as ingredients in a number of products such as bakery, chocolate and confectionery, culinary products, animal feeds or even in recombination processes to manufacture liquid products such as UHT or evaporated milk. Note that infant formula is addressed in Chap. 25.

### *23.6.1 Significant Organisms*

#### 23.6.1.1 Hazards and Controls

Epidemiological data suggest that *Salmonella* is the only significant hazard that needs to be controlled specifically during the manufacture of dried dairy products. Other hazards such as *S. aureus* or *B. cereus* or the presence of preformed staphylococcal enterotoxins are normally only present sporadically at very low levels or occur as the result of an isolated major breakdowns of GHP. Low levels ($<10^2$ CFU/g) of *S. aureus* and *B. cereus* do not represent a risk to human health as long as the products are not mishandled after reconstitution and before consumption. Mishandling (holding time and temperature) would allow growth and toxin formation.

*Cronobacter* spp. is a concern in infant formula, which is addressed in Chap. 25. ICMSF is not aware of any specific risk assessment performed on dry dairy products other than in infant formula.

#### 23.6.1.2 Spoilage and Controls

Due to the extremely low water activity of the dry products ($a_w$ = 0.3–0.4), spoilage is not relevant.

### *23.6.2 Microbial Data*

Table 23.4 summarizes useful testing for dried dairy products. Refer to the text for important details related to specific recommendations.

**Table 23.4** Testing of dried dairy products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | Develop good supplier relationships for critical dry mix ingredients to ensure their safety. Requirements for such ingredients need to be equivalent to those for finished products to ensure its compliance. Depending on the confidence level of the supplier testing is performed either for acceptance or as monitoring |
| In-process | High | Test product residues at critical operations and intermediate product for *Salmonella* and Enterobacteriaceae. Typical guidance levels:<br>• Enterobacteriaceae – same requirements as finished products<br>• *Salmonella* – absent in any of the samples |
| Processing environment | High | Test for *Salmonella* and Enterobacteriaceae in relevant areas. Typical guidance levels:<br>• Enterobacteriaceae – ≤100 CFU/g or sample<br>• *Salmonella* – absent |
| Shelf life | Low | Not applicable for a dry product |
| End product | High | Test for indicators for on-going process control and trend analysis. If aerobic colony counts are consistently much lower, then internal limits should be adjusted accordingly |

| | | | | Sampling plan & limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Dry milk powders | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $10^4$ | $10^5$ |
| | Enterobacteriaceae[c] | ISO 21528 | 5 | 5 | 2 | <3 | 9.8 |

| | |
|---|---|
| Low to high | When in-process and environmental results show negative results, testing of smaller numbers of samples for verification is usually sufficient. However, testing end products for *Salmonella* for lot acceptance is relevant when environmental data indicate potential for contamination or when effectiveness of control measures seems impaired (e.g., construction, wet cleaning) |

| | | | | Sampling plan & limits/25g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Dry milk powders | *Salmonella* | ISO 6785 | 12 | 20[d] | 0 | 0 | |

[a] Alternative methods may be used when validated against ISO methods.
[b] Refer to Appendix A for performance of these sampling plans
[c] Most probable number (MPN)
[d] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 23.6.2.1 Critical Ingredients

Depending on the products manufactured, ingredients such as caseinates, whey powder and other milk derivatives, vitamins, trace elements and minerals or lecithin may be added during processing. Certain ingredients, such as milk derivatives, have a known history of presence of *Salmonella* and are therefore considered as high risk ingredients. While ingredients added before the heat treatment do not represent an issue, those added after the kill step (usually termed "dry mix ingredients") represent a risk and therefore need to fulfill the same microbiological requirements as the finished product.

Sampling and testing of dry mix ingredients for *Salmonella* and indicators, such as Enterobacteriaceae, at the reception is recommended but this practice alone cannot ensure their safety. Sampling and testing regimes are therefore usually adapted according to the level of risk and to the confidence level of the supplier (see Chap. 6). Careful selection of the supplier, in particular for the high risk ingredients, clear communication of the needs and their reasons, audits to ensure that

all the necessary control measures and verifications are in place are important elements ensuring that ingredients will comply with the requirements.

Testing of wet mix ingredients submitted to a subsequent heat-treatment is usually done only to verify that products are manufactured under GHP, thus minimizing the risk of ingress of *Salmonella* into the plant.

#### 23.6.2.2 In-Process

Direct testing of intermediate products is normally not recommended. However, in-process samples play an important role to demonstrate and confirm that the control measures are effective. Such sampling plans need to include representative samples taken after the drying step up to the filling operation. Typical samples are the first powder manufactured, the first filled product, and samples from product contact surfaces where accumulation of residues or lumps may take place, which could indicate the presence of condensation on product contact surfaces and thus the potential for growth in microenvironments. Such sampling points are sifter tailings from the after dryer/after cooler or from tipping stations of intermediate products and filling machines. Additional details are provided in Chap. 4.

While the sampling frequency needs to be adapted to the situation in the factory, such samples must fulfill the same microbiological requirements as the finished product, both for *Salmonella* and indicators such as Enterobacteriaceae.

#### 23.6.2.3 Processing Environment

Since the major cause of presence of *Salmonella* or increased levels of Enterobacteriaceae in finished products is recontamination from the processing environment, sampling and testing of environmental samples plays a key role in verifying the effectiveness of the preventive measures. Testing is done both for *Salmonella*, the relevant pathogen, and for Enterobacteriaceae, as an indicator for the effectiveness of GHP. It should be noted that testing for Enterobacteriaceae alone is not suitable since even low levels do not necessarily guarantee the absence of the pathogen.

#### 23.6.2.4 Shelf Life

Shelf life testing is not relevant for dry products because the low water activity prevents growth.

#### 23.6.2.5 End Product

ICMSF (1986) proposed different 2-class plans for dry milk at the port of entry, either for normal or for high-risk populations. Additionally, 3-class plans were proposed for aerobic colony counts and coliforms for these products. In the absence of knowledge on the processing conditions, this proposal is still valid. However, considering that the final use of the dry milk powder is frequently not known, the most stringent criterion is usually applied by default.

Enterobacteriaceae now represent the indicator of choice and have been used in different regulations, e.g., EC (2005) along with more stringent limits reflecting improved control measures implemented during the last 20–30 years. This book includes criteria for Enterobacteriaceae instead of coliforms, recognizing that some regions may still use coliforms because of the long history of this group as an indicator for dairy products.

Requirements for dry dairy ingredients other than milk powders may be less stringent due to the fact that they are used as raw materials in other products and are either submitted to heat treatments or the requirements of finished products are different.

For manufacturers applying integrated sampling plans with in-process and environmental samples, a low level of end product testing for *Salmonella* is performed as verification only. Positive results in either in-process or environmental samples indicate an increased risk of contamination of the finished product and should trigger a change in the sampling regime. For example, increased testing according to regulatory requirements or up to 20×25 g for release purposes to demonstrate compliance of the product may be appropriate. Depending on the usage, e.g., designed for sensitive consumers, testing of 60×25 g may be considered instead.

## 23.7 Ice Cream and Similar Products

Ice cream can be divided into four main categories according to the main ingredients used: (1) ice cream made exclusively from milk products; (2) ice cream containing vegetable fat; (3) sherbet ice cream containing fruit juice, milk and milk solids non fat, and (4) water ice manufactured from water, sugar, fruit juices or concentrates. The composition of different products is regulated by international or national legislations. Only industrially produced ice cream is covered.

### *23.7.1 Significant Organisms*

#### 23.7.1.1 Hazards and Controls

Most outbreaks have been related to homemade and artisanal ice cream prepared with raw ingredients (e.g., eggs), inadequate heat treatments, contaminated by infected handlers or insufficiently cleaned equipment. Industrially manufactured ice cream has been involved in outbreaks due to *Salmonella*. Although no epidemiological link has been demonstrated, presence of *L. monocytogenes* has lead to several recalls. Ice cream was included in risk assessments for *L. monocytogenes* in ready-to-eat foods and it was concluded that the risk of listeriosis due to ice cream was very remote (FAO/WHO 2004a, b). Regulatory requirements in different countries may require consideration of this organism.

The vegetative pathogens that may be present in the raw ice cream mix are readily killed by the pasteurization step. Processing conditions are usually similar to those applied for cream to take into account the composition of the ice cream mix, in particular the increased fat or total solid content. Pathogen presence in finished products is typically due to postpasteurization contamination from the processing environment or from the addition of contaminated ingredients.

#### 23.7.1.2 Spoilage and Controls

The frozen nature of this product prevents microbiological spoilage.

### *23.7.2 Microbial Data*

Table 23.5 summarizes useful testing for ice cream and similar products. Refer to the text for important details related to specific recommendations.

**Table 23.5** Testing of ice cream for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | It is important to develop good supplier relationships for critical dry mix ingredients to ensure their safety. Requirements for such ingredients should be equivalent to those for finished products (see below) to ensure compliance. Depending on the confidence level of the supplier testing is performed either for acceptance or as monitoring |
| In-process | High | Routine in-process testing is recommended at critical steps of the process. Testing for Enterobacteriaceae provides important information on the hygiene status of processing lines and levels of exceeding those established for the finished product should trigger testing for *Salmonella* |
| Processing environment | Low | In cases where regulatory requirements exist, testing of environmental samples for *L. monocytogenes* (absence in the samples taken) is recommended<br>*Listeria* spp. can be used as a hygiene indicator – while the absence is certainly the target, low levels up 10 CFU/g may be acceptable but need to be interpreted according to observed trends over time<br>Testing for Enterobacteriaceae is not recommended with the exception of areas maintained dry (suggested target values: $10^2$–$10^3$ CFU/g) |
| Shelf life | – | Not applicable |
| End product | High | Testing for Enterobacteriaceae provides important information on the hygiene status of processing lines. High levels may then trigger investigative sampling for pathogens. Testing for *Salmonella* can be limited to verification as long as in-process and environmental results are showing the absence of deviations |

| | | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| | High | Ice cream and similar products | Enterobacteriaceae | ISO 21528-2 | 2 | 5 | 2 | 10 | $10^2$ |
| | Low | | | | | Sampling plan and limits/25 g[b] | | | |
| | | | *Salmonella* | ISO 6785 | 11 | *n* | *c* | *m* | *M* |
| | | | | | | 10[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

### 23.7.2.1 Critical Ingredients

While the basic ice cream mix is pasteurized, ingredients such as fruits, nuts, cookies, chocolate chips or chocolate coating may be added after the heat process.. The significant hazard associated with such ingredients is *Salmonella*. The microbiological quality of these ingredients must be equivalent to those for the finished products. For this reason, the same approach as described in Sect. 23.6.2.1 applies with respect to the selection of supplier and sampling and testing procedures.

### 23.7.2.2 In-Process

Samples taken at critical steps along the processing line play an important role in determining the effectiveness of preventive measures to control recontamination after the heat treatment. Samples are typically taken from the mixing and maturation tanks, at the fillers or after hardening tunnels. Particular attention needs to be paid to build-up of residues or condensation spots where growth may be possible under certain circumstances.

Testing in-process samples for Enterobacteriaceae provides relevant information as to the adherence to GHP and levels above 10 CFU/g indicate poor hygiene practices, such as insufficient cleaning of maturation tanks or poor practices during rework handling, etc.

#### 23.7.2.3 Processing Environment

In the environmental sampling plan, it is important to include areas that may contribute to contamination of processing lines or exposed product to verify the effectiveness of the hygiene control measures. Considering the humidity and temperature in such processing environments, it is likely that potential harborage sites for *Listeria* spp., including *L. monocytogenes* may be present. Therefore, when regulatory requirements for *L. monocytogenes* exist, sampling and testing programs are normally focused on these microorganisms. Detection of high levels and widespread occurrence of *Listeria* spp. are indicative of ineffective control measures, which should be addressed.

#### 23.7.2.4 Shelf Life

Microbiological shelf life testing is not relevant for frozen products.

#### 23.7.2.5 End Product

Enterobacteriaceae is an effective and simple tool to determine the hygiene status of drier parts of the line and increased levels (>10 or $10^2$ CFU/g) are indicative of an increased risk of presence of *Salmonella*, thus triggering testing of this pathogen in end products. In countries where regulatory requirements for *L. monocytogenes* exist, testing against criteria can be performed, the frequency depending on the level of control during manufacturing.

## 23.8 Fermented Milk

Fermented milk for commercial use is manufactured from heat treated full, skimmed or partially skimmed milk, or from reconstituted powdered milk. Products can be flavored or plain. This section discusses yogurt, mild yogurt, kefir, acidophilus milk, kumys and traditional concentrated fermented milks such as stragisto (strained yogurt), labneh, ymer and ylette. Numerous traditional products are prepared at home or manufactured and distributed locally or regionally. In all fermented milk products, lactose present in the milk is transformed by lactic acid producing bacteria causing a concomitant drop in pH. Typical sensory characteristics of different products, such as texture or taste, are characteristic of the specific lactic microbiota or mixtures thereof. Details are provided in ICMSF (2005).

### *23.8.1 Significant Organisms*

#### 23.8.1.1 Hazards and Controls

Fermented milk manufactured from raw milk will contain microorganisms originating from the raw milk and that can survive the fermentation process. This may include pathogens such as *Brucella* spp., *Mycobacterium bovis* and pathogenic *E. coli* that has an increased tolerance to organic acids.

Such products are usually home-made or are limited to local or regional distribution. Control over such pathogens may be enhanced through the stringent requirements described in Sect. 23.2; however, absolute control using such techniques may not be possible.

Most fermented milk is manufactured using milk heated to temperatures of up to 90°C for several minutes. Spore formers such as *B. cereus* or *C. perfringens* may survive this process; however, germination and outgrowth is controlled through fermentative acidification that produces a rapid pH drop below levels that permit growth of these microorganisms. Fermentation, and resulting acid production, is considered as a control measure for all fermented milk. It is therefore essential to avoid inhibition of fermentation caused by the presence of inhibitory substances such as antibiotics or phages, which may significantly delay the drop of pH below an established limit. Screening of milk using rapid tests is routinely used to detect and reject raw milk containing antibiotics before it enters the process.

Recontamination of the fermented milk with pathogens through the addition of ingredients such as pasteurized fruit concentrates or pulps, heat treated pastes or syrups, nuts, chocolate, or natural or artificial flavors is usually a minor problem due to the nature of these ingredients and the fact that they are added to the already acidified base.

#### 23.8.1.2 Spoilage and Controls

Due to the low pH of the fermented milks, microbiological spoilage is restricted to acid tolerant microorganisms, mainly yeasts and molds (Ledenbach and Marshall 2009). Products manufactured with raw milk have a shorter shelf life because spoilage microorganisms may be present in the milk used. Control measures to avoid or minimize spoilage issues are based on the application of GHP, with a focus on hygienic design of manufacturing lines, hygienic measures applied during handling of packaging material, the appropriate protection of exposed product, in particular during the filling operation, etc.

Refrigeration may extend the storage period but cannot completely inhibit cold tolerant yeasts and molds. Control focuses on GHP procedures to avoid introduction of these spoilage microorganisms from the environment into products, particularly those made from heat treated milk, and on use of high quality ingredients. Ingredients such as fruit pulps or concentrates are prone to harbor yeasts or molds, and this is best controlled through supplier acceptance programs and the application of GHP during handling of the fruit containers. For more details on fruit pulps or concentrates, refer to Chap. 13.

### *23.8.2 Microbial Data*

Table 23.6 summarizes useful testing for fermented milk. Refer to the text for important details related to specific recommendations.

#### 23.8.2.1 Critical Ingredients

Raw milk can be considered the most critical ingredient and the initial microbiota depends on the hygiene practices from production to use by the manufacturer of fermented milk. Details on controls for raw milk are described in Sect. 23.2.

Fruit concentrates or pulps may introduce yeasts and molds if not properly managed. See Chap. 13, for additional information.

**Table 23.6** Testing for microbiological safety and quality of fermented milks made from heat treated milk

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | Testing for the presence of inhibitory substances in the milk is important and should be applied as acceptance test. Inhibitory substances should be absent or below detection limits for validated commercial test kits |
| | High | Starter cultures should meet specifications, including lack of phage contamination |
| | High | Good supplier relationships for critical ingredients such as fruit pulps or concentrates are important to ensure absence of spoilage microorganisms such as yeasts. Testing depends on the confidence level in the supplier – either for acceptance or as monitoring. Alternative testing methods such as $CO_2$ levels in container head-space may be an option when yeast is a concern |
| In-process | Low | Routine microbiological testing is not recommended<br>Investigative testing for spoilage issues can be useful to determine the root cause and implement corrective action |
| | High | Monitoring the pH drop is essential and can be done continuously or at regular intervals<br>Pre-operational visual inspection after cleaning is important to minimize spoilage issues and can be complemented by rapid hygiene tests such as ATP determinations |
| Processing environment | Low | Routine microbiological testing is not recommended<br>Investigative testing for spoilage issues can be useful to determine the root cause and implement corrective action |
| Shelf life | Medium | Depending on the products, accelerated storage tests (e.g., 5 days at 25°C for molds) or keeping quality tests over the entire shelf life may provide useful information on the hygiene status of lines. In such cases, the number of samples taken should be representative of the manufacturing lines and results are best evaluated using trend analysis |
| End product | Low | No regular testing recommended |

#### 23.8.2.2 In-Process

Routine determination, either continuous or periodic, of the pH during fermentation is an important element in monitoring this control measure. Lines used to manufacture fermented milks are wet cleaned using Clean in Place (CIP), clean out of place (COP) or combinations of these. Pre-operational visual inspections are useful to verify the effectiveness of cleaning. Such inspections can be complemented by rapid hygiene tests such as ATP determination.

Routine microbiological testing for pathogens is not recommended. However, testing can be very useful to detect build-up of spoilage microorganisms such as gas-forming lactic acid bacteria (e.g., *Leuconostoc* spp.), yeasts and molds. Samples are best taken from critical pieces of equipment such as intermediate storage tanks, balance tanks, fillers etc.

#### 23.8.2.3 Processing Environment

Routine environmental monitoring for hygiene indicators such as Enterobacteriaceae is not recommended for fermented milk due to the nature of processing environments, which are frequently wet cleaned. When issues occur, investigative sampling and testing for spoilage microorganisms will provide useful information to determine root causes.

