Improving the safety of fresh fruit and vegetables

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Contents

Part II Managing risks

19 Combined preservation techniques for fresh fruit

S. M. Alzamora, S. N. Guerrero, University of Buenos Aires, Argentina, A. López-Malo, J. Welti-Chanes, E. Palou and A. Argaiz, University of The Americas, Mexico, and M. S. Tapia, Central University of Venezuela, Venezuela

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1

Pathogens in vegetables

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1.1 Introduction

The health benefits of increasing the proportion of vegetables in the daily diet is well recognized and strongly promoted. However, in recent years the number of foodborne illness outbreaks linked to raw vegetables has increased dramatically (Bean and Griffin, 1990; Centers for Disease Control and Prevention (CDC), 1990; CDC, 2000; Table 1.1). This trend can be partly explained by increased consumption rates, improved surveillance, global/centralized distribution chains and an increase in the proportion of vulnerable people within the population (Food and Drug Administration (FDA), 2001; Sewell and Farber, 2001). However, the most significant factor is the lack of an effective decontamination step at any point in the salad vegetable production chain. Therefore, any contamination acquired in the field can be potentially carried through to consumption. This is especially relevant considering that the prevalence of virulent human pathogens in the environment has increased owing to intensification of animal production, in addition to changes in manure and sewage management practices (International Commission on Microbiological Specifications for Foods (ICMSF), 1998).

In the following chapter an overview of the human pathogens associated with vegetables will be described. Potential sources of human pathogens and persistence in the environment will also be covered. A description of the interaction of human pathogens with growing plants will be outlined. A detailed description of the internalization of human pathogens into the inner plant tissue will be provided. The implications of the association of human pathogens with vegetables will be described and future directions briefly discussed.

Table 1.1 Reported outbreaks of foodborne illness associated with vegetables

Human pathogen	Number of outbreaks		
	1970s	1980s	1990s
Salmonella	4	7	18
Escherichia coli O157:H7	0	0	9
NLV	1	3	1
Protozoan	0	2	3
Total (including others)	26	65	84

Adapted from CDC, 2000.

1.2 Human pathogens associated with vegetables

Vegetables represent a complex commodity that encompasses sprouts (seedlings), leaves, tubers and roots of plants. Crops can be cultivated in open fields, greenhouses and increasingly by soil-free hydroponic systems. In terms of retail, vegetables can be sold intact or minimally processed to provide a ready-to-eat product.

Vegetables can be contaminated at any point in the chain, so that potentially they may harbor a diverse range of human pathogens (Table 1.2). However, from reviewing the incidence of foodborne illness associated with vegetables, the human pathogens of primary concern continue to be *Salmonella*, *Escherichia coli* O157:H7, *Shigella,* Norwalk-like viruses (NLV) and pathogenic protozoa (Table 1.1). In terms of trends, it is noteworthy that *Salmonella* continues to be the main human pathogen associated with vegetables. However, the incidence of *E. coli* O157:H7, enteric viruses and protozoan on vegetables is increasing (Table 1.1).

There can be difficulty in establishing which specific vegetable types carry a greater risk of being contaminated by human pathogens. One approach is to assess which vegetables are commonly implicated in foodborne illness outbreaks. However, this has a disadvantage with respect to the volume of product produced (i.e. market volume) and different cultivation systems. In addition, when outbreaks of foodborne illness occur, raw vegetables are typically overlooked as the source of pathogens in favor of more suspect foods such as meat.

A further method for establishing the range of human pathogens associated with vegetables is to perform large-scale sampling trials. For example, the US Food and Drug Administration (FDA) performed a survey of 1028 vegetables and fruit to determine the incidence of *Salmonella, Shigella* and *E. coli* O157:H7 in domestic produce. The vegetable types included in the survey were broccoli, green onions, celery, cilantro and lettuce. *Shigella* was recovered from green onions (3/93 positive). *Salmonella* was recovered from lettuce (1/142) and cilanto (1/85). No *E. coli* O157:H7 was detected in any

of the samples tested (FDA, 2003). When a similar survey was performed on imported vegetables, *Salmonella* was recovered from cilanto (16/177 positive), lettuce (1/116), celery (1/84) and green onions (1/180). *Shigella* was recovered from celery (2/84), lettuce (1/116) and green onions (1/180) (FDA, 2002). Subsequent investigations identified violations of good agricultural practice (GAP) on all farms supplying contaminated produce.

The FDA survey illustrates the key limitations in relying on the frequency of human pathogens in vegetables to establish relative risk. Human pathogens can be considered opportunistic contaminants and occur sporadically in vegetables. It is tempting to conclude from the FDA data that products such as lettuce or green onions carry a greater risk of being contaminated by human pathogens compared to broccoli. However, variation in cultivation practices and the different degrees to which GAP is adhered to will have an impact on the sanitary quality of vegetables. That is, broccoli would be likely to be contaminated to the same degree as lettuce if cultivated under poor sanitary conditions. Despite the problems in identifying which vegetable types pose the most risk, in terms of food safety, trends are starting to emerge. In broad terms, root vegetables have a higher carriage rate of human pathogens compared to leafy vegetables such as lettuce (Heisick *et al*., 1989; Prazak *et al*., 2002). However, in relative terms, lettuce has been implicated in a greater number of foodborne illness outbreaks compared to vegetables such as carrots (Table 1.3). From all the vegetable types implicated in foodborne illness outbreaks, sprouted seeds remains the most significant food safety risk (Table 1.3). There have been numerous outbreaks of foodborne illness associated with sprouts. The largest outbreak was recorded in Japan where radish sprouts contaminated with *E. coli* O157:H7 resulted in 6000 confirmed cases of illness (Itoh *et al*., 1998). However, *Salmonella* is more commonly implicated in foodborne illness outbreaks associated with sprouts compared with pathogenic *E. coli* and other pathogens (Tables 1.2 and 1.3).

The main reason why sprouted seeds represent such a hazard can be attributed to the high temperature (25–30 °C) and humidity employed during the sprouting process (Hara-Kudo *et al*., 1997). In almost all cases the origin of the pathogens could be traced to the seed used in sprout production. How the seed is contaminated still remains unclear but application of fecally contaminated irrigation water to seed-producing plants is an obvious route. There has been a sustained effort to develop seed decontamination methods to inactivate human pathogens on seeds prior to sprouting. However, despite the diverse array of sanitizers tested, none have been proved to be totally effective (Table 1.4). Indeed, the currently recommended 20 000 ppm calcium hypochlorite cannot be relied upon as a seed decontamination method to inactivate either *E. coli* O157:H7 or *Salmonella* (National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 1999; Canadian Food Inspection Agency (CFA), 2001, Taormina and Beuchat, 1999; Weissinger and Beuchat, 2000). Therefore, sprouted seeds will continue to represent a significant food safety risk until an effective seed decontamination treatment is available.

Table 1.2 Examples of vegetables from which human pathogens have been previously recovered

Pathogen	Product
Aeromonas	Alfalfa sprouts, asparagus, broccoli, cauliflower, celery, lettuce, pepper, spinach
Bacillus cereus	Alfalfa sprouts, watercress, mustard sprouts, soybean sprouts
Campylobacter jejuni	Green onions, lettuce, potato, pepper, spinach
Clostridium botulinum	Cabbage, pepper, garlic, potato, carrots
E. coli O157:H7	Alfalfa sprouts, cabbage, celery, watercress, lettuce, cabbage
Listeria monocytogenes	Bean sprouts, cabbage, chicory, eggplant, lettuce, potatoes, radish, lettuce
Salmonella	Alfalfa sprouts, artichokes, beet leaves, celery, cabbage, cauliflower, eggplant, endive, fennel, green onions, lettuce, mung bean sprouts, mustard cress, pepper, salad greens, spinach
Shigella	Celery, lettuce, green onions
Staphylococcus	Alfalfa sprouts, carrot, lettuce, onions sprouts, radish
Vibrio cholerae	Cabbage, lettuce
Enteric viruses (NLV, *hepatitis A*)	Lettuce, green onions, watercress
Protozoa	Lettuce, onions, green onions

Adapted from Beuchat, 1996 and FDA, 2001.

Table 1.3 Number of significant cases of foodborne illness linked to vegetables from 1990 to 1995

Vegetable	Number of foodborne illness outbreaks					
	Salmonella	*E. coli* O157:H7	*Listeria monocytogenes*	Enteric viruses	Protozoan	Others
Sprouted seed	33	7	0	0	0	2
Lettuce	2	7	1	4	2	3
Cabbage	1	0	1	0	0	1
Root vegetables	0	2	2	1	2	6

Adapted from FDA, 2001.

Table 1.4 Efficacy of seed decontamination methods to inactivate *Salmonella* on seeds destined for sprouting

Sanitizer	Residual *Salmonella* after treatment ($\log cfu\, g^{-1}$)	Log Reduction	[a]Germination (%)
Sodium hypochlorite			
200 ppm	2.55	0.72	98.4
Calcium hypochlorite			
200 ppm	3.15	0.04	95.6
20 000 ppm	1.12	1.95	91.6
Acidified sodium chlorite			
1200 ppm	1.59	1.43	94.5
Tsunami (peroxyacetic acid + hydrogen peroxide)			
1060 ppm	1.46	1.50	88.5
Vortex (peroxyacetic acid)			
1060 ppm	1.28	1.62	90.4
Hydrogen peroxide			
80 000 ppm	0.10	3.22	96.2
Sodium triphosphate			
5.0 ppm	1.33	1.99	93.1
Calcium hydroxide			
(1 ppm)	0.35	2.84	91.2
Calcinated calcium			
(1 ppm)	0.2	2.88	90.7
[b]Acetic acid			
(5% v/v)	0.98	1.74	46.7
[b]Lactic acid			
(5% v/v)	<0.3	2.98	56.8
[b]Citric acid			
(5% v/v)	<0.3	2.98	81.4
No treatment	3.27	0	93.3

Alfalfa seed was inoculated with a cocktail of six *Salmonella* serovars (Montevideo, Infantis, Anatum, Cubana, Stanley). Inoculated seeds were then treated for 10 min in the test sanitizer and subsequently homogenized by stomaching. Serial dilutions were prepared and plated onto TSAN agar plates and the remaining sample enriched for *Salmonella*.

[a] Approximately 100 seeds were removed and germinated on filter paper at 30 °C to access germination yield.

[b] High log count reduction attributed to carry-over of organic acid to agar plates used for enumeration.

Adapted from Weissinger and Beuchat, 2000.

1.3 Characteristics of pathogens recovered from salad vegetables

1.3.1 Pathogenic *Escherichia coli*

Non-pathogenic (generic) *Escherichia coli* is a normal inhabitant of the gastrointestinal tract of humans and animals. However, some *E. coli* strains have now acquired virulence factors enabling them to cause disease of the gastrointestinal, urinary or central nervous system. Pathogenic *E. coli* can be subdivided into five different categories based on the type of clinical condition they cause although all share common linkages (Table 1.5).

All pathogenic *E. coli* strains follow a similar strategy of infection by colonizing the intestinal mucosal cells. The mode in which illness occurs varies between the different pathogenic *E. coli* types. ETEC and EaggEC produce

Table 1.5 Description of pathogenic *E. coli*

Pathogenic *E. coli*	Symptoms	Infectious dose	Implicated in vegetable
EHEC	A range of symptoms can occur including diarrhea, severe abdominal cramps and vomiting. Other more serious symptoms include bloody diarrhea, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and death.	2–100 cells	Yes
EPEC	The symptoms for young children can include watery diarrhea, fever, vomiting and abdominal pain. In adults, the symptoms include severe diarrhea with nausea, vomiting, abdominal cramps and fever.	Unknown	No
EIEC	Includes chills, fever, headache, muscular pain, abdominal cramps and diarrhea. These symptoms are very similar to those for foodborne disease caused by *Shigella*.	$>10^6$	Yes
ETEC	Symptoms include watery diarrhea with fever, abdominal cramps, malaise and vomiting.	$>10^8$	Yes
EAggEC	Persistent diarrhea in young children	Unknown	Yes

enterotoxin, EIEC invade the epithelial cells with EPEC and EHEC adhering to the cell and modifying cellular activity.

Although all pathogenic *E. coli* represent a significant health risk, those belonging to the EHEC group are of most concern especially *E. coli* O157:H7 (Hill *et al*., 1991; Holmberg *et al*., 2004). The reason for the high virulence of EHEC is through the production of shiga-like toxins (verotoxin or verocytotoxin). The genes for shiga toxin are believed to have been horizontally transferred to *E. coli* from *Shigella* via bacteriophage. There are two toxins (encoded by *Stx 1* and *Stx 2*) that act by cleaving a single adenine residue from 28S rRNA belonging to the 60S ribosomal subunit. This apparent trivial alteration in the 28S rRNA sequence has significant consequence in terms of host cell function. This can lead to bloody diarrhea that can be accompanied by renal failure (HUS syndrome).

Although *E. coli* O157:H7 is considered to be the most significant EHEC strain, it must be noted that other non-O157 shiga-toxin producing types such as O111, O145, O113, O103, O91, O26 and O104 also exist (Bower *et al*., 1989; Alexandre and Prado, 2003). Collectively all *E. coli* possessing toxin genes are categorized as shiga-toxin *E. coli* or STEC. However, the presence of *stx* genes is only one component of an array of virulence factors required to cause illness (McNally *et al*., 2001). It is interesting to note that *E. coli* O157 and non-O157 serotypes associated with animals contain only half the virulent factors compared to those of clinical isolates (Johnson *et al*., 2001; Ide *et al*., 2003; Ritchie *et al*., 2003). Therefore, the most virulent STEC *E. coli* have a tendency to be harboured by humans or introduced to animals in contact with sewage (Griffin and Tauxe, 1991; Johnson *et al*., 1996; Tarr and Neill, 1996).

The main source of *E. coli* O157:H7 is from the manure of ruminants (cattle, sheep) and sewage (Chapman *et al*., 1993; Kudova *et al*., 1996). It is estimated that 11% of cattle harbor *E. coli* O157:H7 although estimates of the carriage of total STEC populations is unclear.

ETEC, EIEC and EAggEC have previously been recovered from contaminated vegetables (Beuchat, 1996). ETEC, EIEC and EAggEC are a major cause of diarrhea, especially in infants. Although transmission can be through fecally contaminated food or water, the main route is via person–person contact (Smith *et al*., 1997; Spencer *et al*., 1999; Anon, 2000b). EPEC is almost exclusively transferred via person-to-person contact although sporadic cases of foodborne illness have been attributed to this pathogenic *E. coli* group (Levine and Edelman, 1984; Wu and Peng, 1992).

1.3.2 *Shigella*

Shigella sonnei has been implicated in several vegetable-related foodborne illness outbreaks although normally associated with person-to-person contact. *Shigella* causes dysentery in susceptible hosts with typical

symptoms being diarrhea, abdominal pain, vomiting and fever. Generally, foodborne shigellosis involves a short incubation time (7–36 hours), but symptoms can persist for 3–14 days. As few as 10–100 organisms have been shown to cause illness and secondary complications can occur.

Although *E. coli* O157:H7 and *Shigella* share pathological traits, the latter is less tolerant to environmental stress. Therefore, in the majority of cases, infected food workers are considered the primary source of *Shigella.* However, outbreaks of foodborne illness associated with lettuce contaminated at pre-harvest with *Shigella* have occurred. The most notable was an outbreak involving contaminated iceburg lettuce imported from Spain into the United Kingdom, Norway and Sweden. Subsequent investigations identified that contaminated irrigation water was the probable source of the pathogen (Tauxe, 2004).

1.3.3 *Salmonella*

The genus *Salmonella* includes over 2700 serovars with approximately 200 being associated with human illness. *Salmonella* is commonly carried within the gastrointestinal tract of wild animals, poultry, pigs and humans. *Salmonella enteritidis* and *Salmonella* typhimurium are the main serotypes implicated in foodborne illness. However, *Salmonella* recovered from vegetables typically belong to less common serotype groups (FDA, 2001). Salmonellosis is characterized by diarrhea, fever, abdominal cramps and vomiting usually lasting 4–7 days (Anon, 2000a). The case-fatality rate in industrialized countries is less than 1% (Anon, 2000b).

There is concern with regard to the distribution of multi-drug resistant *Salmonella* within the food chain. It is commonly believed that the exploitation of antibiotics as animal growth promoters has led to the prevalence of resistant servors. It is now emerging that the main source of antibiotic resistance is in humans with the strains being introduced by direct contact with animals being less significant (Snary *et al*., 2004). The possible use of antibiotics to suppress plant pathogens has been considered as a possible route by which *Salmonella* and other human pathogens can acquire resistance. Although this may seem unlikely it is interesting to note that streptogramin-resistant *Enterobacter faecium* has been previously isolated from bean sprouts (Snary *et al*., 2004).

Similarly to *E. coli*, the main transmission route of *Salmonella* to vegetables is through fecal contamination, cross-contamination and food handling. *Salmonella* have been isolated from a broad range of vegetables especially sprouted seeds (Beuchat, 1996; Wells and Butterfield, 1997; Mahon *et al*., 1996; O'Mahony *et al*., 1990). An interesting feature of *Salmonella* associated with vegetables (and other environmental sources) is the tendency to have low virulence compared to those isolated from clinical sources (Tauxe, 1997). Evidence is accumulating to suggest that genes present within *Salmonella* enhance the survival of the pathogen outside the

host environment. Significantly, mutants of *Salmonella* lacking such genes have higher virulence than their parent strain (Winfield and Groisman, 2003; Shelobolina *et al*., 2004). Therefore, a number of *Salmonella* appear to have enhanced their survival in the environment at the expense of virulence. However, this does not imply of course that *Salmonella* associated with vegetables represents a low risk.