#### 23.8.2.4 Shelf Life

Accelerated shelf life tests (5 days at 25°C) or keeping quality samples may be used for certain products as a monitoring tool to assess the overall level of hygiene and the incidence of spoilage. Considering the short shelf life of these products, results are usually used only as monitoring and evaluated using trend analyses.

#### 23.8.2.5 End Product

End product testing is not routinely conducted because monitoring of the fermentation in-process provides the most actionable information.

## 23.9 Cheese

Similar to fermented milks, cheese can be manufactured using raw or heat treated milk. Heat treatments vary in intensity, ranging from thermization to pasteurization. Heat treatments may be applied as a bactericidal step or as a step intended only to reduce enzymatic activity that may otherwise affect the whole process. Irrespective of whether cheese is manufactured using raw or processed milk, it is important to use a milk of good quality to obtain high quality cheese.

Considering the variety of cheeses manufactured worldwide, the reader is referred to ICMSF (2005) for details on categorization and characteristics of different cheeses. Different regulatory approaches for cheese standards are in place in different regions.

### *23.9.1 Significant Organisms*

#### 23.9.1.1 Hazards and Controls

The initial microbiota of raw milk is discussed Sect. 23.2 and the presence of low levels of certain pathogens cannot be excluded. Control measures to minimize the incidence, which is particularly important for raw milk cheese, can be achieved through the programs outlined under Sect. 23.2.1. The effect of different heat treatments has also been discussed in previous sections.

For raw milk cheeses, acidification throughout the initial phases of manufacture up to early stage of ripening are key steps in cheese making and play an important role in the control of pathogens. Several pathogens have been shown to die off during these steps. This is due to the combined effect of low pH, the addition of salt in certain cheeses, the length of the ripening period (which has an impact on the water activity), as well as temperature conditions during ripening and has been described for several pathogens.

However, in some raw milk and artisanal cheeses, certain pathogens such as enterohemorrhagic *E. coli* may survive or even multiply. Where such control measures are not applied due to the nature of the cheese, particular attention must be paid to the procurement of the milk used to ensure, as far as possible, the absence of pathogens such as *Brucella* or *L. monocytogenes*. Special programs are necessary to achieve such limits and examples exist in countries with a traditional raw milk cheese production such as France. Codex Alimentarius (2009) also provides guidelines for primary production of milk, and additional provisions for the production of milk used for raw milk cheese and other products.

#### 23.9.1.2 Spoilage and Controls

Spoilage issues related to cheeses made from either raw or heat treated milk are very similar. It might be caused by original ripening microorganisms such as yeasts and molds. In many cases, bacterial contamination stems from the environment, frequently water before the cheese is packed or otherwise handled. Spoilage is characterized by important changes in visual or sensory changes of the products, in particular when cheeses are sliced or portioned and repacked for sale. Control over spoilage is achieved through strict adherence to hygiene measures during handling and ripening of the cheeses as well as by maintaining appropriate conditions.

Early and late blowing (i.e., excessive gas production) are particular situations associated with the growth of gas forming yeasts or bacteria such as *Bacillus subtilis* and *Clostridium tyrobutyricum* and other related species. Control over such types of spoilage is achieved by the application of strict hygiene measures during milking and avoidance of silage feed for the production of milk for hard cheeses. In certain countries, routine testing for clostridia is performed for acceptance of the milk used for the manufacture of these types of cheeses.

### *23.9.2 Microbial Data*

Table 23.7 summarizes useful testing for cheese. Refer to the text for important details related to specific recommendations.

#### 23.9.2.1 Critical Ingredients

Milk is considered as the critical ingredient, both for the presence of pathogens and spoilage microorganisms such as clostridia. However routine testing for specific microorganisms is rarely applied. As with other fermented milk products, the presence of inhibitory substances should be avoided.

Other ingredients used to manufacture certain cheeses, such as spices or herbs, may be critical and may be a source of pathogens such as *Salmonella* or *L. monocytogenes*. Such ingredients need to be identified during the hazard analysis performed in HACCP. Selection of appropriate suppliers is the preferred option and testing for lot acceptance is not recommended.

#### 23.9.2.2 In-Process

Depending on the type of cheese and the significant hazards identified during the hazard analysis, sampling of product residues, product contact surfaces can be a useful tool to detect pathogens and implement appropriate corrective measures. Examples are *L. monocytogenes* for soft-cheeses and *Salmonella* for cheddar cheese which have been at the origin of outbreaks due to post-process contamination.

Conversely, the aging process of certain cheese may provide pathogen inactivation over time. This is particularly important for *S. aureus* because if the fermentation is slow, this pathogen may grow, produce toxin, and die off during aging. Monitoring proper acidification is a useful tool to ensure that the process is under control in lieu of pathogen testing if properly validated. *E. coli* may also die off during fermentation and the aging process in certain cheeses. If *E. coli* is used as an indicator of process control, especially for raw milk cheeses, it is important to understand the optimum time and conditions in the fermentation and aging processes to conduct sampling and testing. Pathogenic strains of *E. coli* tend to be more acid tolerant than generic *E. coli* and may survive when the indicator dies.

**Table 23.7** Testing of cheeses for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | *Raw milk cheese only*: A good supplier relationship is important, targeting the absence of *Salmonella,* EHEC and *L. monocytogenes* or other pathogens that may survive cheese making |
| In-process | High | Monitor pH during acidification of the curd to detect slow fermentation. In-process testing for *S. aureus* may be relevant if acidification does not proceed as anticipated using criteria listed in the end product section (see text) |
| | High to low | For cheeses that support the growth of *L. monocytogenes* and for raw milk cheeses, testing residues and product contact surfaces may be important to verify the effectiveness of the preventive measures implemented. Pathogens of concern vary by cheese type. Typical guidance levels:<br>• *L. monocytogenes* and *Salmonella* – absent |
| Processing environment | High to low | Significant hazards and routes of contamination vary by type of cheese, and testing the processing environment may be useful to assess the effectiveness of control measures taken. If appropriate, typical guidance levels are:<br>• *L. monocytogenes* and *Salmonella* – absent |
| Shelf life | Low | Testing may be conducted to determine the fate of pathogens during ripening and storage of cheese. Routine testing, however, is not recommended |
| End product | | Testing for *E. coli* or *S. aureus* is useful to verify process control and hygiene conditions for certain cheese types. Upper limits (M) may vary depending on the extent of heat-treatment but high levels may trigger investigative sampling for pathogens, including EHEC, or staphylococcal enterotoxins (see text) |

| | | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| High | Fresh cheese | *S. aureus*[c] | ISO 6888-1 | 8 | 5 | 1 | 10 | $10^2$ |
| High | Raw milk cheese | *S. aureus*[c] | ISO 6888-1 | 7 | 5 | 2 | $10^3$ | $10^4$ |
| Low | Cheese from mildly heated milk or ripened | *S. aureus*[c] | ISO 6888-1 | 7 | 5 | 2 | $10^2$ | $10^4$ |
| Medium | Cheese made from pasteurized milk | *E. coli* | ISO 16649-2 | 4 | 5 | 3 | 10 | $10^2$ |
| Low | Cheese: no growth | *L. monocytogenes* | ISO 11290-2 | NA[d] | 5 | 0 | $10^2$ | – |
| | | | | | Sampling plan and limits/25 g[b] | | | |
| | | | | | *n* | *c* | *m* | *M* |
| High | Cheese: Growth supported | *L. monocytogenes* | ISO 11290-1 | NA[d] | 5[e] | 0 | 0 | – |
| Medium or low | Cheese from raw or mildly heat-treated milk | *Salmonella* | ISO 6785 | 10 | 5[e] | 0 | 0 | – |

[a]Alternative methods may be used when validated against ISO methods
[b]Refer to Appendix A for performance of these sampling plans
[c]*S. aureus* enterotoxin tests may be used in lieu of counts or if criteria are exceeded
[d]NA = Not applicable due to use of Codex (2007) criteria
[e]Individual 25 g analytical units (see Sect. 7.5.2 for compositing)

#### 23.9.2.3 Processing Environment

Testing for *L. monocytogenes* in soft cheese processing environments is important to verify the effectiveness of implemented hygiene control measures. Absence of the pathogen in any sample should be the target. *Listeria* spp. can be used as indicator for the presence of the pathogen. Usually, higher levels ranging between 10 and $10^2$ CFU/g, are acceptable depending on the location in the processing facility. Levels may vary and limits need to be established individually depending on the specific cheese and requirements in the region.

#### 23.9.2.4 Shelf Life

The shelf life of cheese varies considerably depending on the type. Fresh cheese may have a very limited shelf life, while hard ripened cheeses may be aged for over a year. It is prudent for a manufacturer to understand the shelf life and the general microbial ecology of the product they produce. In some instances, microbial pathogens may die off during the aging process as discussed in Sect 23.9.2.2. Routine shelf life testing is not recommended, but for certain cheese, understanding the microbial changes with time is useful in a general sense.

#### 23.9.2.5 End Product

Because of the great diversity of cheese types produced in many regions; as well as production, consumption and distribution practices, it is difficult to recommend specific universally applicable testing for all cheese types. Regulations typically focus on coagulase positive staphylococci or *S. aureus* because of the potential for toxin formation. Generic *E. coli* is sometimes used for certain cheese types (e.g., those made with raw or lightly heated milk) as verification of control measures. Levels of these microorganisms may reduce during the aging process, thus levels chosen by governments may focus on the worst case. For example, European standards (EC 2005) indicate that samples should be taken at the point in the process where the highest levels are anticipated, while Canadian (HPFB 2008) and Australian/New Zealand (FSANZ 2001a, b) standards do not specify a sampling time. This may explain why different levels are listed in these standards.

Table 23.7 provides ICMSF recommendations for testing that may be considered for certain cheese products. It is important to consider local production, use and consumption patterns in establishing criteria for a specific application. For example, for cheeses that support growth of *L. monocytogenes,* testing of finished products may be performed as part of the verification program to demonstrate control over this pathogen. Depending on the shelf life of the product, release maybe based on analytical results. For fresh cheeses with a rather short shelf life, this may not be feasible and testing would be limited to monitoring and trend analysis, if conducted at all.

Quantitative tests for *E. coli* in cheese made from heat treated milk or *S. aureus* for certain cheese types is useful to verify process control and hygiene conditions (see Table 23.7). However, levels of these microorganisms are likely to decline during the aging process, thus in-process testing provides more useful information to evaluate the safety of the product. Additionally, well established cheese production practices may be validated to demonstrate a reliable reduction of potential pathogens, as well as inhibition of toxin formation, during the process. A prudent cheese manufacturer would evaluate these parameters in their HACCP study and with sufficient testing may be able to justify the use of measurements such as acidification in lieu of routine microbiological testing.

Upper limits (*M*) for *S. aureus* may vary depending on the extent of heat-treatment but high levels (e.g., $>10^5$/g) may trigger investigative sampling for staphylococcal enterotoxins. Similarly, high levels of *E. coli* may trigger testing for other pathogens, including pathogenic *E. coli* not included in Table 23.7. This depends on the type of cheese, manufacturing conditions and behavior of specific pathogens, and may be limited to verification when in-process and environmental results demonstrate the absence of deviations.

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# Chapter 24
# Shelf-Stable Heat Treated Foods

## 24.1 Introduction

Shelf-stable heat treated foods include a wide variety of products, such as vegetables, fruit, fish, meat, milk and dairy products, ready meals, soups and sauces. For specific details on shelf-stable milk and milk products see Chap. 23. Shelf-stable products are characterized by their stability during extended storage at ambient temperatures and they have a long history of safe use. Commercial sterility of shelf-stable food means the condition achieved by the application of heat, alone or in combination with other treatments, to render the food free from microorganisms capable of growing in the food under normal ambient conditions of distribution and storage. Shelf-stable heat treated foods have been traditionally subjected to one of three processes:

- The food is placed in a pack which is hermetically sealed, subjected to a thermal process to render it commercially sterile and then cooled (e.g., retort canning)
- The food is subjected to a continuous in-line thermal process for commercial sterility, cooled and then packed aseptically into sterile packs that are then hermetically sealed with a sterilized closure in an atmosphere free of microorganisms (e.g., UHT aseptic processing)
- The food is subjected to a continuous in-line thermal process for commercial sterility, filled hot into suitable packs which are then hermetically sealed (sometimes in a steam environment) and then often inverted for a specific time or subjected to a hot environment to pasteurize the head space and pack (e.g., acidified sauce processing)

Specialized commercial sterilization processes based on ohmic heating, microwave technology and other technological developments are gradually finding wider adoption.

Microbiological testing plays an important role in the control of thermal processing. However, the majority of process controls are physical in nature aimed at ensuring that the correct thermal process is delivered, rapid cooling is achieved and packages are hermetically sealed. This chapter will not deal with these critical aspects of thermal processing and the reader is directed towards more definitive texts on the subject (NFPA 1995; Larousse and Brown 1997; Holdsworth and Simpson 2007).

## 24.2 Significant Organisms

### *24.2.1 Hazards and Controls*

The heat processes applied to shelf-stable foods are sufficient to kill all vegetative microorganisms. Of the remaining bacterial spores *Clostridium botulinum* and *Bacillus cereus* are potential food safety

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_24,

hazards. There are also certain other related species of the same genus that may contain the same toxin genes but these would tend to have similar thermal resistance.

*C. botulinum* is a spore forming bacterium that, under certain conditions, can grow in foods and produce a potent neural toxin. *C. botulinum* is the major hazard in shelf-stable foods that have a suitable pH, nutrients, and water activity in the absence of oxygen. Low-acid shelf-stable foods provide this favorable environment. When a product is acidified to a pH of 4.6 or less, inhibition of the germination of *C. botulinum* spores is assured. Consequently pH 4.6 is considered the "cut off" point defining low-acid (pH >4.6) and acid/acidified foods (pH ≤4.6) (Codex Alimentarius 1993). However, processors should be aware that the growth of certain bacilli and molds in under-processed acid/acidified shelf-stable foods could cause an increase in pH to a point at which *C. botulinum* could start to grow and produce toxin (Odlaug and Pflug 1979; Montville and Sapers 1981; Wade and Beuchat 2003; Evancho et al. 2009). Details on the physiological aspects of *C. botulinum* have been described (ICMSF 1996) and ecological aspects in food commodities have been reviewed (ICMSF 2005).

*B. cereus* and some *Bacillus* spp. can produce enterotoxins that cause vomiting and diarrhea. However, these microorganisms are more heat sensitive than *C. botulinum* and thermal processes necessary to remove more thermotolerant spoilage bacilli are usually sufficient to remove *B. cereus*. Consequently this microorganism is rarely a problem in shelf-stable heat treated foods although care should be taken to ensure that susceptible ingredients are managed in such a way as to avoid the development of pre-formed toxins that might survive the thermal process.

In addition to direct microbiological hazards, histamine can be a specific hazard associated with the use of temperature abused fish during the heat processing of shelf-stable foods involving scombroid fish species (see Chap. 10).

### *24.2.2 Spoilage and Control*

Thermotolerant spore forming microorganisms can cause spoilage of commercially sterile foods under certain circumstances. These spore formers are more heat resistant than *C. botulinum*. Thermophilic spores of aerobic bacteria (e.g., *Geobacillus stearothermophilus*) and anaerobic bacteria (e.g., *C. thermosaccharolyticum*) have been associated with spoilage of low-acid shelf-stable foods. *Desulfotomaculum nigrificans* has also been associated with sulfide-spoilage of canned vegetables. However, these microorganisms are only problematic for shelf-stable foods that are distributed and stored at high ambient temperatures or those that are not cooled sufficiently quickly after thermal processing.

Certain acidophilic spore formers that are also thermotolerant have been the cause of spoilage of acid and acidified shelf-stable foods. *B. coagulans* var *thermoacidodurans*, *C. pasteurianum* and *C. butyricum* are the most common examples. The spores of these microorganisms are more heat sensitive than the spores of thermophilic microorganisms but acidified and acid foods usually receive less severe thermal processes than low-acid shelf-stable foods. Post-process contamination by these microorganisms and lactic acid bacteria may also be problematic in poorly controlled hot-fill and hold processes applied to acidified foods.

For certain shelf-stable fruit products the ascospores of molds can survive thermal processes and cause spoilage. Generally the low oxygen content of fruits in hermetically sealed containers prevent outgrowth of ascospores. However, certain *Byssochlamys* spp., *Talaromyces* spp. and *Eupenicillium* spp. have been associated with spoilage of shelf-stable fruits and fruit-based products as they are more tolerant of low oxygen concentrations. More detailed reviews of the ecology of these spoilage microorganisms are available (ICMSF 2005).

## 24.3 Process Control

This section addresses microbiological testing only as it applies to process control in the production of shelf-stable foods.

### *24.3.1 Packaging Integrity*

Even when an adequate heat process has been applied, the integrity of hermetically sealed containers used for shelf-stable foods is critical to safe processing and requires constant surveillance by the packaging manufacturer and the packaging user. Controls on packaging materials and finished containers are predominantly physical and should focus on routine inspection systems that examine and measure the integrity of packaging materials and the seals made during pack formation (e.g., can seam breakdown inspection, monitoring of flexible pack sealing parameters).

Microbiological testing of packaging integrity is only appropriate in certain circumstances. These types of tests are costly and specialized, and should not be considered on a routine basis. For example, microbiological challenge testing may be appropriate during the commissioning of new aseptic processes or when it is necessary to investigate during process failures. During challenge testing (biotesting) packs are immersed in an aqueous suspension of appropriate spoilage bacteria. If subsequent incubation of the packs results in spoilage due to the challenge microorganism then problems with pack integrity are likely.

### *24.3.2 Heating and Cooling*

The aim of commercial sterilization is twofold. It renders the food free from any viable microorganisms (including spores) of public health significance and more generally, it inactivates microorganisms capable of reproducing in the food under normal ambient temperature conditions of storage and distribution. The development of a scheduled thermal process is a specialized undertaking beyond the scope of this chapter. However, the routine measurement, control and documentation of thermal processes are critical for the sustained, safe production of shelf-stable foods.

Low-acid products with pH above 4.6 and $a_w$ greater than 0.85 are traditionally subjected to at least a heat process commonly known as the "botulinum cook," which is an integrated thermal process equivalent to 2.5 min at 121.1°C (250°F), also referred to as a $F_0 = 2.5$. Depending on the reference values ($D$ and $z$-values for the spores) used to perform calculations or regulatory requirements, a value of $F_0 = 3.0$ is usually considered the minimum process required to protect public health with respect to low-acid shelf-stable foods. However, in practice thermal processes are often more severe than this to kill spore forming spoilage microorganisms.

Thermal processes applied to acid and acidified foods (pH ≤4.6), foods with low $a_w$ (≤0.85), those that contain curing agents or foods that have other combinations of intrinsic factors that prevent the growth of *C. botulinum*, depend upon the particular microbiological hazard being addressed.

Shelf-stable heat treated foods should be cooled to below 45°C as quickly as possible to prevent the germination and outgrowth of thermophilic spores that would survive the heat process. Cooling is most often achieved by indirect contact with potable cold water containing free residual chlorine or another suitable sanitizer. The microbiological quality of this water is important because it is a potential source of contamination for the sterilized food by, for example, direct ingress into warm hermetically sealed containers or by ingress through fissures in damaged cooling sections of continuous process in-line heat

exchangers. It should be noted that, the spores of bacteria are much more resistant to chlorine than vegetative cells, and *Clostridium* spores are more sensitive to chlorine than *Bacillus* spores. A free residual chlorine level of 2–5 mg/L is usually sufficient to reduce the number of bacteria and their spores although account should be taken of water pH, temperature and level of organic material as these affect the effectiveness of chlorination (Moir et al. 2001).

### *24.3.3 Hygienic Handling of Packs*

Hygienic handling of shelf-stable heat treated food packs post-heating is important. Cross contamination, post-heating, may result from a combination of a leakage route into the pack, water and the presence of microorganisms. All these factors must be controlled during hygienic handling of shelf-stable foods. Consequently packs should be dried as soon as possible after heating, they should be subject to minimal handling and should be stored in a hygienic location until they reach ambient temperature. Cans are particularly susceptible to microbial ingress during cooling as their mechanical seals (seams) are weak after heat treatment and a vacuum is formed in the can as it cools. Hence, microorganisms can be pulled into the can through the seams if they are allowed to remain wet and not handled hygienically. In addition, shelf-stable foods must be handled carefully at all times to avoid mechanical damage to packs and containers that may breach the hermetic seal and allow contamination of the food.

## 24.4 Microbiological Data

Scheduled thermal processes that are applied to manufacture commercially sterile foods are designed to cope with typical microbial loads that are representative of products produced under good hygienic practice and good manufacturing practice conditions. Consequently it is important that excessive spore loads are avoided or failure of the thermal process may ensue, leading to spoilage or food safety problems in the finished product. However, in general shelf-stable foods contain microorganisms at such low numbers that direct microbial testing of post-heat treated product is rendered meaningless. The key to consistent safe production of shelf-stable foods is good process control within a well designed food safety management system based on the principles of HACCP. Table 24.1 summarizes useful testing for shelf-stable, commercially sterile products; however, many important details are included in the following discussion.

### *24.4.1 Critical Ingredients*

Certain ingredients, such as sugars, starches, spices and cereals, can carry large numbers of mesophilic and thermophilic bacterial spores. It may be necessary to adopt microbiological criteria for the acceptance of ingredient lots to ensure that spore loads are maintained below concentrations that can be eliminated by the thermal process. Other ingredients like vegetables may also be considered critical by some processors. Buyer-supplier agreements and ingredient specifications are important means of control. These may be supplemented with ingredient testing as appropriate. Specifications may also depend on the final storage and distribution temperatures of the products and need to be more stringent for thermophilic spore formers when product is distributed or stored at high ambient temperatures. Cereals and their derivatives contain spores, including flat sour and other thermophilic spore forming bacteria (Brown 2000). Some spices are potentially prolific sources of very heat resistant spores of bacteria,

**Table 24.1** Testing of shelf-stable heat processed foods for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Medium | Test for bacterial spores in starches, sugars, cereals and spices (Sect. 24.4.1) if confidence in the supplier is low. Typically the concentration of heat resistant thermophilic spores and mesophilic spores in ingredients should not increase the spore load in the product prior to heat treatment above $10^2$/g and $10^6$/g respectively (see text) |
| | | Test scombroid fish species for histamine only if it is possible to store the fish in a way that prevents spoilage prior to results being available (see Chap. 10, for criteria) |
| In-process | Low | Test cooling water for potability. Frequency depends on water source, use and control of sanitizers |
| Processing environment | Low | Periodic testing is recommended for the following:<br>• Hygiene monitoring of critical pre-thermal process production steps that may allow proliferation of heat resistant spore formers<br>• Understanding microbiological ecology of new or modified process lines |
| | Medium | Validation and verification of cleaning with particular emphasis on hygiene monitoring of post-thermal process lines prior to pack drying. May be used in conjunction with rapid hygiene monitors like ATP and testing of cleaning-in-place (CIP) water |
| Shelf life | Low | Not applicable for finished product but may be necessary to validate open pack shelf life |
| End product | – | Routine direct microbiological testing of end product is not recommended using traditional microbiological testing methods |
| | | Useful data depends very much on the product, packaging and distribution of products. Potential testing may include some of the following: |
| | High | Investigation of spoilage incidents. Investigation protocols should have steps in place to determine if the issue is related to under-processing, thermophilic spoilage or post-process contamination via cooling water and/or pack failure |
| | Medium | Verifying certain processes involving post-heat process hermetic sealing of containers may be achieved by pack incubation testing of a proportion of finished product packs (see text). Typical incubation conditions are:<br>• 30–37°C for 10–14 days to detect mesophilic spoilage<br>• 50–55°C for 5–7 days to test for thermophilic spoilage (for products exposed to high temperatures long term)<br>• 25–30°C for 10–14 days for mesophilic spoilage (acid or acidified products) |
| | Medium | For some products and packaging types, 10% of the incubated packs may be opened and examined for spoilage by appropriate chemical and microbial means (see text) |
| | Medium | For scombroid fish species, when GHP/HACCP status of the product is unknown, testing for histamine may be appropriate (see Chap. 10) |

including thermoduric flat sour microorganisms, putrefactive anaerobes and "sulfide stinkers" (Krishnaswamy et al. 1973; McKee 1995; Freire and Offord 2002; Hara-Kudo et al. 2006). The microbial population of refined sugar consists of mesophilic or thermophilic, aerobic or anaerobic *Bacillus* spp. or *Clostridium* spp. (Hollaus 1977; de Lucca et al. 1992; Hollaus et al. 1997). Certain sugar syrups can be potential sources of these spores.

Microbial criteria for ingredient starches and sugars have been successfully applied to reduce the potential for spoilage of canned products in temperate regions (NCA 1968; Smittle and Erickson 2001). Sample preparation, including specific heat treatments, has a significant impact on numbers of spores detected, therefore it is important to consult Smittle and Erickson (2001) and associated methods in that text in application of these criteria. The criteria adapted from NCA (1968), which are based on five samples per lot, can be summarized as follows:

- Total thermophilic, aerobic spores – average ≤125 spores/10 g; no sample >150 spores/10 g using the method of Olson and Sorrells (2001)
- Flat sour spores – average ≤50 spores/10 g; no sample >75 spores/10 g using the method of Olson and Sorrells (2001)
- Thermophilic anaerobic spores – present in ≤3 of 5 samples; no sample with ≥4 of 6 tubes contain spores using the method of Ashton and Bernard (2001)
- Thermophilic anaerobic spores with hydrogen sulfide production ("sulfide spoilers") – present in ≤2 of 5 samples; no sample with >5 spores/10 g using the method of Donnelly and Hannah (2001)

There are no standard specifications for spores in cereals and spices. In setting specifications, the amount of ingredient in the finished product should be considered. In general spices should not be contaminated to a level that raises the spore load in the product prior to heat processing greater than $10^2$/g for thermophilic spores and greater than $10^6$/g for mesophilic spores. This could also be used as a general guideline for cereals.

For certain shelf-stable fruit products or juices it may also be necessary to set specifications to limit the numbers of ascospores of heat resistant molds such as *Byssochlamys* spp. However, this is only necessary for mild processes where excessive levels of such ascospores would be conducive to spoilage. For information relevant to spores of *Alicyclobacillus* spp. in fruit concentrates, refer to Chap. 20.

### *24.4.2 In-Process*

Correct heat processing, prevention of recontamination after heat processing and product formulation where applicable, are the important factors for controlling microbial safety and spoilage of shelf-stable products. Perishable ingredients used to make shelf-stable heat treated foods must be handled carefully prior to processing to prevent incipient spoilage. Storage and handling times and temperatures must be controlled. Processors should also ensure that upstream raw materials and intermediate products are handled appropriately in the event of a line breakdown to prevent cross contamination and/or the growth of microorganisms.

Cooling water can be microbiologically tested at suitable intervals to verify compliance with potable water standards but the frequency of testing depends on individual manufacturing circumstances.

### *24.4.3 Processing Environment*

In general product contact surfaces in processing equipment should be clean and the cleaning validated and verified. Rapid hygiene monitoring methods, such as ATP measurement can be useful in certain situations to verify cleaning monitored by visual inspection or other means. For in-line thermal processes, cleaning can be verified by monitoring of the cleaning-in-place (CIP) cycle: sanitizer concentrations, contact times and temperatures are all important parameters to evaluate.

Certain areas of the pre-thermal process production line may need special attention if they are prone to colonization by mesophilic or thermophilic spore forming bacteria (e.g., vegetable blanchers). Conditions in these areas can allow for selection and build-up of certain spore formers and lead to the contamination of the foodstuff to an extent that the processing conditions are insufficient to reduce them to acceptable levels. Routine microbial monitoring of these areas using an appropriate procedure may be necessary. In addition, routine microbiological monitoring of the process line post-thermal process and prior to pack drying is recommended as these are critical areas in which packs are susceptible to cross contamination.

### *24.4.4 Shelf Life*

Microbiological establishment of shelf life is not relevant for shelf-stable foods; however, it may be necessary to specify a post-opening shelf life to enable the safe usage of the food by the consumer. Appropriate shelf life may be established using a combination of microbiological testing and predictive modeling where available (FSAI 2005).

### *24.4.5 End Product*

Routine direct microbial testing of shelf-stable products is not recommended. The primary means of ensuring the safety and suitability of commercially sterilized foods lies in the process control undertaken as part of the food safety management system based on the principles of HACCP. However, new products or new processes may benefit from a certain level of end product examination during development (e.g., inoculated pack studies, incubation tests etc.). End product testing may also be useful in diagnosing spoilage problems. More information on testing to determine the cause of spoilage is provided by Rangaswami and Venkatesan (1959) and Denny and Parkinson (2001).

Deibel and Jantschke (2001) discussed methods to test for commercial sterility. Opinions differ concerning the value of incubating and testing of finished products after processing. For single batches incubation and testing may only reveal gross processing problems such as under processing or widespread post-process contamination. However, incubation and testing can provide useful information on the overall performance of the processing line over prolonged periods of time when combined with trend analysis. This type of testing may detect underlying issues leading to sporadic non-sterile units.

In certain countries product incubation may be a legal requirement. Unless this is the case, routine incubation testing is not generally recommended but may be useful periodically to verify functioning of the process controls. However, aseptic processes, hot-fill and hold processes, and retort processing of jars are particular processes where incubation of finished product is used routinely. Incubation testing is also useful during commissioning and validation of new heat processes and during investigation when problems with the thermal process are suspected.

If incubation testing is undertaken, representative samples of the product batch for low acid foods should be incubated at 30–37°C for 10–14 days to detect mesophilic spoilage. When low acid products may be exposed to high temperatures during storage and distribution it may also be useful to incubate samples at 50–55°C for 5–7 days to test for thermophilic spoilage. If thermophilic spoilage is suspected, testing should also be conducted to rule out the presence of mesophilic spore formers, to make sure that the issue is not related to under processing, and that the organisms detected are strict, rather than, facultative thermophiles. For high acid or acidified foods, incubation at 25–30°C for 10–14 days is recommended for mesophilic spoilage (Campden BRI 2001; Deibel and Jantschke 2001).

Quantities incubated may vary depending on the type of process, the batch size and product characteristics. For aseptic products, samples usually represent a combination between random samples and

event samples taken after specific events such as start-up of the production line, changes of packaging materials, and stops due to processing incidents. For retorted products, the number of samples is usually much less and limited to a few units per retort. Ideally the sample size should be calculated statistically to be capable of detecting a given level of spoilage. However, it should be noted that if contamination levels are below 1% then the number of packs that must be examined becomes very large.

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# Chapter 25
# Dry Foods for Infants and Young Children

## 25.1 Introduction

The microbiological safety of infant formulae received much attention since the publication of ICMSF (2005) because of the emergence of *Enterobacter sakazakii* as significant opportunistic pathogen. Numerous taxonomic studies on isolates of *E. sakazakii* lead to the reclassification into a new genus, *Cronobacter*, encompassing several closely related species (Iversen et al. 2008). Codex Alimentarius (2008) agreed to change *E. sakazakii* to *E. sakazakii* (*Cronobacter* species) in the Code adopted in 2008. This change is widely accepted, thus the term *Cronobacter* spp. is used throughout the chapter.

Three FAO/WHO expert consultations (2004, 2006, 2008) developed recommendations for appropriate control measures for *Cronobacter* in infant formulae. This lead to the revision of the Codex Alimentarius Commission Code of Hygienic Practices for Powdered Formulae for Infants and Young Children as well as microbiological criteria (Codex Alimentarius 2008). This chapter addresses the Codex Alimentarius Commission recommendations for powdered infant formulae as well as infant cereals, which have different manufacturing and microbial issues.

Powdered follow-up formulae are not discussed in detail in this chapter, but equivalent recommendations to those described for infant formulae apply, with the exception of *Cronobacter* spp., which is not relevant for infants >6 months (FAO/WHO 2008).

## 25.2 Powdered Infant Formulae

Definitions for infant formulae vary between different countries. The EC directive 91/321/EEC (EC 1991), several amendments as summarized in the directive 2006/141/EC (EC 2006a), and Codex Alimentarius (2008) define infant formulae as foodstuffs intend for a particular nutritional use by infants up to 6 months of age. In these documents, products for infants >6 months are categorized as follow-up (or follow-on) formulae. In contrast, the United States does not distinguish between the two age groups and products are categorized as infant formulae (0–12 months). Additional products such as fortifiers added to expressed human milk and special formulae designed to meet the nutritional requirements of very low-birth weight babies suffering from nutritional deficiencies and associated medical conditions are included in this group of products.

The composition, quality and labeling requirements for powdered infant formulae are laid down in either national or international regulations. Examples are the Codex Alimentarius Commission (2008) standard for infant formulae, the Infant Formula Act in the United States (FDA 2004), and the European directive (EC 2006a). Other national regulations exist and they may differ in their definitions and requirements.

International Commission on Microbiological Specifications for Foods (ICMSF),
*Microorganisms in Foods 8*, DOI 10.1007/978-1-4419-9374-8_25,

Products discussed in this section are usually manufactured using the same technologies and the same type of equipment and processing lines. Other powdered dairy based products, such as follow-up formulae, products for children between 12 and 36 months or even for adults, are produced on similar lines and equipment but differ in terms of regulatory microbiological requirements. However, if such products are manufactured on the same line as infant formulae, the most stringent requirements need to be maintained to ensure the proper performance of the processing lines and compliance of the infant formulae to established criteria.

Infant formulae are also manufactured as concentrated sterilized or as ready-to-feed ultra high temperature (UHT) products. These are not discussed in the following sections, but the principles and comments outlined in Chap. 23, Sect. 3, for similar dairy products are valid.

## *25.2.1 Significant Organisms*

### 25.2.1.1 Hazards and Controls

*Salmonella* has historically been recognized as the relevant pathogen for this category of products. More recently, *Cronobacter* spp. was linked to rare but severe cases of disease and several cases were linked to the consumption of contaminated powdered formulae for infants (FAO/WHO 2004, 2006, 2008).

Other Enterobacteriaceae such as *Citrobacter freundii* or *C. koseri* have been reported to cause occasionally meningitis in neonates. The role of infant formulae as a source of these microorganisms has been reviewed and it was determined that causality is plausible but not yet demonstrated (i.e., category B) by FAO/WHO (2004, 2006). *Staphylococcus aureus* or *Bacillus cereus* may be occasionally present at low levels and risk evaluations or assessments have been performed (FAO/WHO 2004, 2006; Anonymous 2004a; EFSA 2005). FAO/WHO Expert consultations classified both in the category C, i.e., causality less plausible or not yet demonstrated. *S. aureus* and *B. cereus* do not represent a direct threat to the health of infants and it is generally accepted that low levels are acceptable and will not lead to illness as long as the product is prepared and handled according to the recommendations. Limits corresponding to these assessments (<50 or 100 CFU/g) are included in several regulations (e.g., EC 2007).

Formulae for special dietary purposes, human milk fortifiers, infant formulae, as well as follow-up formulae, are manufactured according to one of the three process types (see FAO/WHO 2004, 2006, 2008).

1. *Wet-mix processes* during which all unprocessed raw materials and separately processed ingredients are handled as a liquid intermediate product, which is heat treated, dried and then further handled up to the filling stage. In this process, no further additions are done after the heat-treatment and, in particular, not after the drying step.
2. *Dry-mix processes* during which all separately processed ingredients are dry-blended to obtain the final product, which is then further handled up to the filling stage. The process may include and combine different mixing steps to obtain the final recipe.
3. *Combined processes* during which part of the unprocessed raw materials and part of the ingredients are processed according to the wet-mix process to obtain a base powder. This base powder is considered as an intermediate product and then further used for the manufacture of different finished products by the addition of separately processed ingredients.