1.3.4 *Campylobacter*

Campylobacter jejuni is a normal commensal of the gastrointestinal tract of poultry, pigs and cattle. Human carriers also represent a significant vehicle by which the pathogen can be transferred to foods. *Campylobacter* is notoriously fastidious and has very specific growth conditions. The bacterium can survive for short periods outside the host environment but not to the same extent as *Salmonella* and *E. coli* (Stanley *et al*., 1998). However, despite such fragility *C. jejuni,* and to a lesser extent *C. coli*, has been the main cause of gastroenteritis for several years (Tauxe, 1992; Allos and Taylor, 1998; Anon, 2000a, b). This is likely to be due to the low infectious dose (<500 cells) required to cause symptoms in susceptible hosts.

C. jejuni invade and become established in epithelial cells of the lower intestine whereupon a cholera-like toxin is secreted. The main symptom associated with the disease is perfuse diarrhea, which can last between 2–14 days, although it is rarely life threatening. There has been an increase in the recovery of antibiotic resistant *C. jejuni* from human isolates although not from animals (Piddock, 1999).

C. jejuni have been recovered from vegetables especially root crops (Federighi *et al*., 1999). However, evidence to date suggests that the main source of *Campylobacter* recovered from vegetables occurs via cross-contamination events in food service outlets and the domestic environment (Evans *et al*., 2003).

1.3.5 *Listeria monocytogenes*

Unlike typical enteric bacteria, *Listeria monocytogenes* has adapted to survive in both the host and non-host environment. Because *L. monocytogenes* is widely distributed in nature, the pathogen is a common contaminant of vegetables especially root crops (Farber and Peterkin, 1991).

The virulence of *L. monocytogenes* is often underestimated considering the pathogen causes serious illness frequently resulting in death (Anon, 2000a). Susceptible groups include pregnant women, the elderly and immunocompromised. Listeriosis is not a typical foodborne illness as it is characterized by a variety of syndromes (from mild flu to meningitis). Infectious doses depend on host susceptibility and incubation periods can range from 3 days to 3 months. *L. monocytogenes* strains that can survive for extended periods in the field environment typically have lower virulence

compared to isolates implicated in clinical cases of listeriosis (Wiedmann, 2003). Therefore, similarly to other human pathogens, it appears that certain strains of *L. monocytogenes* have traded pathogenicity for the ability to tolerate environmental stress.

1.3.6 *Aeromonas hydrophila*

Aeromonas are widely distributed in the environment, especially in water, but can occur in human feces (Monteil and HarfMonteil, 1997). Two distinct types of gastroenteritis have been associated with *A. hydrophila*, a cholera-like illness with a watery diarrhea and a dysenteric illness characterized by loose stools containing blood and mucus. The ability of *Aeromonas* to cause illness depends on the presence and expression of virulence factors. Although the bacterium is frequently recovered from water in high densities (10^5–10^9) very few strains have the capacity to cause illness in humans. This has been related to the lack of complete virulence factors and high tolerance of the host. Therefore, although widely distributed on vegetables the significance for food safety remains unclear.

1.3.7 Endospore-forming bacteria

Spores of *Clostridium botulinum* and *Cl. perfringens* can be found both in soil and vegetables. With the advent of MAP packaging and preservation of vegetables in oil the prevalence of these obligate anaerobes is increasing (Peck, 2002).

Bacillus cereus is an aerobic spore-forming bacterium that is widely distributed in soil and on plant material. Therefore, its occurrence in vegetables is not uncommon especially leafy vegetables and sprouts (Kim *et al*., 2004).

1.3.8 Enteric viruses

Enteric viruses only reproduce within the human host and all follow the fecal–oral route of transmission (Cliver, 1997). The most significant characteristic of enteric viruses is the ease by which they can be transferred from person to person and the low infectious dose (<20 particles) required to cause illness (Bidawid *et al*., 2000). Enteric viruses are also very stable with resistances to environmental stresses comparable to those associated with bacterial endospores (Meng and Gerba, 1996).

The majority of foodborne illnesses associated with enteric viruses are short lived and not life threatening (Kurdziel *et al*., 2001). Tracing sources of enteric viruses is problematic owing to the lack of routine detection techniques combined with the fact that very few viral foodborne illness cases are actually reported (Richards, 1999). In comparison to direct person-to-person contact, vegetables represent a relatively insignificant source of enteric viruses (Koopmans and Duizer, 2004). However, both hepatitis A

and Norwalk-like viruses (NLV) have been implicated in cases of foodborne illness associated with contaminated vegetables (Scipioni *et al*., 2000; Fiore, 2004). In such outbreaks the crops had been directly exposed to sewage or had been handled by infected workers (Watanabe *et al*., 2002).

1.3.9 Human pathogenic protozoa

Human pathogenic protozoa such as *Giardia*, *Entamoeba*, *Toxoplasma*, *Sarcocystis*, *Isopora*, *Cryptosporidium*, *Eimeria* and *Cyclospora* can be transferred via fecally contaminated water or vegetables (Armon *et al*., 2002). Similarly to enteric viruses, protozoa require a suitable host for replication but can persist within non-host environments for significant time periods (Slifko *et al*., 2000). The main source of human protozoan is from direct contact with humans as opposed to indirect routes such as foods (Slifko *et al*., 2000). All of these human pathogenic protozoa cause diarrhea-like symptoms, except *Toxoplasma* which causes fetal damage and glandular fever-like syndrome (Dumetre and Darde, 2003).

1.4 Sources of contamination in the vegetable production chain

1.4.1 Transmission of human pathogens in manure, water and soil

To cause contamination of vegetables, enteric bacteria have to be introduced into the production chain at some point. Obviously, direct fecal contamination of vegetables just prior to consumption represents the greatest risk. However, through adequate sanitation and handling this risk can be reduced if not eliminated. Of greater concern is the introduction of contamination during crop cultivation, as many of the potential sources cannot be readily controlled or identified.

Enteric pathogens on vegetables are generally believed to be in survival mode as opposed to actively growing. Previously it has been considered that enteric bacteria such as *E. coli* only survive for 2–3 days after being excreted by the animal host. However, such generalizations are inappropriate considering that *E. coli* populations within the gastrointestinal tract of animals can consist of over 1000 distinct types (strains/genotypes) (Gordon and Cowling, 2003). More significantly, the *E. coli* associated with animals have a broad range of survival abilities within non-host environments. It has also been found that those genotypes that dominate the enteric environment have relatively poor survival outside the host environment (Whittam, 1989). Evidence obtained to date would also suggest that strains of *Salmonella* (Winfield and Groisman, 2003; Hurd *et al*., 2004) and *E. coli* O157 : H7 also exhibit a range of survival abilities during the transition from the gastrointestinal tract to the environment. This has naturally complicated studies

attempting to determine the relative survival of enteric pathogens in the environment. In this respect it is likely that many studies previously performed (especially with laboratory strains) underestimated the tolerance of pathogens to environmental stresses, hence their persistence in manure, soil and water.

Manure

Sewage effluents (of animal or human origin) represent the most significant source of human pathogens recovered in water, soil and vegetables (Cieslak *et al*., 1993). Manure is predominantly used in organic cultivation systems but less so by conventional growers (US Department of Agriculture (USDA), 2001). Although organic produce is thought to represent a significance risk with regard to carriage of enteric pathogens, no data have been reported to confirm this view (Johannessen *et al*., 2004).

The application of untreated manure or sewage to growing crops is a direct route by which vegetables can be contaminated (Jones, 1980). For example, in 1993, Cieslak *et al*. isolated *E. coli* O157 : H7 from lettuce cultivated in a garden in which the soil was amended with fresh manure. Under normal conditions the direct contact of manure with vegetables should not occur since a treatment step is applied prior to disposal of effluent into soil or water. Manure and sewage waste can undergo a variety of treatments such as composting, aerobic and anaerobic digestion, alkaline stabilization, conditioning, dewatering and heat drying. The Environmental Protection Agency (EPA) specify that biosolids derived from manure treatment destined for general fertilizer must have fecal coliform counts <1000 cfu g^{-1}, *Salmonella* <4 cfu g^{-1} and enteric viruses at <4 plaque-forming units per gram biosolids (Mechie *et al*., 1997). In the main, proper manure sewage/manure treatment is sufficient to reduce levels of human pathogens to an acceptable level. The treatment required to assure adequate reduction of enteric pathogens in manure remains a debatable issue. Current treatment regimes are based on studies that evaluated the survival of endogenous or artificially introduced human pathogens into manure held under different conditions. Therefore, persistence of human pathogens in manure tends to vary significantly. However, it is widely accepted that the survival of bacterial and viral human pathogens is dependent on temperature, solid content, pH, bacterial concentration, aeration and holding time (Deng and Cliver, 1995; Ajariyakhajorn *et al*., 1997). *E. coli* O157 : H7 can survive in high moisture content bovine manure for over 70 days at 5 °C which compares to 49 days at 30 °C (Wang *et al*., 1996). The persistence of *Salmonella* in manure is also favored under low temperature and high moisture conditions (Mitscherlich and Marth, 1984). However, survival of both pathogens in manure slurry is significantly reduced to below 10 days (Montville and Mathews, 2001).

C. jejuni persistence in manure is comparatively low, being three days in cattle manure and two days in sewage (Mitscherlich and Marth, 1984).