All processes falling under (1) or (3) include a kill step, usually a heat-treatment, allowing for a significant reduction of vegetative microorganisms, often far in excess of 8–10 log-units. The presence of either *Salmonella* or *Cronobacter* spp. in finished products is therefore due to post-process contamination. This may occur either in the wet phase before drying if the line is not hygienically designed

or, as most frequently observed, during the steps after the drying operation up to the filling, which include operations such as transport, intermediate storage and dry-blending operations. Contamination during these steps is either due to the use of contaminated dry-mix ingredients, exposure to contaminated food contact surfaces or from the processing environment itself.

Prevention of post-process contamination can be achieved through the careful selection of suppliers to ensure that all dry-mixed ingredients fulfill the same requirements as the finished product. In terms of contamination from the processing lines and environments, well established hygiene measures such as zoning and minimization of wet cleaning have been shown to allow full control over *Salmonella*. Case studies on recent outbreaks due to contaminated infant formulae have highlighted deviations from well established preventive measures rather than systemic weaknesses of these measures.

Experience with the management of *Cronobacter* spp. has demonstrated that it is not possible to control it to the same extent as *Salmonella*, i.e., it is only possible to minimize its presence and thus the risk of contamination of the finished product (FAO/WHO 2004, 2006, 2008). Such management is only possible by reinforcing the zoning concept and eliminating, as far as possible, water, in particular from cleaning. Details on the different control measures used in the manufacture of powdered infant formulae are provided in Cordier (2007).

#### 25.2.1.2 Spoilage and Controls

Not relevant for infant formulae.

### *25.2.2 Microbial Data*

#### 25.2.2.1 Critical Ingredients

Wet-mix ingredients such as milk powder, whey powder and other milk derivatives are submitted to heat-treatments that provide substantial reductions of vegetative microorganisms. Sampling and testing of such ingredients is only recommended to verify that they are manufactured according to GHP.

Dry-mix ingredients such as lactose, sucrose, oil blends, lecithin, maltodextrin, starches, vitamins and trace elements need to fulfill the same requirements as the finished products. Careful selection of the suppliers, in particular for the high-risk ingredients (both for *Salmonella* and *Cronobacter* spp.), clear communication of the needs and their reasons, and audits to ensure that all the necessary control measures and verifications are in place, are important elements of ensuring that these ingredients will comply with the established requirements. Sampling and testing of such ingredients for *Salmonella* and *Cronobacter* spp. as well as for Enterobacteriaceae as hygiene indicators upon receipt is recommended, but cannot, as a stand-alone measure, ensure ingredient safety. Sampling and testing regimes are therefore usually adapted according to the level of risk and to the confidence level of the supplier (see Chap. 6).

#### 25.2.2.2 In-Process

In-process samples play a key role in verifying the effectiveness of the control measures and in demonstrating control over recontamination. Effective sampling plans need to include representative samples taken along the processing line, from the drying step to the filling of the finished product. This would include the first powder manufactured at start up, the first filled product as well as samples from product contact surfaces where an accumulation of residues or lumps will occur. Examples of such sampling points are sifter tailings (after dryer/after cooler, above filling machines) or fines

recovered in cyclones which could be indicative of the build-up of microorganisms. Additional details are provided in Chap. 3. These samples should in principle adhere to the same microbiological limits as the finished product.

#### 25.2.2.3 Processing Environment

The major cause of the presence of *Salmonella*, *Cronobacter* spp. or Enterobacteriaceae in finished products is recontamination from the processing environment. Sampling and testing of environmental samples therefore plays a key role in verifying the effectiveness of the control measures. Testing is done for *Salmonella*, as well as for Enterobacteriaceae as an indicator of the effectiveness of GHP.

It should be noted that Enterobacteriaceae play a dual role as an indicator. With respect to *Salmonella,* low levels of Enterobacteriaceae do not necessarily guarantee the absence of the pathogen and it is therefore necessary to test directly for the pathogen. In the case of *Cronobacter* spp., however, there is a much closer link and direct testing for *Cronobacter* spp. will not necessarily provide additional management information. Investigative testing for *Cronobacter* spp. in combination with molecular typing (e.g., ribotyping) may be useful for mapping of the microorganism throughout a plant.

In the past, environmental levels of Enterobacteriaceae of $10^2$–$10^3$ CFU/g or swab samples were not of direct concern with respect to recontamination with *Salmonella*, as long as the pathogen was not present in the processing environment. In the case of *Cronobacter* spp., however, experience has shown that a much tighter control of Enterobacteriaceae to levels consistently below 10 CFU/g, is important to minimize recontamination. Increases above this level, and in particular above $10^2$ CFU/g, lead almost invariably to increased rates of contamination of the finished product and thus to a concomitant increase of the risk of the presence of *Cronobacter* spp. above acceptable levels.

#### 25.2.2.4 Shelf Life

Microbiological shelf life testing is not relevant for these products.

#### 25.2.2.5 End Product

ICMSF (1986) previously proposed a 2-class plan for *Salmonella* and 3-class plans for coliforms and aerobic mesophilic counts as criteria for infant formulae at the port of entry. For other pathogens such as *S. aureus* and *B. cereus* no specific recommendations were included, but the comment was made that levels of up to $10^2$ CFU/g were still acceptable if testing was done. Most of these recommendations have been included in existing regulatory requirements, including Codex Alimentarius (1991).

For manufacturers applying integrated sampling plans with in-process and environmental samples; however, end product testing for *Salmonella* is usually performed as verification only. Positive results of either in-process or environmental samples indicating an increased risk of its presence in the finished product should trigger a change in the sampling regime, i.e., testing of up to $60 \times 25$ g analytical units for release purposes may be appropriate under such conditions (see Table 25.1).

During the revision of the Codex Alimentarius Code of Hygiene for Infant Formulae, the ICMSF proposed a 2-class plan for *Cronobacter* spp. based on the FAO/WHO risk assessments (FAO/WHO 2004, 2006). This 2-class plan was adopted by Codex Alimentarius (2008) and is applied or considered in several other national regulations.

**Table 25.1** Testing of powdered infant formulae for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | It is important to develop good supplier relationships for critical dry-mix ingredients to ensure their safety. Requirements should be equivalent to those for finished products (see below). Depending on the confidence level in the supplier, test either for acceptance or as monitoring |
| In-process | High | Routine in-process testing is recommended at critical steps of the process. Requirements include:<br>• *Salmonella* – absent in any sample ≥25 g<br>• *Cronobacter* spp. – absent in any sample ≥10 g<br>• Enterobacteriaceae – absent in any sample of ≥10 g |
| Processing environment | High | Due to its widespread occurrence at very low levels, routine testing for *Cronobacter* spp. is not recommended. It may be considered for mapping of the situation in the plant or for investigation. Routine testing for *Salmonella* and Enterobacteriaceae is recommended<br>• *Salmonella* – absent<br>• Enterobacteriaceae – <10 CFU/g |
| Shelf life | – | Not applicable |
| End product | | Test for indicators for on-going process control and trend analysis |

| | | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Product | | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Infant formula | High | Aerobic colony count | ISO 4833 | 2 | 5 | 2 | $5\times10^2$ | $5\times10^3$ |
| | | | | | Sampling plan and limits/10g[b] | | | |
| | High | Enterobacteriaceae | ISO 21528-1 | NA[c] | 10[d] | 2 | 0 | – |

When in-process and environmental results are negative for *Salmonella*, testing a limited number of samples for verification is usually sufficient. When these data indicate a potential for contamination or when the effectiveness of control measures seems impaired (e.g., construction activities, wet cleaning), test according to recommendations below

Considering that *Cronobacter* spp. is much more widespread and even if controlled to very low levels in the environment, testing according to the plans below for lot acceptance is recommended

| | | | | | Sampling plan and limits/25 g[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Product | | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Infant formula | Low to High | *Salmonella* | ISO 6785 | 15 | 60[e] | 0 | 0 | – |
| | | | | | Sampling plan and limits/10 g[b] | | | |
| | High | *Cronobacter* spp. | ISO TS 22964 | 14 | 30[d] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] *NA* Not applicable. Codex Alimentarius (2008) criteria are recommended
[d] Individual 10 g analytical units (see Chap. 7, Sect. 5.2 for compositing)
[e] Individual 25 g analytical units (see Chap. 7, Sect. 5.2 for compositing)

For indicators, a change from coliforms to the more precisely defined Enterobacteriaceae is recommended based on the outcome of the two expert meetings (FAO/WHO 2004, 2006). Much more stringent requirements than the criteria in the former Code of Hygiene (i.e., for coliforms $n=5$, $c=1$, $m<1$ CFU/g, $M=20$ CFU/g) are considered appropriate to reflect the increased risk of contamination with *Cronobacter* spp. Such stringent criteria (i.e., for Enterobacteriaceae $n=10$, $c=0$ or 2, $m=0$ in 10 g samples) have been implemented in the EU (EC 2007) and in other countries.

An expert consultation reviewed existing scientific and technical information on the relevance of *Cronobacter* spp. for follow-up formulae and based on the lack of evidence, criteria were limited to *Salmonella* and Enterobacteriaceae, with no limits set for *Cronobacter* spp. (FAO/WHO 2008).

## 25.3 Infant Cereals

Cereal-based foods for infants and young children are weaning foods that are gradually introduced from the age of 4 to 6 months as part of a diversified diet. Typically, they do not represent the sole source of nutrition. Numerous traditional cereal-based weaning foods exist around the world and several publications address their microbiological status (e.g., Livingstone et al. 1992; Potgieter et al. 2005; Badau et al. 2006; Wagacha and Muthomi 2008). This chapter addresses industrially manufactured dehydrated infant cereals.

The definition of cereal-based products for infants and young children varies in different countries, including the age of introduction (Cuthbertson 1999; Agostoni et al. 2008).

Infant cereals are usually manufactured by heating a cereal soup before further processing. The major ingredients of cereal soups are flour, either from a single cereal or mixtures, and water. Other ingredients such as maltodextrin, sugars, milk solids, starches, honey, fruit or vegetable pulps, and cocoa, may also be used.

After the heat-treatment, which varies by manufacturer and desired sensory quality, the soup is further processed on roller-dryers. During this processing step, the soup is evenly distributed in a thin film on rotating heated drums. This causes an immediate evaporation of the water and the creation of a thin dry film of product, which is scraped off the drum onto a conveyor. Although high temperatures are reached during this step, drying is not considered a controlled killing step, as the product characteristics and water activity changes rapidly, which affects the kill rates. These products can also be manufactured by extrusion.

The cereal film is then milled to obtain a powder or small flakes with a defined particle size. This base powder can be stored before further processing, either by filling or mixing with additional dry ingredients such as vitamins, trace elements, fruit or vegetable powders, flakes or pieces, etc. The number and type of ingredients added generally changes (e.g., size of particulates) according to the age of the consumer, which ranges from infants to young children or toddlers.

### *25.3.1 Significant Organisms*

#### 25.3.1.1 Hazards and Controls

Control measures described in Chap. 15, apply and should be implemented with more stringency because the susceptibility of infants may be greater than that for the general population and thus regulatory limits for infant cereals may be more stringent than for cereal-based products for adults (e.g., EC 2006b). *Salmonella* is the only relevant bacterial pathogen for this product category and a few outbreaks have been documented (Rushdy et al. 1998). Other microorganisms such as *S. aureus*, *B. cereus* or *Cronobacter* spp. may occasionally be present at low levels. No outbreaks related to

these microorganisms have been reported in relation to infant cereals and they do not represent a direct threat to the health of infants. It is therefore generally accepted that low levels are acceptable and will not lead to illness, as long as the product is prepared and handled according to the recommendations.

*Salmonella* control is achieved through heat-treatments that are designed to achieve appropriate sensory qualities of the cereal soups. The times and temperatures provide substantial reductions of vegetative pathogens (usually in excess of 20 log cycles) and even some spore formers are inactivated. For the latter, reductions of 3–8 log cycles are achievable, depending on the conditions applied.

Mycotoxins may represent significant hazards in infant cereals, as for other cereal-based products. Contaminated products were detected regularly in a Canadian survey (Lombaert et al. 2003); however, a similar survey in the UK rarely detected mycotoxins and positive samples were below the regulatory limits (Anonymous 2004b). Control is achieved through careful selection of suppliers. Testing upon receipt depends on confidence in suppliers.

#### 25.3.1.2 Spoilage and Controls

Not relevant as after the drying, all processing steps are dry and microbial spoilage does not occur.

### *25.3.2 Microbial Data*

Recommended testing of powdered infant cereals for microbiological safety and quality is summarized in Table 25.2 and summarized below.

#### 25.3.2.1 Critical Ingredients

Wet-mix ingredients such as those described above are submitted to heat-treatments allowing for substantial reductions of vegetative microorganisms. Sampling and testing of such ingredients is only recommended to verify that they are manufactured according to GHP.

Dry-mix ingredients must meet the same requirements as the finished products. Careful selection of suppliers, especially for the high-risk ingredients; clear communication of the needs and their reasons; and audits to ensure that all the necessary control measures and verifications are in place are important elements of a supplier program. Sampling and testing of such ingredients for *Salmonella* and Enterobacteriaceae as a hygiene indicator, is recommended, but cannot ensure safety as a stand-alone measure. Sampling and testing regimes are usually adapted to the level of risk and the confidence in the supplier (see Chap. 6).

See Chap. 15, for relevant mycotoxin tests for different grains. Visual tests for fungal growth, insect infestation and wet spots are appropriate. Test flour or grains prior to milling for appropriate mycotoxins if confidence in the supplier is low.

#### 25.3.2.2 In-Process

In-process samples play a key role in verifying the effectiveness of the control measures and in demonstrating control over recontamination. Effective sampling plans include representative samples from the processing line, including the roller drying step, the milling step and filling packages of the finished product. Examples include the first powder manufactured at start up, the first filled product, and samples from product contact surfaces where accumulation of residues or lumps occurs.

**Table 25.2** Testing of powdered infant cereals for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | High | *Salmonella* requirements for dry-mixed ingredients should be equivalent to those for finished products (see below). Test either for acceptance or as monitoring<br>Test flour or grains prior to milling for appropriate mycotoxins if confidence in the supplier is low |
| In-process | High | Routine in-process testing is recommended at critical steps of the process. The requirements should be absence of *Salmonella* in any sample of ≥25 g and Enterobacteriaceae in 1 g or 0.1 g (depending on the age of the consumer, the strictest applying for the range 6–12 months) |
| Processing environment | High | Routine testing of environmental samples for *Salmonella* (absence in the samples taken) and Enterobacteriaceae (levels of 100 CFU/g as target) is recommended |
| Shelf life | – | Not applicable |
| End product | High | Test for indicators for on-going process control and trend analysis. Choose Enterobacteriaceae and aerobic colony count levels according to the age range and the composition of products (see text) |

| | | | | Sampling plan and limits/g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Infant cereals | Aerobic colony counts | ISO 4833 | 2 | 5 | 2 | $1\times10^3$ $-5\times10^3$ | $1\times10^4$ $-5\times10^4$ |
| | Enterobacteriaceae | ISO 21528-1 or ISO 21528-2 | 5 | 5 | 2 | 0–10 | $10–10^2$ |

| | | In situations when in-process and environmental results show negative results for *Salmonella*, testing of small numbers of samples for verification is usually sufficient. When environmental or in-process data indicate a potential for contamination or when effectiveness of control measures seems in doubt (e.g., construction, wet cleaning), testing of up to $60\times25$ g or equivalent for release is advisable |
|---|---|---|

| | | | | Sampling plan and limits/25g[b] | | | |
|---|---|---|---|---|---|---|---|
| Product | Microorganism | Analytical method[a] | Case | *n* | *c* | *m* | *M* |
| Infant cereals | *Salmonella* | ISO 6579 | 15 | 60[c] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Individual 25 g analytical units (see Chap. 7, Sect. 5.2 for compositing)

Examples of sampling points are sifter tailings (at the mill(s), above filling machines) or fines recovered in cyclones that could be indicative of the build-up of microorganisms. Additional details are provided in Chap. 3. These samples should, in principle, fulfill the same microbiological limits as the finished product.

#### 25.3.2.3 Processing Environment

The major cause of the presence of *Salmonella* or Enterobacteriaceae in finished products is recontamination from the processing environment. Sampling and testing of environmental samples therefore plays a key role in verifying the effectiveness of the control measures. Testing is done for *Salmonella*, as well as for Enterobacteriaceae as indicator for the effectiveness of GHP.

Environmental levels of Enterobacteriaceae of $10–10^2$ CFU/g or swab are considered achievable, and *Salmonella* should be absent in any of the samples taken.

#### 25.3.2.4 Shelf Life

Microbiological shelf life testing is not relevant for these products.

#### 25.3.2.5 End Product

ICMSF (1986) previously proposed a 2-class plan for *Salmonella*, and a 3-class plan for coliforms and aerobic colony counts as criteria for infant cereals, which were handled in the same category as infant formulae. For other pathogens such as *S. aureus* and *B. cereus* no specific recommendations were included, but the comment was made that levels of up to $10^2$ CFU/g were acceptable. Most recommendations made at that time were included in existing regulatory requirements, including the Codex Alimentarius Commission.

Infant cereals were excluded from the scope of the Codex Code of Hygiene for Infant Formulae in 2008. Criteria for salmonellae, as included in the previous Code (Codex 2006) and proposed by ICMSF, are still relevant but based on current knowledge, the application of criteria for hygiene indicators different than those for infant formulae is justified. Consideration should also be given to the age group, as products are consumed up to 3 years of age (sometimes older) as part of a diversified diet. For hygiene indicators such as aerobic colony counts and Enterobacteriaceae, less strict limits than those for infant formulae are certainly warranted when an increasing number of ingredients are used and products are intended to be consumed by older children.

For manufacturers, applying integrated sampling plans with in-process and environmental samples are routine; however, end product testing for *Salmonella* is usually performed only as verification. Positive results for either in-process or environmental samples indicate an increased risk of presence in the finished product, and should trigger a change in the sampling regime, i.e., testing of up to $60 \times 25$ g analytical units for release purposes may be appropriate under such conditions.