Enteric virus can persist in sewage for up to four months under low temperatures and high moisture (Enriquez *et al*., 2003).

Irrigation water

Sewage spills, run-off from concentrated animal production facilities, storm-related contamination of surface waters, illicit discharge of waste and other sources can all potentially introduce both bacterial and viral human pathogens into irrigation water (Armon *et al*., 1994; Vernozy-Rozand *et al*., 2002). Irrigation water used in crop production represents one of the most significant sources of contamination in vegetable production. Salad crops irrigated with water contaminated with sewage were responsible for numerous cases of typhoid fever and hepatitis A in Santiago, Chile (Alcayaga, 1993). Lettuce irrigated with contaminated water can accumulate *E. coli* O157:H7 over repeated exposures. As can be expected, lettuce plants exposed to contamination seven days or less prior to harvest represent a greater risk than contaminated irrigation water introduced early in the cultivation period (Solomon *et al*., 2003).

Recycling of municipal wastewater for irrigation purposes has been implemented in several countries such as Australia, Germany, Israel, Spain, Holland and the USA. However, several studies have illustrated that this practice may increase the risk of introducing human pathogens (Exall, 2004). For example, onions and garlic cultivated with treated municipal wastewater harbored unacceptable limits of *Salmonella* and *E. coli* at harvest (Fasciolo *et al*., 2002; Bennett *et al*., 2003).

The method of applying irrigation water can also enhance the introduction of human pathogens to growing vegetables (NACMCF, 1999). The various irrigation modes used for vegetables include gravity (flood) irrigation, spray irrigation, drip/trickle irrigation and subirrigation (FDA, 2001). Many factors, such as water availability and cost, soil type, slope, depth of water table, economics and cropping rotations, determine the mode of irrigation rather than food safety issues. Flood and spray irrigation represent the greatest risk as any contamination within the water is directly deposited onto the edible leaves of crops (FDA, 2001).

There have been numerous studies performed to determine the persistence of human pathogens within irrigation water (Vaz da Costa-Vargas *et al*., 1991; Shuval, 1993; Armon *et al*., 1994; Bastos and Mara, 1995; Gallegos, 1998; Downs *et al*., 1999; Ait Melloul and Hassani, 1999; Takayanagui *et al*., 2000; Table 1.6). There are difficulties in relating the relative persistence of enteric pathogens in water since no standardized experimental protocol is followed. In addition, the occurrence of viable but non-culturable (VBNC) populations of pathogens can further complicate survival values (Mitscherlich and Marth, 1984). For example, *Salmonella* species (including *S.* Typhimurium DT 104), introduced into autoclaved river water, decreased from 8 log to 5 log cfu ml^{-1} over a 45-day period at 23 °C (Santo Domingo *et al*., 2000). However, when the VBNC cells were examined, less than 1 log

Table 1.6 Survival of human pathogens in water

Pathogen	Notes	Temperature (°C)	Survival	Reference
E. coli O157	Sterile municipal water	8	91 days	Wang and Doyle, 1998
	Sterile municipal water	25	49 days	Wang and Doyle, 1998
	Sterile well water	15	1 log reduction in 70 days	Artz and Killham, 2002
	Well water	15	65 days	Artz and Killham, 2002
	Sterile well water	15	10 days	Artz and Killham, 2002
Salmonella	Sterile municipal water	23	2 log reduction after 45 days	Santo-Domingo *et al*., 2000
	River water	23	3 log reduction after 45 days	Santo-Domingo *et al*., 2000
	Sterile well water	18	152 days	Mitscherlich and Marth, 1984
Campylobacter	Sterile municipal water	4	8–28 days	Terzieva and McFeters, 1991
	Sterile municipal water	37	22 hours	Terzieva and McFeters, 1991
Yersinia enterocolitica	Sterile spring water	4	446 days	Karapinar and Gonul, 1991
	River water	16	6 days	Chao *et al*., 1988
	Groundwater	30	10 days	Chao *et al*., 1988
Rotavirus	Groundwater	15	2 log reduction in 5 days	Gerba, 1999

of reduction was observed. Lower temperatures also extended the persistence of enteric pathogens in water (Okafo *et al*., 2003). In general the persistence of *Salmonella* is greater than that of *E. coli* O157 : H7 which in turn persists for longer times than *Campylobacter*.

Survival studies of enteric pathogens are typically performed in sterilized (autoclaved or filtered) water samples. Although this facilitates enumeration of the introduced pathogen it does not provide an assessment of survival in natural environments. In this respect it is interesting to note that survival of enteric pathogens in non-sterile water is significantly shorter owing to the activity of protozoan (Artz and Killham, 2002). However, protozoan can form protective niches for enteric pathogens thereby enhancing persistence under certain conditions (Terzieva and McFeters, 1991).

The persistence of *E. coli* O157 : H7 has also been found to vary depending on the source of water. Artz and Killham (2002) evaluated the survival of *E. coli* O157 : H7 in water sourced from four different wells. The authors reported that in two water samples *E. coli* O157 introduced at levels of 10^7 cfu ml^{-1} were reduced to below the level of detection within 10 days regardless of whether the water had been sterilized or not. Although not confirmed, this low level of persistence was attributed to the presence of antimicrobial ions such as copper. The study underlines the difficulties encountered when attempting to predict the survival of enteric pathogens in water.

Giardia cysts persist for a shorter period in irrigation water compared to *Cryptosporidium* oocysts. A study by Olson *et al*. (1999) showed that temperatures as low as −4 °C inactivated *Giardia* cysts in water while *Cryptosporidium* oocysts remained viable for >12 weeks. At 4 °C *Giardia* cysts retain viability for 11 weeks while *Cryptosporidium* oocysts again survive for longer periods. At 25 °C *Giardia* cysts were inactivated in water within 2 weeks but *Cryptosporidium* oocysts survived for >10 weeks.

Soil

Soil is a natural habitat for several human pathogens such as *B. cereus*, *Cl. botulinum* and *Cl. perfringens*, *L. monocytogenes* and *Aeromonas* (Lund, 1992). Such bacteria have adapted to survival in soil with spores persisting for indefinite periods. The persistence of enteric pathogens in soil is dependent on several factors. For example, the survival of *E. coli* is prolonged in clay soils where absorption of cells by the soil particles provides protection against protozoa (Roper and Marshall, 1978). Persistence of enteric pathogens is also extended in moist soils at cool temperatures (Bolton *et al*., 1999; FDA, 2001). *Salmonella* has higher persistence in soil compared to *E. coli* O157 : H7. When *S.* Typhimurium was inoculated at 8 $\log_{10}$ cfu g^{-1} into moist soil, stored at 20 °C, less than 2 log reductions in numbers were observed after 45 days (Guo *et al*., 2002). However, under natural environmental conditions, *S.* Typhimurium introduced via hog manure only persisted for 14 days (Baloda *et al*., 2001). *Campylobacter* is less persistent compared to both *E. coli* O157 and *Salmonella* but nevertheless can be recovered 20 days after introduction into soil (Mitscherlich and Marth, 1984).

In addition to stress, enteric bacteria also have to compete with natural endogenous microflora to become established in the soil environment. In this

respect it has been perceived that enteric pathogens would struggle to obtain nutrients and/or be inactivated by antimicrobials (e.g. antibiotics) formed by resident microflora. However, Ibekwe and Grieve (2004) reported that introduction of *E. coli* O157:H7 into soil increases the diversity of microbial populations. This would suggest that enteric pathogens, rather then being integrated into soil microflora, can actually modify the microecology. Whether this effect enhances persistence has yet to be elucidated.

The survival of human pathogens in soil can be enhanced by being integrated into the rhizosphere in plants. For example, *E. coli* O157:H7 introduced onto the roots of rye grass or alfalfa plants persist for greater time periods compared to when introduced into soil alone (Gagliardi and Karns, 2002). However, this effect appears to be plant specific since the persistence of *E. coli* O157:H7 was not observed on the roots of clover or hairy vetch. Extended persistence of *L. monocytogenes* in soil when associated with radish or parsley, but not carrots, has also been reported (Al-Ghazali and Al-Azawi, 1990; Van Renterghem *et al*., 1991). Therefore, when considering the persistence of human pathogens within soil, plant type in addition to environmental conditions have to be considered.

Enteric viruses can persist for up to four months in subsurface soil layers. In contrast viruses on the surface are typically inactivated within days by the antimicrobial effects of ultraviolet (UV) light. Under heavy rainfall viruses can be spread over wide areas (>150 m) especially when introduced into water courses (Santamaria and Toranzos, 2003).

While *Giardia* is sensitive to freezing of soil, *Cryptosporidium* is resistant. Olson *et al*. (1999) reported that *Giardia* cysts in soil were inactivated after 7 days at –4 °C, but *Cryptosporidium* could survive for >12 weeks. However, persistence of both protozoa was reduced to 8 weeks at 4 °C and 4 weeks at 25 °C (Olson *et al*., 1999).