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# Chapter 26
# Combination Foods

## 26.1 Introduction

Commercially prepared ready-to-cook or ready-to-eat (RTE) foods are widely available throughout the world. A combination food product contains major ingredients from more than one commodity groups and interactions of the ingredients may create conditions for microbial growth that are different from the inherent properties of each ingredient. This must be considered for safety and stability. Examples of combination foods include meat and vegetable pot pies, seafood and meat salads, dried soups, dessert pies, flavored ice cream, egg rolls, dim sum, enchiladas, filled pasta, sandwiches, pizza and many other dishes. It is not possible to provide a complete list of all combination foods. Therefore, general considerations are outlined for this broad category and a more specific example is provided for filled or topped dough products.

## 26.2 General Considerations

A wide range of processes are used to produce these foods, which may be offered for sale as perishable, semi-preserved, refrigerated, frozen or shelf-stable products. Relatively minor changes in formulation, especially postprocessing addition of condiments such as grated cheese, sesame seed, ground spices or chocolate frosting, may alter the microbiota of these products to a degree that different microbiological criteria would apply for apparently similar products. The interface between two product groups may also influence the effectiveness of traditional preservations. For example, a high moisture, acidified filling used in a low moisture, neutral cake product may provide sufficient neutralization of the acid and adequate moisture to support growth of certain microorganisms at the product interface. This is very product specific and must be addressed during product design.

Several product chapters in this book contain examples of combination foods that are traditionally associated with a particular commodity, such as ice cream in the milk products chapter, pasta with cereal products chapter etc. Others do not fit exclusively into one commodity group. Refer to the chapter(s) on relevant microorganisms for the commodities used in the combination product.

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## 26.3 Microbial Data

Some of the most important microbiological data for combination foods should be collected during the product development process to identify significant microorganisms for the product in its intended distribution, storage and preparation conditions. As previously discussed, combining different foods may alter the anticipated microbial ecology of a product. Studies should be conducted to determine if there is something unique about the microbiological profile of the product when the food components are combined, compared to what is typically encountered when the foods are handled separately. Validation of the formulas (recipe), processes, shelf life and end use is important for combination foods.

### *26.3.1 Critical Ingredients*

For most combination foods, the quality of the raw materials is of paramount importance for the quality and safety of the end product. Setting microbiological criteria for the end products may be less effective than testing the raw ingredients or in-line samples for the purpose of reducing the potential hazard to the consumer. For example, the total colony count may not be indicative of adherence to GHP for combination foods if they contain fermented product ingredients. Similarly, coliform or Enterobacteriaceae counts may not be useful indicators for products that contain raw vegetables.

Associations among ingredients may facilitate growth of pathogens or spoilage microorganisms that were under control in the ingredients separately. For example, yeast in dried fruit may contribute to spoilage of yogurt and must be managed through ingredient specifications. Such implications should be evaluated during design of the product to ensure that the final product will meet shelf life expectations (see Sect. 26.3.4).

### *26.3.2 In-Process*

A few commercially processed combination foods have been incriminated in outbreaks of foodborne illness. Most outbreaks have occurred because of postprocessing time-temperature abuse, improper storage or mishandling by the preparer before serving. While hazards that may be introduced in commercial food preparation are the same as those that would be present in the home, the magnitude of the risk is much greater in a commercial setting because of the greater number of people exposed to the commercial product. Additionally, increased handling associated with assembling the product provides an opportunity for product contamination. This is especially important for products that are assembled after individual components are cooked.

### *26.3.3 Processing Environment*

Post process contamination can also occur with combination foods. The general considerations for environmental verification and control described in Chap. 4 apply to combination foods. For example, a facility should consider environmental monitoring for *Listeria* spp. if it produces a refrigerated product, especially if it supports the growth of the microorganism under its intended distribution, storage and use. Environmental monitoring for salmonellae may be appropriate for products that are ready to cook but may receive only a mild heat treatment by the consumer (e.g., microwave meals, pot pies etc.). This is likely to reduce the ultimate risk to the consumer.

### *26.3.4 Shelf Life*

The shelf life of combination foods depends on many factors, including the ingredients, storage conditions, water activity, pH, processing, packaging etc. Associations among ingredients may facilitate growth of pathogens or spoilage microorganisms that were under control in the ingredients separately. For example, the interface between a low pH, high $a_w$ filling and a low $a_w$ cake may support growth and toxin production by *Staphylococcus aureus* even when the individual ingredients do not support growth. Similarly, preservatives in an aqueous ingredient may migrate to a fatty phase when mixed with a high fat ingredient, which may subsequently allow growth of spoilage microbiota or pathogens. When the potential for microbiological spoilage or safety issues exists, the manufacturer should establish shelf life based on an understanding of the potential for these issues to develop.

For some products, quality attributes or spoilage will occur well before potential safety issues, for others pathogen growth prior to use may be a concern. Challenge studies may be appropriate for combination products, especially those with long shelf life. Recommendations for conducting such studies have been published (NACMCF 2009).

### *26.3.5 End Product*

Because of the wide variety of products that can exist in this category, no standardized criteria can be recommended. However, GHP and HACCP are typically the measures for the control of hazards present. Frequently, verifying the effectiveness of these programs is best evaluated through in-process and environmental testing. For certain product categories, criteria may be defined based on available data when there is a history of a microbiological issue and when testing may be useful to prevent this issue.

An example of considerations for a more specific category of combination foods is addressed in the following section on filled and topped dough products.

## 26.4 Topped or Filled Dough Products

A wide variety of topped and filled baked or cooked cereal products were addressed in the previous publication (ICMSF 2005), including cakes, pies, tarts, doughnuts, sweet buns, pizza, lasagna, ravioli or dumplings, egg rolls, bao zi, empanadas, enchiladas and others. This reference can be consulted for a more detailed discussion of the microbial ecology and appropriate controls for these products. Fillings and toppings can include a wide variety of raw ingredients such as meats, fish, cheese, cream, nuts, vegetables, fruits, and their pastes and jams. They may be precooked, but some fillings and toppings are added to dough without cooking and are cooked with the dough.

### *26.4.1 Significant Organisms*

#### 26.4.1.1 Hazards and Controls

Those of potential concern are fillings or toppings with sensitive ingredients such as products of animal origin (e.g., meat, fish, milk, eggs), especially if they are inappropriately cooked. The presence and potential for growth of pathogens in the fillings and toppings depends on the composition, the degree of cooking and the amount of handling before they are used. Thorough cooking and

hygienic handling of cooked filling and topping is important. Use of pasteurized egg is effective in reducing the potential for *Salmonella* contamination, particularly when cooking of the finished product may not be sufficient to eliminate the hazard.

GHP during processing is essential to reduce contamination from the environment and equipment, cross contamination from other raw ingredients and subsequent growth of microorganisms in cooked foods. Sanitary cleaning procedures, temperature control, records for applicable cooking and cooling, and operational practices of workers should be routinely examined and reviewed. For uncooked ingredients added to the cereal shells and cooked to produce the final products, temperature control is critical. In an *S. enteritidis* outbreak in Japan, 96 school children became ill by consuming undercooked dessert buns served in a school lunch. Leakage at the edge of an oven was strongly suspected to cause undercooking of the dessert buns that contained contaminated eggs (Matsui et al. 2004). Other details of the recommended practices are described in the previous publication (ICMSF 2005).

Refer to appropriate product category chapters to understand the hazards associated with various fillings based on their ingredients.

#### 26.4.1.2 Spoilage and Controls

In general, dough products with fillings or toppings may be more susceptible to microbial growth than unfilled products due to a potential increase of $a_w$ and pH, as well as potential nutrient changes as a consequence of the filling or topping process. Spore formers that survive heat treatments may grow in some final products if formulation or temperature control is not used. Fungi and spoilage bacteria may contaminate the product during the filling and topping process via the equipment or from the environment. Temperature control of fillings, toppings and final products that support microbial growth is essential for both safety and spoilage control. Basic hygiene control of the processing area and filling equipment is critical.

### *26.4.2 Microbial Data*

#### 26.4.2.1 Critical Ingredients

Refer to appropriate product category chapters to understand hazards and appropriate tests associated with various fillings.

#### 26.4.2.2 In-Process

For process control, routine monitoring would be appropriate for the fillings and toppings that are RTE after being added to baked shells, especially if they support microbial growth. For cooked materials, aerobic colony counts and Enterobacteriaceae would be appropriate indicators. Aerobic colony count may also be appropriate for certain uncooked fillings, especially if temperature control is not practiced and there is potential for growth in the filling during the time of production.

#### 26.4.2.3 Processing Environment

Monitoring the environment to identify potential harborage sites for *Salmonella* is recommended to verify sanitation conditions of the facility and to prevent occasional contamination of the intermediate or final products from the environment. For RTE products that support the growth of *Listeria monocytogenes*, environmental sampling is recommended.

### 26.4.2.4 Shelf Life

The shelf life of the products depends on the composition of fillings and toppings and intended distribution and storage conditions. Appropriate refrigeration, freezing, modified atmosphere packaging and use of preservatives will influence the shelf life of individual products. For products filled after cooking, it is important to assess the level of control at the interface between the filling and the dough. It has been demonstrated in some products that growth may be inhibited in subcomponents

**Table 26.1** Testing of topped or filled dough products for microbiological safety and quality

| Relative importance | | Useful testing |
|---|---|---|
| Critical ingredients | Low to high | Test for mycotoxins if confidence in ingredient flour is low<br>Test sensitive ingredients with no subsequent kill step for *Salmonella* if confidence in supplier is low |
| In-process | High | For cooked fillings or toppings, test appropriate product residues and in-line samples to verify adequacy of processing and lack of recontamination. Appropriate tests depend on the type of product and process involved. Refer to text |
| Processing environment | High | Test for *Salmonella* in the processing plant environment as appropriate (see text). Typical levels encountered:<br>• *Salmonella* – absent |
| Shelf life | High | Examine $a_w$, pH and atmosphere condition for products with long shelf life that rely on these parameters for stability |
| End product | Low | Testing for pathogens is not recommended during normal operation when GHP and HACCP are effective as confirmed by relevant tests above. When above testing or process deviations indicate a possible safety issue, these sampling plans may be considered if the pathogen listed is identified as a potential hazard for the specific product through hazard analysis |

| Product | Microorganism | Analytical method[a] | Case | Sampling plan & limits/g[b] n | c | m | M |
|---|---|---|---|---|---|---|---|
| Frozen RTE dough products with low acid or high $a_w$ fillings or toppings | *S. aureus* | ISO 6888-1 | 9 | 10 | 1 | $10^2$ | $10^4$ |
| | *L. monocytogenes*[c] | ISO 11290-2 | NA[d] | 5 | 0 | $10^2$ | – |
| | | | | Sampling plan & limits/25 g[b] | | | |
| | *Salmonella* | ISO 6579 | 12 | 20[e] | 0 | 0 | – |
| | *L. monocytogenes*[f] | ISO 11290-1 | NA | 5[e] | 0 | 0 | – |
| Frozen or refrigerated ready-to-cook dough products with low acid or high $a_w$ fillings or toppings | | | | Sampling plan & limits/g[b] | | | |
| | *S. aureus* | ISO 6888-1 | 8 | 5 | 1 | $10^2$ | $10^4$ |
| | | | | Sampling plan & limits/25 g[b] | | | |
| | *Salmonella* | ISO 6579 | 10 | 5[e] | 0 | 0 | – |

[a] Alternative methods may be used when validated against ISO methods
[b] Refer to Appendix A for performance of these sampling plans
[c] Products do not support *L. monocytogenes* growth under intended use (e.g., consumed in the frozen state, or thawed and consumed within lag time)
[d] *NA* not applicable due to use of Codex criteria
[e] Individual 25 g analytical units (see Sect. 7.5.2 for compositing)
[f] Product supports *L. monocytogenes* growth under intended use (e.g., thawed and refrigerated for substantial time)

(e.g., filling and cooked dough), but growth may occur at the interface. The combination of cooking and modified atmosphere packaging may provide conditions that support growth of pathogenic spore formers depending upon $a_w$ and pH. Validation of the intended shelf life of the product is important to assure safety under intended use and distribution conditions.

#### 26.4.2.5 End Product

Microbiological testing may be useful for some topped or filled dough products, but not for others. ICMSF previously proposed criteria for *S. aureus* and salmonellae for dough products containing fillings and toppings that have $a_w \geq 0.85$, pH ≥ 4.6 or supporting growth of pathogenic microorganisms (ICMSF 1986). Since the earlier publication, automation of some manufacturing processes may reduce the potential risk presented by *S. aureus* if extensive handling by workers is eliminated (e.g., machine assembled pasta in place of hand-formed pasta). Additionally the potential risk presented by *L. monocytogenes* for refrigerated RTE products, especially those supporting growth of the microorganism, must be considered. Table 26.1 summarizes the relative importance of testing topped or filled dough products. Selection of specific microorganisms as well as product attributes (pH, $a_w$, preservatives, etc.) and process controls (e.g., time and temperature) depends on the particular product. Therefore the recommendations in Table 26.1 are general in nature and need to be modified based on the results of a thorough hazard analysis.

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# Appendix A
# Sampling Considerations and Statistical Aspects of Sampling Plans

## Types of Attributes Sampling Plans

ICMSF (1974) first established guidance on the use of sampling plans and microbiological criteria for foods in international trade. This book and a previous update (ICMSF 1986) continue to use this framework, which has also been adopted by Codex Alimentarius and others. The plans are *attributes* sampling plans for which the results of tests applied to the samples are used only to classify the individual test samples as *acceptable* or *defective* in a two-class plan, or *acceptable*, *marginally acceptable* or *defective* in a three-class plan (Fig. A.1) according to some specified condition, or attribute, of the sample. The decision to accept or reject the product is based on the number of test sample results in each class. The microbiological criteria define the acceptability of a product or a food lot, based on the absence or presence or number of microorganisms or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot of the product (Codex Alimentarius 1997). A full description of the statistical basis and operation of these plans has been described (ICMSF 2002) and a summary is provide below.

### *Basic Statistics of Sampling*

In attributes sampling, the overall quality of a lot or batch of product is assessed on the proportion of units in the lot that have the specified attribute or satisfy the specified condition. In food microbiology the attribute specified is frequently the absence of a pathogen in a specified amount of product. An acceptable product satisfies the criterion of absence (i.e., a "negative" result), whereas a defective product is one that is found to contain the microorganism (usually called a "positive" in presence/absence testing). If many of the microorganisms are present in the food sampled, a positive result is expected for most tests. However, if few microorganisms are present, fewer tests are expected to yield a positive result.

Imagine that ten sample units of a food from a lot are tested using an appropriate laboratory procedure for the presence of a specific microorganism. If the microorganism is not detected in any of the analytical units then the whole lot of food is considered to be acceptable relative to this microorganism. However, if the microorganism is detected in one or more samples, the whole lot is rejected. This plan is described by $n = 10$ (number of sample units drawn) and $c = 0$ (maximum allowable number of positive results).

It is possible that a plan will occasionally accept a defective lot (i.e., consumer's risk). There is no way to avoid some degree of error in acceptance and rejection decisions unless the entire lot is tested, in which case no edible food is left. The risk of the wrong decisions can be reduced by testing more

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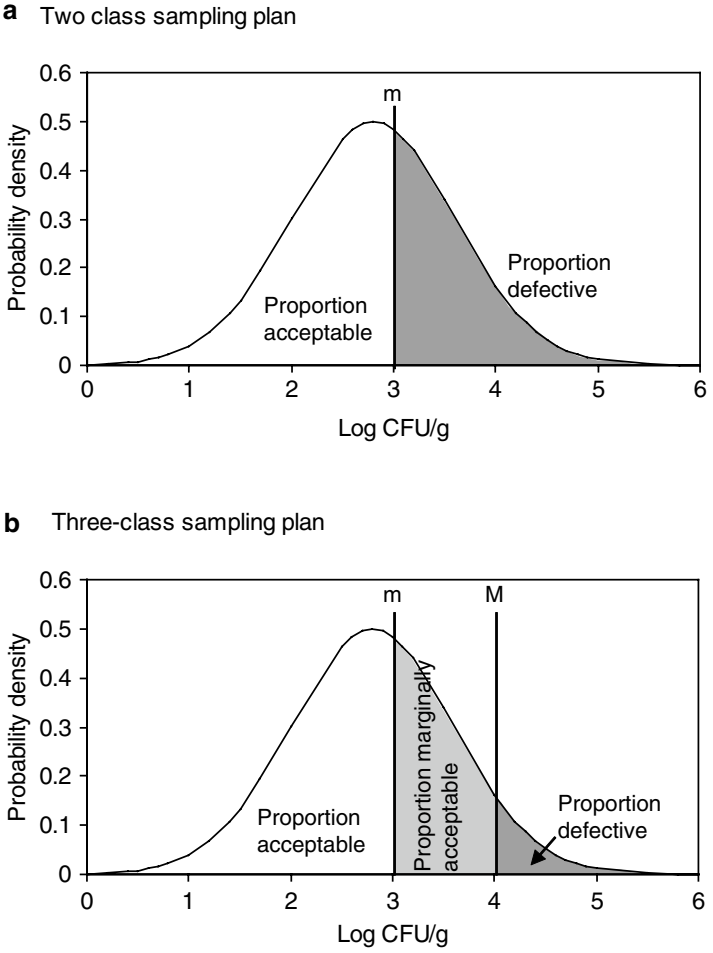


**Fig. A.1** Relationship between: (**a**) acceptable and defective log concentrations for a two-class plan ($m=3$ log CFU/g) and (**b**) acceptable, marginally acceptable and defective concentrations for a three class plan when $m=3$ log CFU/g, $M=4$ log CFU/g and the distribution of organisms has geometric mean = 2.8 and standard deviation 0.8

sample units; i.e., a larger value for $n$. In theory, the chance of a wrong decision based on sampling can be reduced to any desired level by making n sufficiently large but, in practice, a compromise is made between large n (many sample units) and reduced chance of making an erroneous evaluation of the status of the lot, and small n (few sample units) and higher probability of a wrong decision.

An operating characteristic function describes the performance of a sampling plan. The function relates the probability of acceptance, $P_a$, which is the expected proportion of times that the results will indicate that the lot is acceptable, for a given number of samples from that lot that are examined for the defect, and for a true rate, or proportion, of defective units in the lot as a whole.