1.4.2 Pre- and post-harvest handling

The harvesting and processing of vegetables involves significant handling by agricultural workers. Humans represent a significant source of virulent pathogens that can be readily transferred to vegetables and subsequently to the consumer (NACMCF, 1999). It is estimated that over 50% of all foodborne illness cases can be linked to poor food handling practices. Direct transfer of virulent pathogens from meat to salad vegetables is commonly encountered in the kitchen environment. Pathogens can also be introduced via soil attached to root crops and subsequently transferred to contact surfaces, in addition to other vegetables.

The most significant risk associated with handling is the possible introduction of enteric viruses (Richards, 2001; Koopmans *et al*., 2003). A high profile hepatitis A outbreak associated with green salad onions was reported in the USA in 2003. The initial outbreaks were centered on a restaurant in Pennsylvania, which resulted in 575 cases of hepatitis A and one death. Although the restaurant was initially identified as the

source of the outbreak, subsequent investigation linked other cases in Tennessee and Georgia. Further inspection of the farm in Mexico was later identified as the most likely source of the virus (Anon, 2004). Interestingly, the persistence of viruses such as polio has been shown to be dependent on the vegetable type. When introduced onto lettuce or cabbage a 1 log reduction in polio virus was observed over 8 days. In contrast, viruses introduced onto green onions remained stable for over 14 days (Kurdziel *et al*., 2001).

1.4.3 Processing and washing

Upon harvest the field heat of vegetables is removed to reduce plant autolytic reactions and microbial activity. There are several techniques for cooling crops based on air, water, ice and vacuum-based systems. Water and ice cooling is cheap and can be readily applied in the field environment. Of course if the water or ice is contaminated this would be a direct route for introducing human pathogens. Vacuum cooling requires no water and is performed within a contained chamber. However, human pathogens can be potentially drawn into the internal plant tissue when the vacuum is released. Indeed, vacuum infiltration is a common technique used by researchers to introduce bacteria into the inner plant tissue. There is currently no evidence to suggest that any specific cooling method enhances or decreases the risk of introducing human pathogens.

Operations such as cutting, slicing, skinning and shredding provide an opportunity for human pathogens to grow on the exudates released, penetrate the inner tissue and cross contaminate subsequent produce (Francis *et al*., 1999). Post-harvest washing of vegetables still remains the key intervention step in the production chain even though this has a very low efficacy (Brackett, 1992; Beuchat and Ryu, 1997; Beuchat, 1999; Carmichael *et al*., 1999; NACMCF, 1999; Taormina and Beuchat, 1999). Sodium hypochlorite is typically used as a sanitizer in post-harvest washing even though it is rapidly sequestered by organic matter (White, 1972). This not only results in limited decontamination efficacy but also can lead to accumulation of contamination within wash tanks. Additionally, if the water temperature is lower than that of the vegetable it is possible that microbes will be drawn into the inner plant tissue. To overcome the problem of infiltration it is recommended to use warm water for washing produce (FDA, 1998). However, this has the adverse effect of warming the produce thereby accelerating plant autolysis and growth of spoilage microbes or even human pathogens.

1.5 Interaction of human pathogens with growing vegetables

Once introduced into vegetables human pathogens have to persist through to consumption in order to cause foodborne illness. Of course

any pathogen introduced into vegetables just prior to consumption would represent a greater risk than those contaminating the plant in the field.

The food safety risk associated with contamination introduced to growing salad vegetables remains an active research area. In broad terms human pathogens can persist on the surface of leaves, within the rhizosphere (roots) or within the plant tissue. In the following section the relative persistence of human pathogens on and within growing plants is described. Again, the use of laboratory strains and variation in experimental protocols make comparisons between studies problematic.

1.5.1 Persistence of human pathogens on the phyllosphere of plants

The phyllosphere (or aerial) parts of plants represent a challenge for the survival of microbes. The exposure to high doses of UV, fluctuations in temperature and relative humidity all compromise viability. Bacteria (epiphytes) that exist within the phyllosphere have evolved specialized mechanisms to improve stress tolerance and nutrient acquisition. *Pseudomonas* spp form the predominant bacterial population recovered on the leaves of plants (Lund, 1992; Lindow and Brandl, 2003). Epithytic pseudomonads produce fluorescent or pigmented compounds that afford protection against UV. The hydrophobic waxy cuticle of plants can inhibit the movement and accessibility of nutrients to bacterial cells. However, biosurfactants produced by the majority of epithytic *Pseudomonas* spp decrease the water tension enabling relatively free movement across the leaf surface to nutrient sources and natural openings such as stomata. *Pseudomonas* are also known to release a toxin called syringomycin that can produce holes in the plant cell membrane allowing access to intracellular nutrients without necessarily resulting in disease symptoms.

In contrast to pseudomonads, many human pathogens have no specialized mechanisms to enhance persistence in the phyllosphere. The association of human pathogens with biofilms formed by resident epithytes is considered to enhance survival on leaf surfaces. It has been estimated that 10–40% of the total bacteria on the surface of parsley and broad-leaf endive are associated with biofilms (Morris *et al*., 1998; Morris and Monier, 2003). However, the limited studies performed with *E. coli* or *Salmonella* would suggest that bacterial cells tend to aggregate between the grooves of epidermal cells rather than associate with biofilm structures (Warriner *et al*., 2003a).

There have been relatively few studies investigating the survival of human pathogens on the surface of leaves over long periods. However, studies using *C. jejuni*, *E. coli* and *Salmonella* would suggest that this is significantly lower compared to the rhizosphere (Brandl and Mandrell, 2002; Brandl *et al*., 2004). Nevertheless, as previously outlined, contamination of edible leaves immediately prior to harvest would represent a significant food safety hazard.

1.5.2 Internalization of bacteria through natural openings and damaged plant tissue

It has been demonstrated that human pathogens can also be protected from post-harvest biocidal washing by being located in the subsurface structures of plants such as stomata (Ryall and Pentzer, 1982; Seo and Frank, 1999; Takeuchi and Frank, 2000; Burnett and Beuchat, 2001). *E. coli* O157:H7 inoculated onto lettuce leaves has been shown to survive biocidal washes by being located in stomata, or to a greater extent, when they are able to find entry into the inner part of the leaf through cut edges (Seo and Frank, 1999; Beuchat, 1999). Damaged caused by spoilage bacteria/fungi can also enable human pathogens to enter the inner plant tissue and thereby become protected (Wells and Butterfield, 1997).

1.5.3 Internalization of human pathogens into growing plants

The presence of fungal endophytes within the healthy tissue of vegetables was first described in 1904 (Tan and Zou, 2001). Work performed by Samish and Etinger-Tulczynsha, (1962) suggested that bacterial endophytes also existed within plants although this was disputed for many years (Lund, 1992). However, it has only recently been accepted that bacteria can indeed reside in the internal structures of undamaged plants (Chanway, 1998; Sturz and Nowak, 2000; Elbeltagy *et al*., 2001).

The specific definition of an endophyte remains debatable. Early definitions suggest that an endophyte is any microbe that could be recovered from surface sterilized plant tissue. This was later refined to any microbe that can persist within the internal tissue of plants without causing detrimental or positive effects (Bell *et al*., 1995; Wilson, 1995; Sturz *et al*., 1998; Sturz *et al*., 2000). However, this definition may also be misrepresentative as endophytes are now considered to be involved in suppressing phytopathogens, promoting plant growth and aiding plant nutrition. It should also be noted that the activity of endophytes is dependent on the plant type. For example, an endophyte that appears neutral in one plant type can be growth promoting or even phytotoxic in another (Surette *et al*., 2003).

The endophytic bacterial population of plants is known to be diverse, comprising both Gram positive and Gram negative cells (Bell *et al*., 1995; Quadt-Hallman *et al*., 1997a, b). For example, nitrogen-fixing bacteria from the genus *Azospirillum*, *Herbaspirillum*, *Acetobacter*, *Azoarcus* and *Burkholderia* spp are frequently encountered endophytes in non-legume plants (Baldani *et al*., 1997). Non-nitrogen fixing endophytes include species of *Bacillus*, *Pseudomonas*, *Corynebacterium*, *Micrococcus*, *Erwinia*, *Streptomyces*, *Rhodococcus*, *Microlunatus* and *Luteococcus (*James and Olivares, 1998). Although this list is not comprehensive it provides an indication of the high diversity of bacterial endophyte populations that exist within plants (Weller, 1988; Chanway, 1998; Sturz and Nowak, 2000; Stoltzfus *et al*.,

1997; James and Olivares, 1998; Reinhold-Hurek and Hurek, 1998; Strobel and Daisy, 2003).

Establishment of endophytic populations

Enhancing the growth and resistance of plants through modification of the endophytic microflora has been pursued by several workers (Hallmann *et al*., 1997; Sturz and Nowak, 2000). However, efforts to date to introduce beneficial endophytes into plants have met with little success. Competitive inhibition by endogenous endophyte populations is known to restrict newly introduced strains from becoming established (Hozore and Alexander, 1991). However, additional factors are also likely to affect the ability of a bacterium to become integrated into the endophytic microflora. Indeed, the routes by which endophytes associate with plants remain open to speculation. The introduction of bacteria into the endophytic microflora of plants can occur from populations present within seeds (Clay, 1989; Pleban *et al*., 1995). For example, pseudomonads have been known to infiltrate seeds and become established in the mature plant (Baker, 1972).

However, the significance of endophytic populations being transmitted through generations of plants via seed has yet to be established (Bressan and Borges, 2004). It is thought that the majority of endophytes are selected from the surrounding rhizosphere during the course of seed germination. In this period the seed releases a mixture of carbohydrates and peptides that attract surrounding bacteria (Andrews *et al*., 1982; Joce *et al*., 1990; Hara-Kudo *et al*., 1997; Troxler *et al*., 1997). Bacterial cells can then gain entry into the inner plant tissue via germinating radicals and secondary roots (Agarwal and Shende, 1987; Ndoye *et al*., 1994; Barraquio *et al*., 1997; Hallman *et al*., 1997). Bacteria localized in the apoplastic fluid surrounding the root cells (symplast) are restricted in entering the xylem via the Casparian strip which is a thickened cell wall impregnated with a water-insoluble substance, suberin (Fig. 1.1). However, in emerging seedlings the protective structures are incomplete so enabling bacteria to enter the xylem and become distributed throughout the entire plant (Peterson *et al*., 1981; Kloepper *et al*., 1992; Lamb *et al*., 1996; Troxler *et al*., 1997). Entry of endophytes into the xylem of mature plants is less frequently encountered although it has been reported that the rhizobacterium *Pseudomonas aureofaciens* can become internalized into developed corn plants (Lamb *et al*., 1996). There is evidence to suggest that partial localized degradation of protective structures by plant virus (Brugidou *et al*., 1998) could potentially enable access to the xylem by bacteria. However, typically endophytes are restricted to the roots of plants and to a lesser extent to the aerial tissue.

In addition to physical structures, potential endophytes also have to contend with plant defenses. In broad terms the plant defense mechanisms can be classified as constitutive (pre-formed) or induced (Duff *et al*., 2003).

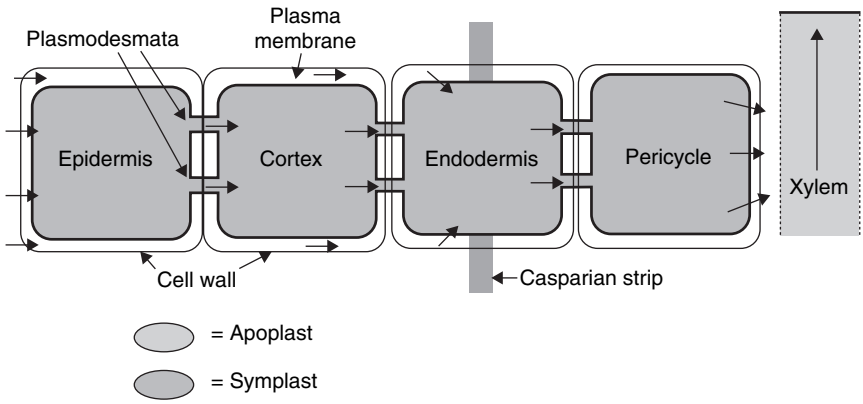

Fig. 1.1 Representation of structures found within plant roots. The apoplast fills the space between the cell wall and plant cells. To enter into the xylem, water must pass through the symplast of the endodermal cells. The movement of bacteria is restricted by the plant cells plasma membranes and the caperian strip within the root.

The constitutive defense essentially suppresses saprophytes or opportunistic phytopathogens. Phenolics, alkaloids, glucosinolates and phytoalexins are all types of chemical agents identified in plants that constitute preformed defenses. The release of antimicrobial proteins by germinating radish seeds has also been reported (Lucas, 1999). Obviously for a microbe to become established in plants it requires resistance to any antimicrobial constituents and/or to prevent activation of inducible defenses (Baker *et al.*, 1997).

Inducible responses (hypersensitive response; HR and systemic acquired resistance; SAR) are commonly a response to phytopathogens whereby the plant detects invasive activity. A strategy developed by some phytopathogens is to avoid activating HR via losing flagella and/or shielding lipopolysaccharides (LPS). Activation of HR and SAR causes localized necrosis to limit the spread of infection. The LPS obtained from numerous bacteria, including generic *E. coli*, can prevent induction of HR but induce localized induced resistance (LIR) (Newman *et al.*, 2001). The LIR leads to the release of antimicrobials within the localized area to suppress saprophytic activity but does not lead to necrosis. This strategy exhibited by plants is to prevent activation of degradative plant defenses when exposed to non-phytopathogens.

Inducible systemic resistance (ISR) is a global defense typically induced by plant growth-promoting endophytic bacteria. Here the plant becomes primed to defend against pathogen attack without leading to programmed cell death.

Human pathogens as endophytes

The internalization of human pathogens into the endophytic microflora of plants has been viewed with skepticism (Lund, 1992). Considering that enteric pathogens are more adapted to the gastrointestinal tract of animals it is difficult to envisage how they could compete with endogenous soil bacteria when colonizing plants. Furthermore, the constitutive plant defenses would be considered sufficient to suppress such saprophytes. However, there is accumulating evidence to suggest that human pathogens can be integrated into the endophytic microflora of plants (reviewed by Warriner *et al*., 2003a). Indeed, it is now emerging that the endophytic microfloras of many plants contain opportunistic animal pathogens such as *Enterobacter amnigenus*, *E. cloacae*, *Stenotrophomonas maltophilia*, *Staphylococcus xylosus*, *Staph. epidermis, B. cereus* and *Ochrobactrum anthropi*. The occurrence of *Staphylococcus* within endophytic populations is surprising considering this is a normal commensal of the skin. However, this underlines that a diverse range of human pathogen types can exist endophytically within plant tissue. However, of most concern are the virulent human pathogens such as *E. coli* O157:H7, *Salmonella*, *Campylobacter* and *L. monocytogenes*.

The ability to utilize the nutrients released by seeds or roots is considered a prerequisite to becoming established in the rhizosphere of plants prior to internalization (Roberts *et al*., 2000). In this respect enteric pathogens, including *Campylobacter*, can actively grow on exudates released by plants (Ji and Wilson, 2002; Warriner *et al*., 2003b, c; Brandl *et al*., 2004).

The resistance of enteric pathogens to plant preformed defenses is starting to emerge. Through various lines of research it is becoming established that Gram negative pathogens use common strategies to invade plant and animal hosts (Buckhout and Thimm, 2003). For example, Type III secretion systems can be found in both plant and animal pathogens. The Type III secretion system is essentially a microtube by which the invading bacterium attaches to the surface of the host cell. Chemicals and proteins are delivered through the Tir III protein to sequester defense mechanisms and reprogram host cell activity. Of course this does not imply that a plant pathogen would cause disease in animals. Indeed, to date only *Pseudomonas auruginosa* PA14 is known to cause disease in both animals and plants (Rahme *et al*., 2000). Nevertheless, it is conceivable that traits in phytobacteria associated with plant interactions could be present in enteric pathogens such as *E. coli* O157:H7. It is known that broad host range bacteriophages that infect *Pseudomonas* and *E. coli* O157:H7 can transfer genetic traits between these two genera (Hendrix *et al*., 1999; Muniesa *et al*., 2004). An example of phytobacteria sharing genes with enteric pathogens has been found in *Ps. syringae* pv. *Maculicola*. The phytopathogen possesses a β-lactamase that protects the bacterium from the preformed defenses of *Arabidopsis*. The gene encoding for the enzyme,

donated as *sax* (**S**urvival on ***A****rabidopsis* e**X**tract), has been identified in a range of *Ps. syringae* pathovars but absent from non-phytopathogenic strains. From comparative homology studies the gene shows a high level of similarity to an uncharacterized gene in *E. coli* O157:H7 (Crooks, unpublished data). Whether the expression of *sax* within *E. coli* O157:H7 enhances persistence within plants has yet to be established.

1.5.4 Internalization of human pathogens in different plant types

Attempting to demonstrate internalization of human pathogens within growing plant tissues is problematic. The traditional approach is to surface sterilize plant material with sanitizers (for example, sodium hypochlorite, peracetic acid, ethanol) and recover the subsequent bacteria. However, spores (fungal and bacterial) and biofilms are resistant to sanitizers leading to false positive results (Reissinger *et al.*, 2001). Penetration of sanitizer into the internal tissue of plants is a further problem and can potentially lead to an underestimation of endophyte numbers. The presence of VBNC can also be encountered when attempting to recover endophytes. This is especially relevant with human pathogens that would ordinarily be present in very low numbers within plant material and then in a stress state.