With a sampling plan taking only one sample ($n=1$), for any defect rate, the probability of sampling a defective unit is simply the same as the true defect rate, and the probability of accepting the batch based on that sample is given as $(1-P_a)$. For example, if the defect rate is 50% there is a one in two chance of selecting a defective unit and, thus, a one in two chance of accepting the batch based on that sample. However, if 10% of units are defective, there is a 10% (one in ten) chance of randomly selecting as the sample one of those defective units and rejecting the batch, but there is a 90% chance of not sampling a defective unit and, therefore, a 90% chance of accepting the batch

based on a single sample. If two samples were taken (i.e., $n=2$), the chance of not detecting a positive in either sample is the product of the probability of not detecting a positive in the first sample and the probability of not detecting a positive in the second sample. For sampling plans with $c=0$, the probability of acceptance for any number of samples is given by the product of the probability of not detecting a positive in the first sample, and the probability of not detecting a positive in the second sample, and the probability of not detecting a positive in the third sample, and so on. This relationship between the true defect rate and the probability of detection (and, thus, of acceptance of the batch) is summarized in the Binomial distribution, which can be described mathematically. In fact, the hypergeometric distribution provides a more correct description of the type of sampling done for product acceptance in food microbiology, but the two distributions are very similar when the total quantity tested is a small proportion of the total size of the lot being assessed, so that the Binomial distribution provides a very good approximation for most realistic sampling schemes. However, for pathogen testing, $c$ is frequently set to 0, especially for ready to eat products. When $c=0$, the probability of acceptance calculated by the binomial is a good approximation to that calculated by the hypergeometric for finite population sizes. Table A.1 illustrates the effect of sample number and true defective rate on the probability of not detecting a defective sample and therefore of determining that the batch is acceptable.

The above probabilities of acceptance can be calculated for any combination of true defect rate, number of samples ($n$) and $c$. This relationship can be plotted as an *operating characteristic* (OC) curve (Fig. A.2). This is often done to be able to quickly calculate the confidence one has in the reliability of the results of a sampling plan, or to calculate how many samples need to be tested to achieve a stated degree of confidence of detecting a lot of unacceptable quality, where the quality is defined by the rate of defective units and the attribute itself.

Since decisions to accept or reject lots are made on samples drawn from the lots, occasions arise when the sample results do not reflect the true condition of the lot. Note that sampling plans with smaller sample sizes have less ability to correctly discriminate between acceptable and unacceptable lots.

**Table A.1** Effect of true rate of defective units and number of samples on probability of lot acceptance for sampling plans with $c=0$

| % defective | 0 | 5 | 10 | 20 | 30 | 50 |
|---|---|---|---|---|---|---|
| Pa (1 sample) | 1.00 | 0.95 | 0.90 | 0.80 | 0.70 | 0.50 |
| Pa (5 samples) | 1.00 | 0.77 | 0.59 | 0.33 | 0.17 | 0.03 |

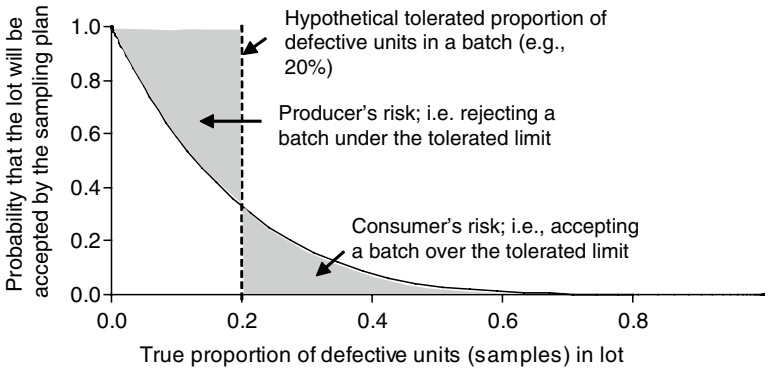


**Fig. A.2** Operating characteristic curve for a sample plan with $n=5$ and $c=0$, illustrating the consumer's risk and producer's risk

The *producer's risk* is the probability of falsely rejecting a lot of acceptable quality, and assumes that there is a small, but acceptable proportion of defective samples. Conversely, the *consumer's risk* describes the probability that a defective lot will be falsely accepted. Consumer's risk, for the purpose of this text, is considered to be the probability of accepting a lot when the actual microbial content is substandard as specified in the sampling plan, even though the samples tested indicate acceptable quality. The consumer's risk is equivalent to the probability of acceptance ($P_a$) *for an unacceptable lot*. The producers risk is the probability of rejection ($1-P_a$) *for an acceptable lot*. Figure A.2 illustrates the producer's risk and consumer's risk as a function of the true defective rate in a batch of product, for a sampling plan of $n=5$ and $c=0$. Producer's risk decreases as the true proportion of defective units decreases, providing incentive for producers to operate well below the tolerated defective level. Consumer's risk associated with a sampling plan decreases as the true proportion of defective units increases because it is more likely that a defective batch will be rejected.

## *Representative Sampling*

When designing a sampling plan it is important to avoid bias in an attempt to have the sample represent the population of the lot as well as possible. Random sampling is one way of achieving this. Consider a lot made up of 10 g blocks called *sample units*, and a decision is made to sample 10 such units. These units should be chosen in a manner that each sample unit in the lot has the same chance of being included among the sample units chosen. In practice it is often difficult to ensure such random samples are drawn, and this can be particularly significant for populations with incomplete mixing or of unknown origin. At the very least, an attempt should be made to draw test material from all parts of the lot.

## *Performance of Microbiological Methods*

The estimates for the performance of sampling plans in this book do not take into account any errors that might arise from the microbiological methods used to determine either the presence or concentration of microorganisms in foods. The errors associated with quantitative microbiological methods, such as colony count techniques, differ from those for qualitative methods, such as presence/absence tests. Errors affecting the quality of data obtained by analytical laboratories have been reviewed by Corry et al. (2007) and Jarvis (2008). The quality of results is characterized by the accuracy of the method, i.e., the ability to provide results equal to, or close to, the real value. Repeatability ($r$) of a method reflects the difference between two single results obtained when the same sample is analyzed by the same analyst under identical analytical conditions. On the other hand, reproducibility ($R$) represents the difference obtained between two laboratories. Laboratory accreditation procedures, and national and international definition and standardization of laboratory methods seek to define the level of uncertainty that can be ascribed to a series of tests (Corry et al. 2007). Organizations such as the International Standards Organization (ISO), Codex Alimentarius and AOAC International attempt to provide measurements of uncertainty associated with methods used for the examination of foods for pathogenic and other microorganisms.

Participation of laboratories in proficiency tests offered by national, professional or commercial organizations also represents an opportunity for improvements in analytical performance and laboratory procedures. The quality control of the media used, control of incubator and water bath temperatures, and improvements in the skills and training of personnel and standardization of laboratory practices play a role (Black and Craven 1990, Peterz 1992, Berg et al. 1994). Proficiency tests

facilitate benchmarking performance of the laboratory and identification of weaknesses that need improvement. Samples provided for proficiency testing have limitations related to the preparation and the viability of the microorganisms added to the sample. Consequently, check samples for proficiency testing are not available for all food matrices. The concentration of pathogens is frequently relatively high and a competitive flora is not always included in check samples. Therefore, such samples may not accurately assess the laboratory's ability to detect very low numbers of injured cells that may occur in actual food samples. The use of reference materials containing very low levels of injured cells may be more useful in assessing laboratory performance and reliability of a method. Reference materials have been developed for various microorganisms (Peterz and Steneryd 1993, In't Veld et al. 1995).

Simplified or alternative methods are often used to cope with large numbers of analyses and to obtain results more quickly. This is legitimate and can accommodate a sudden influx of samples, e.g., environmental samples to detect a source of contamination. Alternative methods that allow a laboratory to analyze a greater number of samples may be more effective in identifying a potential source of contamination than applying cumbersome standard methods that limit the number of samples that can be analyzed. However, if alternative methods are used, it is extremely important to validate the method. This not only allows more results to be obtained sooner, but also guarantees reliability of results. A number of validation procedures exist, ranging from a simple peer review by an expert panel, to thorough procedures based on extensive comparative and collaborative studies (Andrews 1996, Lombard et al. 1996, Rentenaar 1996, Scotter and Wood 1996).

## *Quantitative Performance of Attributes Sampling Plans*

The attribute assessed in attributes sampling plans in food microbiology is frequently based on the presence or absence of the microorganism of concern in a defined quantity of the sample, or series of samples, of the product (e.g., not detected or "negative" in five samples of 25 g each). However, the attribute is sometimes based on whether the concentration of microorganisms in the sample is above or below a limit (e.g., $<10^2$ CFU/g).

It is useful to understand how probable it is that a given sampling plan will detect a certain level of contamination in the product and thus reject a nonconforming batch. This is known as the *performance* of the sampling plan. It has been demonstrated that contamination is often not homogenously distributed within a lot. In other words, single distribution does not characterize the population, but rather a mixture of multiple distributions. At the scale of a lot or between lots, the mean concentration is usually not constant but varies according to a lognormal distribution. However, at the local scale of a sample, the mean concentration can be considered constant, in which case the number of colony forming units (CFU) in a sample varies randomly according to the Poisson distribution.

Frequently, most samples of a contaminated lot will test negative, with only a few testing positive. These few, however, may be capable of causing illness. Therefore, when selecting or designing an attributes sampling plan, the intent is to ensure that the *average* concentration in the batch is sufficiently low so that, within a specified level of confidence that accounts for variation, no samples from the batch contain unacceptable levels.

When an attributes sampling plan is based on a detection of a microorganism in a defined quantity of food, the absence of any positive result is often misinterpreted as demonstrating the total absence of contaminants in the whole lot. A more appropriate interpretation is that presence/absence testing based on enrichment methods involves the same concept as a "most probable number" method, in which replicates of a single dilution of the sample are tested. Thus, absence of a positive result suggests only that the contamination level is below that which the sampling plan is able to reliably detect. The performance or likelihood of a sampling plan to detect a microorganism can be determined

(Legan et al. 2000, van Schothorst et al. 2009). The method described by van Schothorst et al. (2009) is more appropriate for sampling plans involving enrichment and is outlined below.

It may be tempting to infer that a negative result for a sample can be used to calculate the concentration on the basis of simple probability; e.g., absence in 25 g suggests that the concentration is <1 cell/25 g or <0.04 cells/g, and absence in five samples of 25 g infers that the concentration is <0.008 cells/g. This simplistic approach assumes that the cells are homogenously distributed in the lot, and even then at this concentration the probability of detecting a positive in the sample is not 100% but rather only 63%. Variation in the concentration of cells in the lot and random aspects of sampling small particles (cells) in large samples must be considered. Taking more random samples provides more confidence that the results are representative of the entire lot, but cannot guarantee detection.

At the very low pathogen concentration levels typically considered in presence/absence testing, assuming a continuous distribution like the lognormal is inappropriate because organisms are discrete. Discrete distributions like the Poisson are more appropriate because a sample either has no organisms or a countable number of organisms. Even if the cells are evenly distributed throughout the lot, the result is affected by chance events relating to the position of the cell relative to where the material is sampled. Thus, even when the true concentration in a sample is below the acceptable limit, a sample unit could contain a cell and the batch be rejected with a $c=0$ sampling plan. Similarly a series of samples may fail to include a cell even if simple probability would suggest that at the concentration present, a cell would be expected to be detected among the total volume of sample analyzed. This effect is less pronounced when a higher concentration of cells is acceptable, e.g., when the attribute is set at <100 cells/g, as opposed to absent in the sample. This is because the sampling error is larger when fewer items are observed in the sample. In Poisson processes, the standard deviation is equal to the square root of the mean number of target cells/sample. Presence/absence methods are based on the observation of one, or at most, a few cells. Thus, whereas the standard deviation associated with a count of 100 cells is ±10%, for a test involving observation of single cell the standard deviation approaches 100%.

It has been demonstrated that the concentration of microorganisms frequently follows a log-normal distribution in foods (Jarvis 2008). Therefore the normal distribution of log counts can be used to estimate the proportion of defective samples in a lot if the overall geometric mean (the term "mean" refers to geometric mean throughout the rest of this appendix) and standard deviation are known or can be inferred. In reality, the standard deviation can never be truly known. It must be estimated. However, estimates of these values can be used to determine the relative probability of accepting a defective lot of food for a given sampling plan.

A sampling plan can never assess the mean concentration of the entire lot with complete accuracy. It can only estimate the concentration at a selected level of confidence. To assess the performance of a sampling plan, one needs to know the number and size of samples tested, and assume the variability in concentration of cells within the lot. The Poisson effect in sampling can also be accounted for in interpreting the detection threshold of a specified attributes sampling plan. A spreadsheet tool enabling these calculations and including consideration of the Poisson effect is available at www.icmsf.org.

The tool was used to identify the geometric mean that results in a 5% probability of lot acceptance under different sampling plans recommended in this book using a range of standard deviations. The true standard deviation of the distribution of concentration of contaminants in a lot is unknown, thus the tables include a range of distributions of cell concentration for illustration purposes. For example, the standard deviation of the distribution of cell concentrations in a well mixed product such as milk may be lower than that for a product in which ingredient quality or process hygiene could vary over the production run. The standard deviations used apply to distribution of cell concentrations and do not include variation associated with analytical methods.

Table A.2 provides the performance of sampling plans using viable counts and the geometric mean concentration of CFU/g that would be rejected by the sampling plan with 95% confidence is provided.

**Table A.2** Performance of attributes sampling plans in this book for attributes assessed by viable count data

| ICMSF cases | $n$ | $c$ | $m$ | $M$ | Sample size | Geometric mean concentration (CFU/g)[a] at 95% probability of rejection | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | s.d.[b] = 0.25 | s.d. = 0.50 | s.d. = 0.8 | s.d. = 1.2 |
| 2, 5, 7 | 5 | 2 | <1 | 5 | NA[c] | 1.6 | 2.2 | 2.5 | 2.7 |
| 2, 5, 7 | 5 | 2 | <3 | 9.8 | NA | 4.8 | 5.8 | 6.2 | 6.1 |
| 2, 5, 7 | 5 | 2 | <10 | – | NA | 17 | 28 | 51 | 110 |
| 2, 5, 7 | 5 | 2 | 10 | $10^2$ | NA | 17 | 25 | 33 | 39 |
| 2, 5, 7 | 5 | 2 | $10^2$ | $10^3$ | NA | 170 | 250 | 330 | 390 |
| 2, 5, 7 | 5 | 2 | $10^2$ | $10^4$ | NA | 170 | 280 | 480 | 790 |
| 2, 5, 7 | 5 | 2 | $5\times10^2$ | $5\times10^3$ | NA | 830 | 1,300 | 1,600 | 1,900 |
| 2, 5, 7 | 5 | 2 | $10^3$ | $10^4$ | NA | 1,700 | 2,500 | 3,300 | 3,900 |
| 2, 5, 7 | 5 | 2 | $10^3$ | $5\times10^4$ | NA | 1,700 | 2,700 | 4,500 | 6,800 |
| 2, 5, 7 | 5 | 2 | $10^3$ | $10^5$ | NA | 1700 | 2800 | 4800 | 7900 |
| 2, 5, 7 | 5 | 2 | $10^4$ | $10^5$ | NA | 17,000 | 25,000 | 33,000 | 39,000 |
| 2, 5, 7 | 5 | 2 | $2\times10^4$ | $5\times10^4$ | NA | 30,000 | 34,000 | 35,000 | 33,000 |
| 4 | 5 | 3 | 10 | $10^2$ | NA | 23 | 39 | 51 | 57 |
| 3, 6, 8 | 5 | 1 | 2.3 | 7 | NA | 2.9 | 3.2 | 3.3 | 3.3 |
| 3, 6, 8 | 5 | 1 | 10 | $10^2$ | NA | 13 | 16 | 18 | 20 |
| 3, 6, 8 | 5 | 1 | $10^2$ | $2\times10^2$ | NA | 120 | 130 | 120 | 120 |
| 3, 6, 8 | 5 | 1 | $10^2$ | $10^3$ | NA | 130 | 160 | 180 | 200 |
| 3, 6, 8 | 5 | 1 | $10^3$ | $10^4$ | NA | 1,300 | 1,600 | 1,800 | 2,000 |
| 3, 6, 8 | 5 | 1 | $10^4$ | $10^5$ | NA | 13,000 | 16,000 | 18,000 | 20,000 |
| 9 | 10 | 1 | $10^2$ | $5\times10^2$ | NA | 86 | 72 | 54 | 35 |
| 9 | 10 | 1 | $10^2$ | $10^4$ | NA | 86 | 73 | 61 | 46 |
| 9 | 10 | 1 | $10^3$ | $10^4$ | NA | 860 | 730 | 580 | 390 |
| 10[d] | 5 | 0 | $10^2$ | – | NA | 93 | 87 | 80 | 71 |
| 11 | 10 | 0 | $10^2$ | – | NA | 69 | 47 | 30 | 17 |
| NA | 3 | 1 | 10/100 mL | 100/100 mL | 100 mL | 19/100 mL | 33/100 mL | 54/100 mL | 91/100 mL |
| NA | 3 | 1 | 100/100 mL | $10^3$/100 mL | 100 mL | 190/100 mL | 330/100 mL | 540/100 mL | 910/100 mL |

The performance is the geometric mean concentration (CFU/g) at which the sampling plan will reject a lot with 95% confidence

[a] Numerical notation is used for clarity but only two significant figures are inferred

[b] s.d. = standard deviation of log counts

[c] NA = not applicable assuming representative sample of product

[d] Also applicable to Codex criteria for *L. monocytogenes* for products not supporting growth

Table A.3 provides the geometric mean at 95% confidence for attributes plans based on enrichment of samples. These are reported as the number of grams or mL containing, on average, only one cell.

For some cases in the Tables (e.g., cases 2, 5, 8 and sometimes 6) as the standard deviation (s.d.) increases, the geometric mean detected with 95% confidence also increases. Conversely, in other cases (e.g., cases 9–15), as the s.d. increases, the geometric means detected with the same level of confidence decrease. Also in Table A.3 for $n=1$ sampling plans, higher geometric means are required for detection with 95% confidence, while when more samples are taken (cases 10–15) lower geometric means are detected for a higher s.d. This may seem to be contradictory, but this can be explained.