An alternative to culturing techniques is the application of cell labeling, exploiting green fluorescent protein (gfp) in combination with laser confocal microscopy (Dumas *et al.*, 1999; Brandl and Mandrell, 2000). Gfp is a protein originally isolated from the jellyfish *Aequorea victoria*. The key benefit of gfp is the ability to fluoresce under UV light in the absence of an energy source or other cellular co-factors, thereby enabling *in situ* visualization with minimum disruption to cell physiology. The gene encoding for gfp can be readily inserted and expressed in bacterial cells using plasmid vectors. However, for the plasmid to be retained and replicated within the host cell, selective pressure (typically using an antibiotic) needs to be applied. Therefore, when studying plant–microbial interactions over extended periods where selective agents cannot be used, the gfp phenotype can be readily lost. A further limitation to gfp labeling is the need for a high cell density in order to visualize bacteria using confocal microscopy. Clearly if the tagged bacteria are present in low numbers than locating cells within plant tissue is impossible.

A further method to visualize the presence of internalized bacteria is through the use of a glucuronidase (GUS) activity stain. The GUS stain is based on the cleavage of a chromagenic substrate (for example, 5-bromo-4-chloro-3-indoyl-β-D-glucuronide; X-GLUC) that can be directly visualized as a blue/green precipitate within plant tissues. Therefore, if the target cell is present, the chromagen accumulates and hence has greater sensitivity compared to gfp labels. The GUS technique has been used extensively to study plant–bacteria interactions based on *gus* gene insertion into the target bacterium (Sessitsch *et al.*, 1998). GUS activity is not present in plants

or a wide range of bacteria (Wilson *et al*., 1992), although it is expressed in the majority (>96%) of known generic *E. coli* strains (Lee and Hartman, 1989). Warriner *et al*. (2003b, c) have used GUS *in situ* staining to demonstrate the internalization of generic *E. coli* in spinach and bean sprouts.

In the following section the internalization of human pathogens into a range of crop types will be described.

Sprouted seeds

Studies have shown that radish (Hara-Kudo *et al*., 1997; Itoh *et al*., 1998), alfalfa (Joce *et al*., 1990; Ponka *et al*., 1995; Mahon *et al*., 1997; Taormina and Beuchat, 1999) and mung bean (Warriner *et al*., 2003b) sprouts cultivated from seeds inoculated with either *E. coli* O157:H7 or *Salmonella* typically internalize into the plant tissue. Once internalized both pathogens can survive surface sterilization treatment even by potent antimicrobial agents such as $HgCl_2$ or 20 000 ppm sodium hypochlorite.

Gandhi *et al*. (2001) inoculated alfalfa seeds with gfp tagged *Salmonella* Stanley. The bacterium was found in the subsurface areas of the root, hypocotyls and cotyledons of the formed sprouts. A similar distribution of *E. coli* O157:H7 on alfalfa sprouts derived from inoculated seeds has also been reported (Taormina and Beuchat, 1999). However, Charkowski *et al*. (2002) reported differences in the growth and distribution of gfp tagged *S. enterica* and *E. coli* O157:H7 on alfalfa sprouts. *S. enterica* reached a higher ($3.2 \log \text{cfu g}^{-1}$) level compared with *E. coli* ($\log 2.3 \text{ cfu g}^{-1}$) after two days of sprouting. *E. coli* O157:H7 preferentially colonized the roots whilst *Salmonella* colonized the seed coat and roots (Charkowski *et al*., 2002). Itoh *et al*. (1998) inoculated radish seeds with *E. coli* O157:H7 and, by using immunofluorescent microscopy, the bacteria could be visualized on the inner tissue of stoma and beneath the epidermis of the hypocotyls of the sprouts.

Lettuce

Lettuce has been used as a model vegetable to demonstrate the internalization of human pathogens primarily because of its commercial importance. Wachtel *et al*. (2002a) determined the interaction of gfp labeled non-pathogenic and EHEC *E. coli* with lettuce seedlings in an adherence assay. Here 48-hour germinated lettuce seeds were introduced into suspensions of *E. coli* (ca. 10^6 cfu ml^{-1}) and incubated overnight at 20 °C. The pathogenic *E. coli* cells adhered to the roots to a greater extent than those of the non-pathogenic strains tested. The authors suggested that this could be due to the attachment of the bundle forming pili of pathogenic *E. coli* which is implicated in cell wall attachment in animals (Giron *et al*., 1991). However, when additional non-pathogenic strains were tested the attachment was found to be comparable with the O157:H7 strains studied. When lettuce seedlings were viewed under a confocal microscope the bacteria were observed within the deep grooves and tips of seed coats, root hairs

and the emerging radical. The same authors also cultivated lettuce within soil microcosms irrigated with water containing different inoculation levels (10^8, 10^6, 10^4 and 10^2 cfu ml^{-1}) of *E. coli* O157:H7. The planted lettuce seeds were grown in the inoculated soil for up to 10 days and *E. coli* counts associated with the roots, hypocotyl and cotyledon subsequently determined (Wachtel *et al*., 2002a). At the highest dose level, but not at lower cell densities, *E. coli* was associated with the roots of plants by day 3. *E. coli* numbers at the lower doses progressively increased over the cultivation period to reach levels of 10^3–10^4 on roots. Although the majority of *E. coli* was recovered from the roots, lower numbers (ca. 2 log cfu g^{-1}) were associated with hypocotyls and cotyledons of ten-day-old lettuce plants. Evidence that the *E. coli* had become internalized into the inner plant tissue was obtained using confocal laser microscopy. Here, *E. coli* was observed within the vascular system of hypocotyls (Wachtel *et al*., 2002a).

Solomon *et al*. (2002) propagated lettuce seedlings in soil inoculated with gfp labeled *E. coli* O157:H7 (at cell densities of 10^8, 10^6 or 10^4 cfu g^{-1}). At days 3, 6 and 9 samples were taken with the roots separated from the leaves. In this instance the plant samples were washed to remove surface located bacteria and subsequently surface sterilised using a combination of 80% v/v ethanol and 0.1% $HgCl_2$. The authors reported that *E. coli* O157:H7 could be recovered from the internal tissue of seedlings inoculated with the highest cell density (i.e. 10^8 cfu g^{-1}) but not at the lower inoculation levels applied. Fluorescent microscopy of seedlings showed that *E. coli* was present at depths of up to 45 µm below the outer leaf surface. Subsequent experiments using contaminated irrigation water containing 10^7 cfu ml^{-1} *E. coli* O157:H7 was used to water 50-day-old plants. Care was taken to prevent direct contact with the leaves with soil. *E. coli* O157:H7 was recovered from the leaves but as no surface sterilization treatment was performed, it is unclear whether these were internalized (Solomon *et al*., 2002).

Spinach

The interaction of a bioluminescent labeled generic *E. coli* strain with growing spinach plants was reported by Warriner *et al*. (2003c). The study demonstrated that *E. coli* introduced onto the spinach seed could be recovered from the internal and external tissue of subsequent seedlings. By *in situ* glucuronidase (GUS) staining of seedlings the authors confirmed that *E. coli* had become internalized within root tissue and, to a limited extent, within hypocotyls. Spinach plants derived from inoculated seed and cultivated in soil microcosms harbored the *E. coli* strain on the surface and internal root structures. *E. coli* could also be recovered from the surface of spinach leaves but not internally. Twenty-day old plants introduced into inoculated soil microcosms harbored *E. coli* on the surface of roots and leaves but internalization into the inner tissue was restricted (Warriner *et al*., 2003c). Interestingly, when corresponding studies were performed in a

hydroponic cultivation system, the uptake of *E. coli* into the internal root tissue was greater compared to plants cultivated in soil microcosms.

Hydroponic cultivation of crops within contained greenhouses has been considered to afford protection against introduction of human pathogens from contaminated soil and animals. However, the results from Warriner *et al*. (2003c) would suggest that if contaminated irrigation water is used in hydroponic cultivation the risk of plants being contaminated is in fact greater compared to soil-based systems.

Similar studies with spinach plants have also been performed using a bioluminescent strain *E. coli* O157:H7. The root colonization characteristics and persistence were the same as observed for generic *E. coli* (Jablasone *et al*., 2005). Through scanning electron microscopy studies the authors were also able to demonstrate that *E. coli* O157:H7 introduced to seeds preferentially colonized the root junctions of subsequent plants. The root junctions represent sites where nutrient-rich exudates are released thereby enhancing the persistence of the pathogen on roots. However, it should be noted that although *E. coli* O157:H7 can become associated with spinach roots, uptake into the inner leaf tissue was negligible.

S. Typhimurium and *L. monocytogenes* can become established on seedlings in a similar manner to *E. coli*, although no internalization, even into the roots, occurs (Jablasone *et al*., 2005). It was also noted that persistence of both *Salmonella* and *L. monocytogenes* is significantly lower compared to that of *E. coli*. *C. jejuni* has been shown to persist on the roots of spinach or radish plants for over 10 days (Brandl *et al*., 2004). However, the authors did not report if *Campylobacter* can be internalized into the inner plant tissue.

Cabbage

Cabbage crops accidentally irrigated with creek water contaminated with *E. coli* resulted in the organism being recovered from the roots of plants but not the edible leaves (Wachtel *et al*., 2002b). No studies were performed to determine if the *E. coli* had been internalized. From ribotyping studies, six different *E. coli* types (all non-pathogenic) were recovered from cabbage roots. When the different *E. coli* strains were introduced to seedlings and incubated overnight a range of adherence strengths were observed ranging from very high to low (Wachtel *et al*., 2002b). This reinforced the view that the interaction of *E. coli* (and presumably other human pathogens) is strain dependent.