Consider a sampling plan with an acceptable limit of 2 log CFU/mL assessed by a two class sampling plan ($m=M=2$ log CFU/g = 100 CFU/g). Figure A.3a illustrates the probability distribution with s.d. = 0.25 for which 5% of samples are below $m=2$ and 95% are above. The mean of the log normal distribution that satisfies this criterion is 2.41 (geometric mean 260). Thus any batch with a geometric mean ≥260 CFU/mL will be rejected with 95% confidence. If the s.d. is increased to 1.2 (Fig. A.3b) and 5% of the distribution is still below $m=2$, the distribution gets wider, which moves the log mean (3.97, geometric mean 9,300) to the right.

**Table A.3** Performance of attributes sampling plans in this book for attributes assessed by presence/absence (i.e., enrichment) methods

| ICMSF cases | *n* | *c* | *m* | *M* | Sample size | Geometric mean concentration (per g or mL) at 95% probability of rejection[a] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | s.d.[b] = 0.25 | s.d. = 0.50 | s.d. = 0.8 | s.d. = 1.2 |
| 10[c] | 5 | 0 | 0 | – | 10 g | 1 cell in 18 g | 1 cell in 20 g | 1 cell in 22 g | 1 cell in 25 g |
| 10[c] | 5 | 0 | 0 | – | 25 g | 1 cell in 44 g | 1 cell in 49 g | 1 cell in 55 g | 1 cell in 62 g |
| 11 | 10 | 0 | 0 | – | 25 g | 1 cell in 93 g | 1 cell in 120 g | 1 cell in 180 g | 1 cell in 310 g |
| 12 | 20 | 0 | 0 | – | 25 g | 1 cell in 190 g | 1 cell in 270 g | 1 cell in 490 g | 1 cell in 1,200 g |
| 14 | 30 | 0 | 0 | – | 10 g | 1 cell in 120 g | 1 cell in 170 g | 1 cell in 340 g | 1 cell in 980 g |
| 14 | 30 | 0 | 0 | – | 25 g | 1 cell in 290 g | 1 cell in 430 g | 1 cell in 850 g | 1 cell in 2,400 g |
| 15 | 60 | 0 | 0 | – | 25 g | 1 cell in 590 g | 1 cell in 910 g | 1 cell in 2,000 g | 1 cell in 7,400 g |
| NA[d] | 1 | 0 | 0 | – | 100 mL | 1 cell in 27 mL | 1 cell in 13 mL | 1 cell in 5.0 mL | 1 cell in 1.3 mL |
| NA | 1 | 0 | 0 | – | 250 mL | 1 cell in 69 mL | 1 cell in 33 mL | 1 cell in 13 mL | 1 cell in 3.2 mL |
| NA | 5 | 0 | 0 | – | 100 mL | 1 cell in 177 mL | 1 cell in 196 mL | 1 cell in 219 mL | 1 cell in 249 mL |
| NA | 5 | 0 | 0 | – | 250 mL | 1 cell in 440 mL | 1 cell in 490 mL | 1 cell in 550 mL | 1 cell in 630 mL |
| NA | 5 | 0 | 0 | – | 50 mL | 1 cell in 88 mL | 1 cell in 98 mL | 1 cell in 110 mL | 1 cell in 120 mL |

The performance is the geometric mean concentration (grams containing one cell) at which the sampling plan will reject a lot with 95% confidence

[a] Numerical notation is used for clarity but only two significant figures are inferred

[b] s.d. = standard deviation of log counts

[c] Also applicable to Codex criteria for *L. monocytogenes* for products supporting growth

[d] NA = Not applicable because no ICMSF case exists

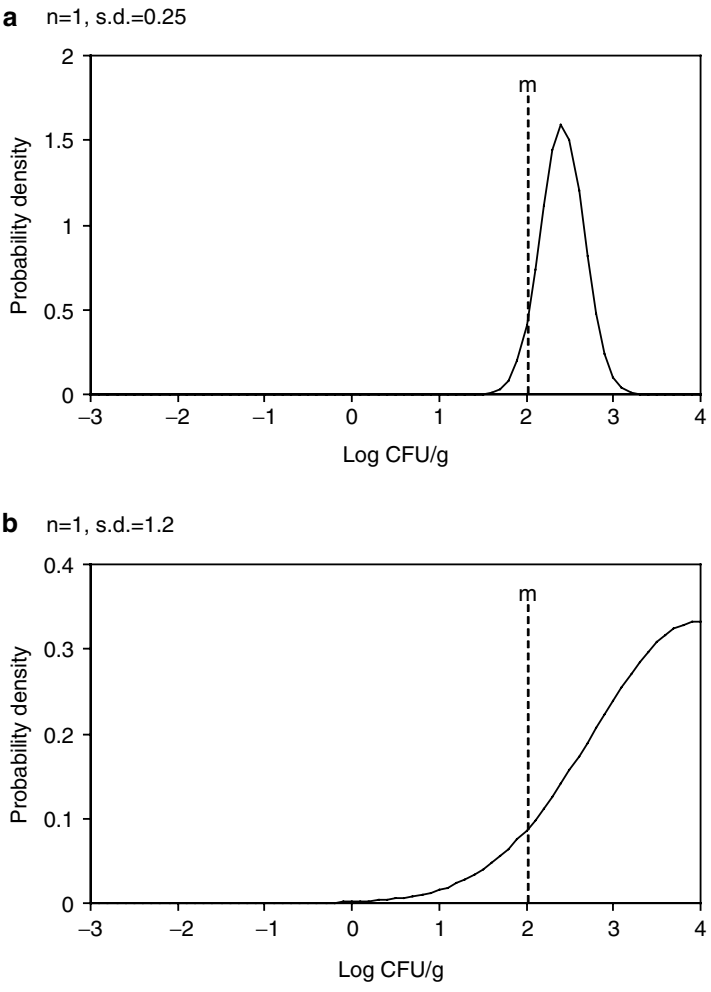


**Fig. A.3** Distributions for a sampling plan with $n = 1$ and $m = 2$ logs for rejection with 95% confidence for (**a**) s.d. = 0.25 (log mean = 2.41 → geometric mean = 260) and (**b**) s.d. = 1.2 (log mean = 3.97 → geometric mean = 9,300)

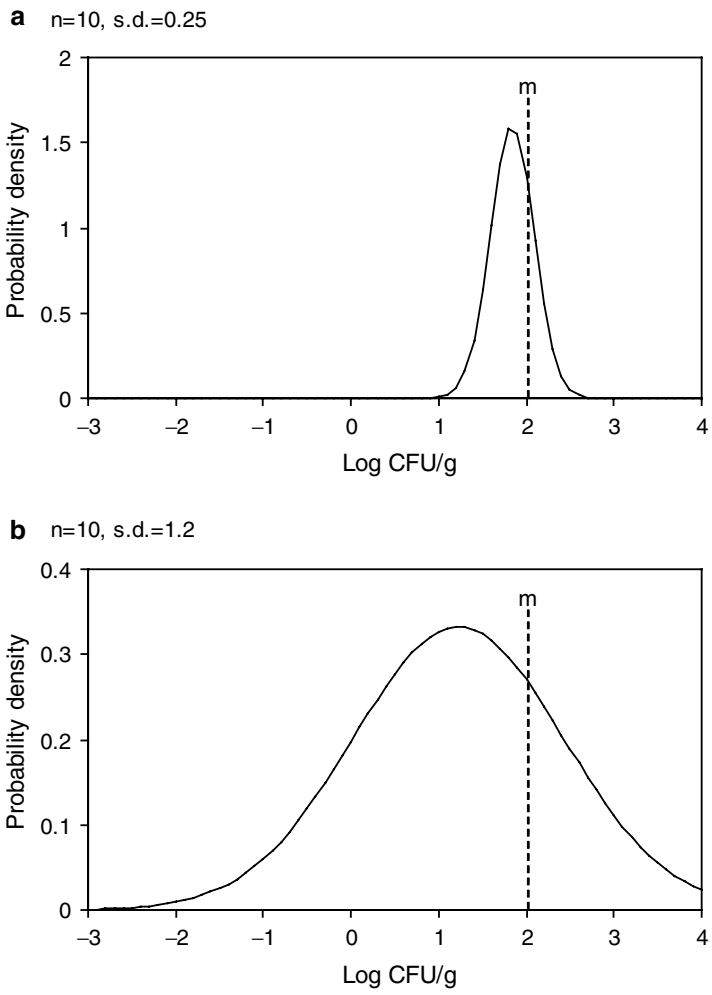


**Fig. A.4** Distributions for a sampling plan with $n=10$ and $m=2$ logs, for rejection with 95% confidence for (**a**) s.d. = 0.25 (log mean = 1.84 → geometric mean = 1.84) and (**b**) s.d. = 1.2 (log mean = 1.22→ geometric mean = 17); m (– –), probability distribution (—)

With $n=10$ and s.d. = 0.25 (Fig. A.4a) the distribution is such that 74% of the data are below $m=2$ (since $0.74^{10}=0.05$, yielding 5% probability not to detect). If the s.d. is increased to 1.2 (Fig. A.4b), the distribution becomes wider, but again 74% of the distribution should be below $m=2$. In this case the geometric mean moves to the left, which reduces the geometric mean detected with 95% confidence.

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# Appendix B
# Calculations for Chapter 2

## Equivalent Effects for Microorganism Level and Variability

The values in Fig. 2.4 (see Chap. 2) can be calculated using the $z$-score. For FSO = 2, the calculation is $x+z \cdot s = 2$, with a mean value $x$, a standard deviation $s$ and with the $z$-scores determined by the probability level. The $z$-score is presented in Table B.1.

The probability lines in Fig. 2.4 can be calculated using the equation $s=(2-x)/z$. For example, the line for a probability of 0.05 in Fig. 2.4 is described by

$$s = (2 - x) / z = (2 - x) / 1.645.$$

In Table 2.1, the mean levels of 1.03, 0.63 and 0.18 and the standard deviation of 0.59 correspond to a probability level of 0.05, 0.01 and 0.001, respectively:

$$\left(2 - 1.03\right) / 1.645 = 0.59 \quad \text{(using the } z - \text{score for 0.05 probability level)}$$

$$(2 - 0.63) / 2.326 = 0.59 \quad \text{(using the } z - \text{score for 0.01 probability level)}$$

$$(2 - 0.18) / 3.09 = 0.59 \quad \text{(using the } z - \text{score for 0.001 probability level)}$$

The effect of reducing the standard deviation can be converted in a log gain by this approach. An equivalent change in level following a reduction of the standard deviation can be determined by the formula $\Delta x = z \Delta s$.

In Table 2.2, a mean of −1.2 with standard deviation of 1.11, results in

$$z = (2 - x) / s = (2 + 1.2) / 1.11 = 2.88.$$

By reducing $s$ in $H_0$ from 0.8 to 0.4, the standard deviation of the overall level reduces from 1.11[1] to 0.87.[2] This yields a "gain" in log mean of 0.69[3] logs. Thus, the extent to which one can move the mean concentration while retaining the same proportion defective depends on both the change in overall standard deviation and on the conformity level set (Table B.1).

[1] $1.11 = \text{sqrt}(0.8^2+0.5^2+0.59^2)$ from Table 2.2

[2] $0.87 = \text{sqrt}(0.4^2+0.5^2+0.59^2)$ from Table 2.5

[3] $0.69 = 2.88 \times (1.11-0.87)$

**Table B.1** $z$-score at various probability levels (one sided test)

| Probability level | $z$-score |
|---|---|
| 0.05 | 1.645 |
| 0.01 | 2.326 |
| 0.005 | 2.576 |
| 0.002 | 2.878 |
| 0.001 | 3.090 |

# Appendix C
# ISO Methods Referenced in Tables

## Rational for Choosing ISO Methods

One of the requirements for accurate articulation of a microbiological criterion is identification of the method used to generate the result. The Commission recognizes that many standard references exist and chose to use ISO methods to be consistent the Codex Alimentarius Commission. Other methods may be used when validated against these methods (Table C.1).

**Table C.1** ISO methods referenced in tables in this book

| Method number | Title |
|---|---|
| ISO 4833:2003 | Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony count technique at 30°C |
| ISO 6222:1999 | Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium |
| ISO 6461–2:1986 | Water quality – Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) – Part 2: Method by membrane filtration |
| ISO 6579:2002 | Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp |
| ISO 6785:2001 | Milk and milk products – Detection of *Salmonella* spp |
| ISO 6888–1:1999 | Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium |
| ISO 7899–2:2000 | Water quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method |
| ISO 7932:2004 | Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration presumptive *Bacillus cereus* – Colony count technique at 30°C |
| ISO 7937:2004 | Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of *Clostridium perfringens* – Colony count technique |
| ISO 9308–1:2000 | Water quality – Detection and enumeration of *Escherichia coli* and coliform bacteria – Part 1: Membrane filtration method |
| ISO 11290–1:1996 | Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection method |
| ISO 11290–2:1998 | Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method |
| ISO 16266:2006 | Water quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration |
| ISO 16649–2:2001 | Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* – Part 1: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide |

(continued)

**Table C.1** (continued)

| Method number | Title |
|---|---|
| ISO 16654:2001 | Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157 |
| ISO 21527–2:2008 | Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 2: Colony count technique in products with water activity less than or equal to 0.95 |
| ISO 21528–1:2004 | Microbiology of food and animal feeding stuffs – Horizontal methods for the detection and enumeration of Enterobacteriaceae – Part 1: Detection and enumeration by MPN technique with pre-enrichment |
| ISO/TS 21872–1:2007 | Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholera* |
| ISO/TS 22964:2006 | Milk and milk products – Detection of *Enterobacter sakazakii* |

# Appendix D
# Objectives and Accomplishments of the ICMSF

## History and Purpose

The International Commission on Microbiological Specifications for Foods (ICMSF, the Commission) was formed in 1962 through the action of the International Committee on Food Microbiology and Hygiene, a committee of the International Union of Microbiological Societies (IUMS). Through the IUMS, the ICMSF is linked to the International Union of Biological Societies (IUBS) and to the World Health Organization (WHO) of the United Nations.

In the 1960s there was growing recognition of foodborne disease, which consequently stimulated greatly increased microbiological testing of foods. This created unforeseen problems in international trade in foods. Different analytical methods and sampling plans of doubtful statistical validity were being used. Furthermore, analytical results were interpreted using different concepts of biological significance and acceptance criteria, creating confusion and frustration for both the food industry and regulatory agencies. In this environment ICMSF was founded to: (1) assemble, correlate and evaluate evidence about the microbiological safety and quality of foods; (2) consider whether microbiological criteria would improve and assure the microbiological safety of particular foods; (3) propose, where appropriate, such criteria; and (4) recommend methods of sampling and examination.

Nearly fifty years later, the primary role of the Commission is to be a leading source for independent and impartial scientific concepts that, when adopted by governmental agencies and industry will reduce the incidence of microbiological foodborne illness and food spoilage worldwide and facilitate global trade.

## *Functions and Membership*

The ICMSF provides basic scientific information through extensive study and makes recommendations without prejudice based on that information. Results of the studies are published as books, discussion documents or refereed papers. Major publications of the Commission are listed in Appendix F. The recommendations of ICMSF have no official status, the official promulgation of such recommendations are nationally the province of governments and internationally the province of the United Nations and its agencies such as WHO and FAO.

The ICMSF functions as a working party, not as a forum for the reading of papers. Meetings consist largely of discussions within subcommittees, debating to achieve consensus, editing draft materials and planning. Most work is done between meetings by the Editorial Committee and members, sometimes with the help of nonmember consultants.

Since 1962, 43 meetings have been held in 24 countries (Australia, Brazil, Canada, Chile, China, Denmark, Dominican Republic, Egypt, England, France, Germany, India, Italy, Mexico, Singapore,

South Africa, Spain, Switzerland, The Netherlands, Uruguay, USA, the former USSR, Venezuela and the former Yugoslavia). During its meetings, Commission members frequently participate in symposia organized by microbiologists or public health officials of the host country.

As this book is published, the membership consists of 17 food microbiologists from 12 countries, with combined professional interests in research, public health, official food control, education, product and process development, and quality control from government laboratories in public health, agriculture and food technology; from universities; and from the food industry (see Appendix E). The ICMSF is also assisted by consultants, specialists in particular areas of microbiology and critical to the success of the Commission (see Consultants, Contributors and Reviews in the front matter of this book). New members and consultants are selected for their expertise, not as national delegates. All work is voluntary without fees or honoraria.

Currently, three Sub-Commissions (Latin American, South-East Asian, China/North-East Asian) promote ICMSF activities among food microbiologists in their regions and facilitate communication worldwide (see Appendix E).

The ICMSF raises its own funds to support its meetings. Support has been obtained from government agencies, WHO, IUMS, IUBS and the food industry, including over 100 food companies and agencies in 20 countries (see Appendix G). Grants for specific projects, seminars and conferences have been provided by a variety of sources. Some funds are received from the sale of its books.

## *Past and Present Work*

Since its founding, ICMSF has had a profound and global impact on the field of food microbiology by addressing such issues as test methods for microorganisms, sampling plans, microbiological criteria, HACCP, risk assessment and risk management. Its activities and recommendations are published as books, scientific and popular papers, opinion papers, proceedings and presentations.

For almost 25 years major ICMSF efforts were devoted to methodology. This resulted in improved comparisons of microbiological methods and better standardization (17 refereed publications). Among many significant findings it was established that, when analysing for salmonellae, analytical samples could be composited into a single test with no loss of sensitivity. This made it practical to collect and analyze the large number of samples recommended in some sampling plans. With the rapid development of alternative methods and rapid test kits, and the ever expanding list of biological agents involved in foodborne illness, the Commission discontinued its program of comparing and evaluating methods, recognizing that issues of methodology were being addressed effectively by other organizations.

The long-term objective of the Commission to enhance the microbiological safety of foods in international commerce was initially addressed through two books that recommended uniform analytical methods (ICMSF 1978), and sound sampling plans and criteria (ICMSF 1974, 1978, 2nd ed 1986). The Commission then developed a book on the microbial ecology of foods (ICMSF 1980a, b) intended to familiarize analysts with processes used in the food industry and microbiological aspects of foods submitted to the laboratory. Knowledge of the microbiology of the major food commodities, and the factors affecting the microbial content of these foods, helps the analyst to interpret analytical results.

At an early stage the Commission recognized that no sampling plan can ensure the absence of a pathogen in food. Testing foods at ports of entry, or elsewhere in the food chain, cannot guarantee food safety. This led the Commission to explore the potential value of HACCP for enhancing food safety. A meeting in 1980 with the WHO led to a report on the use of HACCP for controlling microbiological hazards in food, particularly in developing countries (ICMSF 1982). The Commission then developed a book on the principles of HACCP and procedures for developing HACCP plans

(ICMSF 1988), covering the importance of controlling the conditions of producing, harvesting, preparing and handling foods. Recommendations are given for the application of HACCP from production and harvest to consumption, together with examples of how HACCP can be applied at each step in the food system.

The Commission next recognized that a major weakness in the development of HACCP plans is the process of hazard analysis. It is difficult to be knowledgeable about the many biological agents recognized as responsible for foodborne illness. ICMSF (1996) summarized important information about the properties of biological agents commonly involved in foodborne illness, and serves as a quick reference when making judgments on the growth, survival or death of pathogens.

Subsequently the Commission updated its volume on the microbial ecology of food commodities (ICMSF 1998).

*Microorganisms in Foods 7: Microbiological Testing in Food Safety Management* (ICMSF 2002) introduced the concept of Food Safety Objectives and their use for the establishment of HACCP plans and microbiological criteria. The book gives an up-date of the statistical aspects of sampling and the choice of the "cases" that determine the stringency of sampling plans. *Microorganisms in Foods 7* replaced the first part of *Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications* (1986). It illustrates how systems such as HACCP and GHP provide greater assurance of safety than microbiological testing, but also identifies circumstances in which microbiological testing still plays a useful role. Since the publication of *Microorganisms in Foods 7* in 2002, a number of these important concepts have been adopted by the Codex Alimentarius Commission and included in their procedural manual. Importantly, the new risk management framework has been used to facilitate and expedite the development and communication of risk management options for a number of urgent food safety public health issues internationally. A good example is the Codex standard for the control of *Cronobacter* spp. (*E. sakazakii*), the organism identified as causing illness and death of infants through consumption of infant formula. In this case, the scientific community was able to use the risk management framework to very quickly provide advice to caregivers and other stakeholders to impact positively on the implementation of preventive measures.

In addition to the English version of the *Microorganisms in Foods* Series, most books are also available as a Spanish translation within Latin America. *Microorganisms in Foods 7* will also be available in Mandarin for China and the updated version of *Microorganisms in Foods 6* will be available in Japanese.

More recently, the Commission produced an updated 2nd edition of *Microorganisms in Foods 6: Microbial Ecology of Food Commodities* (2005). The publication describes the initial microbiota and the prevalence of pathogens, the microbiological consequences of processing, typical spoilage patterns, episodes implicating food commodities with foodborne illness, and measures to control pathogens and limit spoilage for 17 major food commodities. As well as updating knowledge on the microbial ecology for each commodity, control measures were presented in a standardized format in line with international developments in risk management and a comprehensive index was also added.

ICMSF has produced a number of other useful publications, aimed at both the scientific community or at interested laymen. Addressing the need for a scientific basis in risk assessment, a working group of the ICMSF published "Potential application of risk assessment techniques to microbiological issues related to international trade in food and food products" (ICMSF 1998). As national governments look to use the tools from epidemiology to evaluate the success and performance of risk management options, the Commission endeavored to articulate the role of epidemiology in risk management in the scientific paper, "Use of epidemiologic data to measure the impact of food safety control programs" (ICMSF 2006). More recently, the Commission has published two further concept papers aimed a examining the implications of the new risk management framework to both the establishment of microbiological specifications (Van Schothorst et al. 2009) and also the validation of control measures in a food chain (Zwittering et al. 2010).

A successful popular press publication is the ICMSF layperson's guide, "A Simplified Guide to Understanding and Using Food Safety Objectives and Performance Objectives," (ICMSF 2005). First issued in English, this guide has been translated into French, Portuguese, Spanish and Bahasa Indonesia. It was intended to inform readers about the new Codex risk management metrics in non-technical language. The guide is also now available on the ICMSF website as an illustrated version suitable as an educational resource.

Many members actively collaborate with FAO and WHO by participating in expert meetings, consultations and Codex working group meetings, and by involvement as expert trainers in capacity building activities. During preparation of this book, ICMSF has been represented on the Codex Committee on Food Hygiene (CCFH) and the Codex Committee on General Principles (CCGP), and several members have represented ICMSF on Codex electronic working groups and regional committees. Several ICMSF concepts and principles have been adopted by Codex Alimentarius, e.g., in several new guidelines and Codes of Hygienic Practice developed by CCFH, or in commodity-specific Codes such as those for milk products or meat products. As this book goes to press, the Commission is providing expert advice within CCFH on Proposed Draft Revision of the Recommended International Code of Hygienic Practice for Collecting, Processing and Marketing of Natural Mineral Waters, the Proposed Draft Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food, as well as the Proposed Draft Guidelines for the Establishment and Application of Microbiological Criteria for Foods.

After nearly fifty years of service, the original objectives of the Commission are even more relevant today given food safety trends and an anticipated doubling demand and international trade in food by 2050. Diseases caused by foodborne pathogens constitute a worldwide public health problem and food exports and imports are a critical factor in both the economic recovery and food security of many countries. Effective global food safety management systems and standards are therefore important from a public health and economic standpoint as national governments seek to protect their consumers while facilitating trade. In an environment of global interdependence in food security, countries cannot solely rely upon their own food safety managements systems and it is therefore essential that food safety standards are based on sound scientific principles and that their equivalency can be demonstrated. It is in this context, that the continued role of the ICMSF as a leading source for independent and impartial scientific advice to international standard setting bodies such as the Codex Alimentarius Commission, national governments and industry will be crucial to the development of equivalent food standards aimed at reducing the burden of global diseases and facilitating international trade in food. The future success of ICMSF continues to depend upon its ability to work effectively with its partners, as well as the efforts of its members and consultants who generously volunteer their time, and those who provide the financial support so essential to the Commission's activities.

See Appendix F, ICMSF Publications, for complete citations for books and publications cited in this section.

## *ICMSF General Conference Sites and Major Sponsors*

| No. | Year | Location | Sponsors |
|---|---|---|---|
| 1 | 1962 | Montreal, Quebec, Canada | Members' agencies |
| 2 | 1965 | Cambridge, UK | Members' agencies; Low Temperature Research Station, Cambridge, UK; Pillsbury Co |
| 3 | 1966 | Moscow, USSR | Members' agencies |
| 4 | 1967 | London, UK | Members' agencies; Unilever Research |
| 5 | 1969 | Dubrovnik, Yugoslavia | Members' agencies; Union of Medical Societies of Yugoslavia; US Department of Health, Education and Welfare, Public Health Service, Centers for Disease Control |

| No. | Year | Location | Sponsors |
|---|---|---|---|
| 6 | 1970 | Mexico City, Mexico | Members' agencies; ICMSF sustaining fund |
| 7 | 1971 | Opatija, Yugoslavia | Members' agencies; Union of Medical Societies of Yugoslavia; US Department of Health, Education and Welfare, Public Health Service; Centers for Disease Control |
| 8 | 1972 | Langford, England | Members' agencies; Meat Research Institute; Agriculture Research Council, UK; ICMSF sustaining fund |
| 9 | 1973 | Ottawa, Ontario, Canada | Members' agencies; Health and Welfare Canada, Health Protection Branch; ICMSF sustaining fund |
| 10 | 1974 | Caracas, Venezuela | Members' agencies; Latin American Congress for Microbiology, International Union of Biological Societies; ICMSF sustaining fund |
| 11 | 1976 | Alexandria, Egypt | Members' agencies; Ministry of Health, Arab Republic of Egypt; US Department of Health and Human Services, Centers for Disease Control; ICMSF sustaining fund |
| 12 | 1977 | Cairo, Egypt | Members' agencies; Ministry of Health, Arab Republic of Egypt; US Department of Health and Human Services, Centers for Disease Control; ICMSF sustaining fund |
| 13 | 1978 | Cairo, Egypt | Members' agencies; Ministry of Health, Arab Republic of Egypt; US Department of Health and Human Services, Centers for Disease Control; ICMSF sustaining fund |
| 14 | 1980 | Stresa,Italy | Members' agencies; Comitato Organizzatore "Total Quality Control Congress"; Regione Piemonte; Regione Lombardia; Provincia di Novara; Banca Popolare di Novara; Fondazione Alivar; Italy Centro Studi Hospes;Terme di Crodo, S.P.A.; ICMSF sustaining fund |
| 15 | 1981 | Chexbres, Switzerland | Members' agencies; Nestlé Products Technical Assistance Co.; ICMSF sustaining fund |
| 16 | 1982 | Anaheim, California, USA | Members' agencies; Silliker Laboratories; ICMSF sustaining fund |
| 17 | 1983 | Sharnbrook, Bedford, UK | Members' agencies; Unilever Research, Colworth Laboratories; ICMSF sustaining fund |
| 18 | 1984 | Berlin, Federal Republic of Germany | Members' agencies; Federal Ministry of Youth, Family Affairs and Welfare; German Research Foundation; Senate of Berlin; Unilever Germany; ICMSF sustaining fund |
| 19 | 1985 | La Jolla, California, USA | Members' agencies; Beatrice Foods; Silliker Laboratories; ICMSF sustaining fund XX 1986 Roskilde, Denmark Danish Meat Products Laboratory; ICMSF sustaining fund |
| 20 | 1986 | Roskilde, Denmark | Danish Meat Products Laboratory; ICMSF sustaining fund |
| 21 | 1987 | Toronto, Ontario, Canada | Members' agencies; Medical research Council Canada; Canada Packers Inc.; ICMSF sustaining fund |
| 22 | 1988 | Dubrovnik, Yugoslavia | Members' agencies; Nestlé Products Technical Assistance Co.; ICMSF sustaining fund |
| 23 | 1989 | Milan, Italy | Members' agencies; Comune di Milano, Camera di Commercio Industria, Artigianato, Agricoltura di Milano; Centrale del Latte di Milano; Egidio Galbani Spa di Milano; Istituto Scotti Bassani di Milano; Nuovo-Criai di Caserta; Ciba-Geigy di Milano; Alfa Laval di Monza;ICMSF sustaining fund |
| 24 | 1990 | Playa Dorada, Dominican Republic | Members' agencies; Pan American Health Organization / World Health Organization; Instituto Dominicano de Tecnología Industrial (INDOTEC); Central Bank of the Dominican Republic; Asociacion de Propietarios de Hoteles y Condominios de Playa Dorada; Secretaría de Estado de Turismo (SECTUR); Nestlé (Dominican Republic); ICMSF sustaining fund |
| 25 | 1991 | Sydney, NSW, Australia | Members' agencies; Australian Institute of Food Science and Technology; ICMSF sustaining fund |
| 26 | 1992 | Taverny, France | Members' agencies; Nestlé France; ICMSF sustaining fund |

(continued)

| No. | Year | Location | Sponsors |
|---|---|---|---|
| 27 | 1993 | Papendal, The Netherlands | Members' agencies; The Netherlands EFFI; Netherlands Society for Microbiology; Netherlands Society for Nutrition and Food Technology; ICMSF sustaining fund |
| 28 | 1994 | León, Spain | Members' agencies; Ministerio de Salud y Consumo; ICMSF sustaining fund |
| 29 | 1996 | Pretoria, South Africa | Members' agencies; ABSA Bank, AECI Aroma & Fine Chemicals; Department of Health; Department of Microbiology-WITS; Gold Star Yeasts; Hartlief Continental Meats; Inspection and Quality Services; Kanhym Fresh Meat; KWV; Labotec, Lever Industrial, S.A.; Society for Microbiology, Sea Harvest Corp.; Separations Scientific, SGS Qualitest; Sun International; 3M South Africa; Toyota S.A. Marketing; Traditional Beer Investments, Black Like Me Products; Boehringer Mannheim; Bull Brand Foods; C.A. Milsch; Dragoco S.A.; Enzymes S.A., Firmenich; First National Bank; Foodtek-CSIR; Foundation for Research Development; Meat Industry Centre (ANPI-ARC); Merck; Nestlé; Nutritional Foods, Quest Intern.; Royal Beech-Nut; Von Holy Consulting, Willards Foods; Woolworths; Xera Tech-The Document Company; Xerox Office Supplies; ICMSF sustaining fund |
| 30 | 1997 | Annecy, France | Members' agencies; Fondation Marcel Mérieux; ICMSF sustaining fund |
| 31 | 1998 | Guaruja, Brazil | Members' agencies; COMBHAL 98; ICMSF sustaining fund |
| 32 | 1999 | Melbourne, Australia | Members' agencies; Public Health and Development Division of the Victorian Department of Human Services; ICMSF sustaining fund |
| 33 | 2000 | Berlin, Germany | Members' agencies; Bundes Institut für gesundheitlichen Verbraucherschutz und Veterinärmedizin; Bund für Lebensmittelrecht und Lebensmittelkunde e.V.; Milchindustrie-Verband e.V.;Kraft Foods, R&D Inc.; ICMSF sustaining fund |
| 34 | 2001 | Annecy, France | Members' agencies; Fondation Marcel Mérieux; ICMSF sustaining fund |
| 35 | 2002 | Pucon, Chile | Members' agencies; VII Congreso Latinamericano de Microbiologia e Higiene de Alimentos, Nov. 2002, Chile; ICMSF sustaining fund |
| 36 | 2003 | Lugano, Switzerland | Members' agencies; Schweizerische Geselschaft für Lebensmittel Hygiene;ICMSF sustaining fund |
| 37 | 2004 | Hangzhou, Shanghai, Beijing, China | Members' agencies; Silliker Group, Corp; bioMerieux China Ltd.; 3M China Ltd; Unilever; DuPong QUALICON; Beijing Sanyuan Foods Co, Ltd.; Zhejiang Provincial Center for Disease Control and Prevention; Zhejiang Gongshang University, College of Food Science, Biotechnology and Environmental Engineering; Shanghai Jiaotong University, Department of Food Science and Technology; ICMSF sustaining fund |
| 38 | 2005 | Wintergreen, USA | Members' agencies; 3M Microbiology; American Society for Microbiology; Ecolab Inc.; Food Products Association; General Mills; Kraft Foods; Masterfoods USA; Cattlemen's Beef Board and the National Cattlemen's Beef Association; Nestlé USA Inc.; Silliker Inc.; Standard Meat Company; US Environmental Protection Agency/Office of Water; US Department of Agriculture/Cooperative State Research; Education and Extension Service; US Department of Agriculture / Food Safety and Inspection Service; US Food and Drug Administration/Center for Food Safety and Applied Nutrition; Risk Assessment Consortium; International Life Sciences Institute; International Association for Food Protection; Institute of Food Technologists; ICMSF sustaining fund |

| No. | Year | Location | Sponsors |
|---|---|---|---|
| 39 | 2006 | Cape Town, Pretoria, South Africa | Members' agencies; Consumer Goods Council of South Africa (CGCSA);South African Association for Food Science & Technology (SAAFoST); 3M; Unilever SA; ICMSF sustaining fund |
| 40 | 2007 | Singapore | Members' agencies; ILSI Southeast Asia Region; Agri-Food & Veterinary Authority of Singapore; ICMSF sustaining fund |
| 41 | 2008 | New Delhi, India | Members' agencies; ILSI India; Ministry of Food Processing Industries, GOI; Agricultural and Processed Food Products Export Development Authority (APEDA); India Council of Agricultural Research (ICAR); India Council of Medical Research (ICMR); National Horticulture Mission (NHM), Ministry of Agriculture; ICMSF sustaining fund |
| 42 | 2009 | Punta del Este, Uruguay | Members' agencies; Latin American Sub-Commission of ICMSF; Uruguayan Society of Food Science and Technology; ICMSF sustaining fund |
| 43 | 2010 | Annecy, France | Members' agencies; Fondation Marcel Mérieux; ICMSF sustaining fund |

# Appendix E
# ICMSF Participants

## ICMSF Members at the Publication of this Book

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| | | | |
|---|---|---|---|
| Dr. A. C. Baird-Parker | UK | 1974–1999 | |
| Dr. M. T. Bartram | USA | 1967–1968 | |
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| Dr. L. Buchbinder* | USA | 1962–1965 | |
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| Dr. R. Buttiaux | France | 1962–1967 | |
| Dr. J. H. B. Christian | Australia | 1971–1991 | Chair 1980–1991 |
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| Dr. M. Ingram* | UK | 1962–1974 | Ex-officio member 1962–1968 |
| Dr. J.L. Jouve | France | 1993–2004 | |

(continued)

| | | | |
|---|---|---|---|
| Dr. M. Kalember-Radosavljevic | Yugoslavia | 1983–1992 | |
| Dr. K. Lewis* | USA | 1962–1982 | |
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| Dr. H. Lundbeck* | Sweden | 1962–1983 | Chair 1973–1980 |
| Dr. S. Mendoza | Venezuela | 1992–1998 | |
| Mrs. Z. Merican | Malaysia | 1992–2004 | |
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| Dr. J. Pitt | Australia | 1990–2002 | |
| Dr. H. Pivnick | Canada | 1974–1983 | |
| Dr. M. Potter | USA | 2003–2009 | |
| Dr. F. Quevedo | Peru | 1965–1998 | |
| Dr. T. A. Roberts | UK | 1978–2000 | Chair 1991–2000 |
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| Dr. G. G. Slocum* | USA | 1962–1968 | |
| Dr. P. Teufel | Germany | 1982–2007 | |
| Dr. F. S. Thatcher* | Canada | 1962–1973 | Chair 1962–1973 |
| Dr. R. B. Tompkin | USA | 1982–2002 | |
| Dr. M. van Schothorst | Switzerland | 1973–2003 | Secretary 1991–2003 |

*Founding member

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### *President*

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# Appendix F
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