A generic *E. coli* strain introduced to germinating cabbage seeds could be visualized in the xylem vessels of 5-week old hydroponically cultivated plants using immunohistological staining (Rafferty *et al*., 2003). Moreover, plants containing endophytic populations of *E. coli* induced the expression of unidentified genes and enhanced chitinase activity. This provided indirect evidence that the ISR had been activated by the presence of internalized *E. coli* (Rafferty *et al*., 2003).

Arabidopsis

Although *Arabidopsis* is not a commercial vegetable crop it provides an ideal model for studying bacterial–plant interactions. *Arabidopsis* has found utility in bacterial–plant interactions owing to its rapid generation time (6–12 weeks), well studied genome, availability of mutant in collections housed at the University of Nottingham, UK and Ohio State University, USA and close similarity of plant defense mechanisms present in commercially important crops (O'Callaghan *et al*., 2001).

Cooley *et al*. (2003) have studied the interaction of *S.* Newport and *E. coli* O157:H7 with *A. thaliana* cultivated in either a gnotobiotic (soil-less) or soil microcosms. In gnotobiotic culture both *Salmonella* and *E. coli* O157:H7 colonized the roots and subsequently the whole plant. Indeed, the seed and chaff harvested from contaminated plants were occasionally contaminated. However, when the same experiments were performed in soil microcosms there was a steady decline in both *E. coli* and *Salmonella* numbers as the plants matured. The decline in both *Salmonella* and *E. coli* O157:H7 numbers on *Arabidopsis* also occurred when a rhizosphere *Enterobacter asburiae* isolate was co-inoculated along with the pathogens. The results from the work would suggest that in soil-free systems (such as those encountered in hydroponic cultivation) the persistence of human pathogens in plants is enhanced because of the absence of a competitive background microflora.

A comparative study on the internalization of a generic *E. coli* and *E. coli* O157:H7 C9490 into a range of *Arabidopsis* ecotypes is provided in Table 1.7. Each bacterium was introduced onto seeds and cultivated for 5 weeks in soil microcosms. The most interesting feature is the low internalization of *E. coli* O157:H7 strain within *Arabidopsis* compared to generic *E. coli*. Whether this represents the *E. coli* O157:H7 triggering of the ISR response or competitive exclusion by endogenous microflora remains unclear.

Root crops

The preferential colonization of roots by human pathogens may imply that crops such as carrots and radish are more likely to be contaminated than leafy vegetables. It is established that as with other plants, root crops contain an endophytic population. However, surprisingly very little work has been performed to identify the potential internalization of human pathogens in such crops. It has been reported that *Salmonella* and *E. coli* O157:H7 can internalize into carrot and radish seedlings cultivated in soil-free microcosms but do not persist over extended cultivation periods (Jablasone *et al*., 2005).

The majority of other studies have focused on surface contamination of root crops cultivated in soil inoculated with human pathogens. For example, Islam *et al*. (2004a, b) found that *E. coli* O157:H7 (introduced at 10^7 cfu g^{-1}) decreased by 2 log cfu g^{-1} over a 3 month period on onions. This

Table 1.7 Growth and internalization of generic and pathogenic *E. coli* within growing *Arabidopsis* plants. Arabidopsis seed was inoculated with either generic or pathogenic *E. coli* and cultivated in seed microcosms for 6 weeks. Plants were harvested and loosely attached bacteria on the surface detached by rinsing in peptone water. Plants were surface sterilized in 2000 ppm sodium hypochlorite and internalized bacteria screened for in the plant extracts

Ecotype	Generic *E. coli* ($\log \text{cfu g}^{-1}$)		*E. coli* O157:H7 ($\log \text{cfu g}^{-1}$)	
	Surface counts	Internal counts	Surface counts	Internal counts
Columbia	4.75	3.97	3.27	ND
CS1398 P2	4.82	3.82	ND	ND
6701-2	4.91	4.15	3.42	ND
1639	5.18	4.22	3.36	ND
CS5917 P1	4.83	4.21	ND	ND
6929-2C	4.75	4.45	ND	ND
6923-2	4.78	4.24	2.65	1.77
1150-4C	4.81	4.29	2.88	1.40
S2223P6	4.49	3.79	3.77	ND
CS920 P3	4.30	2.30	3.49	2.01
1401	4.19	4.02	3.29	ND

ND: Not detected $<1.70 \log \text{cfu g}^{-1}$.
Hora and Warriner, unpublished data.

compared to $3 \log \text{cfu g}^{-1}$ in soil alone. On carrots, *E. coli* O157:H7 populations decreased by $1.7 \log \text{cfu g}^{-1}$ over 48 days. *S*. Typhimurium introduced into soil (10^7cfu g^{-1}) in which radish and carrot seeds were sown could be recovered on the surface of vegetables 203 days ex-planting (Islam *et al*., 2004b).

The results from Islam *et al*. (2004a, b) confirm that enteric pathogens can persist on root crops over extended periods. In this respect it may be considered that vegetables such as carrots would be implicated in a greater number of foodborne illness cases than hitherto recorded. The low incidence of foodborne illness related to root crops is possibly due to several factors. For example, because root crops carry soil, it is likely that produce would be more vigorously cleaned compared to delicate leafy vegetables. In addition, the presence of potent antimicrobial constituents within root crops would inactivate any pathogens present during the course of preparation (Viswanathan and Kaur, 2001).

1.5.5 Internalization of enteric viruses into growing plants

The persistence of enteric viruses on vegetables has been found to be plant dependent (Seymour and Appleton, 2001). For example, hepatitis A persists for longer on lettuce compared to carrot or fennel (Croci *et al*., 2002). The authors also noted that current post-harvest biocidal washing was not

totally effective in removing the virus from artificially inoculated salad vegetables (Croci *et al*., 2002). The possibility that enteric viruses could interact with growing plants in the field has not been considered to any great extent owing to the inability of viruses to multiply in the environment and also their low resistance to UV solar radiation.

The internalization of enteric viruses into the inner tissue of plants has not been conclusively demonstrated but the potential has been realized for several years (Tierney *et al*., 1977; Smith, 1982). Studies have been performed with tomato plants grown in soil irrigated with water containing poliovirus (10^3–10^4 pfu ml^{-1}). Here, the poliovirus was occasionally recovered from leaves of plants (Oron *et al*., 1995). However, the poliovirus application was far higher than typically encountered in the natural environment (0.1–10 pfu ml^{-1}). Therefore, the risk of internalization of enteric viruses was considered to be low (Oron *et al*., 1995).

The internalization of feline calicivirus and MS2 F (+)-specific coliphage into growing cress cultivated in inoculated soil has been reported (Kirkham *et al*., 2002). When the cress was harvested, each virus type could be recovered from the edible portion of plants. The authors did not rely on surface sterilization to prove internalization. Instead, the inoculated soil was overlaid with agar to prevent virus transfer to the upper part of the growing plant (Kirkham *et al*., 2002).

The uptake of viruses may be viewed as a significant risk, although this may provide an opportunity to control human pathogenic bacteria using bacteriophages against, for example, *Salmonella* or *E. coli* O157:H7. However, despite the apparent tolerance of bacteriophage to a range of environmental stresses, the persistence on the roots of growing lettuce or carrots is low (Petterson *et al.*, 2001).

1.6 Implications for control

Views on the survival, persistence and interaction of human pathogens with plants are now changing. Through various lines of research it is established that human pathogens can persist over extended periods in the environment especially when associated with plants. The extent to which human pathogens can internalize into undamaged plant tissue still remains to be elucidated. From studies performed to date it appears that human pathogens can internalize to a greater extent into sprouted seeds compared to mature plants. However, the fact that a diverse range of opportunistic human pathogens are frequently present in the endophytic microflora would imply that internalization of bacteria such as *E. coli* O157 is possible. Although it appears that many human pathogens that internalize into vegetables have low virulence, these should be considered a risk until proven otherwise. This has significant implications for safety strategies, as once internalized any pathogens present cannot be removed by simple

washing. In this respect the only effective control method would be to prevent contamination of vegetables at each point of the chain (farm to fork). In the field environment the irrigation water quality and manure management have to be closely monitored. This is especially relevant in hydroponic cultivation systems where the interaction of human pathogens is greater compared to soil grown crops. In harvesting operations the transfer of enteric bacteria from infected workers has to be addressed. Although post-harvest processing of vegetables cannot ensure removal of pathogens, the sanitary standards of wash water have to be ensured. Importantly, food handlers need to minimize potential cross-contamination events during food preparation.

1.7 Future trends

Current approaches to enhance the microbiological safety of vegetables through greater surveillance and issuing of guidelines will only have limited effectiveness in reducing the levels of foodborne illness. This view is supported by progress towards improving the food safety of sprouted seeds. Even though seed traceability, seed decontamination, screening spent irrigation water for human pathogens and a high standard of sanitation are implemented by sprouters, a number of sprout recalls and foodborne illness cases continue to occur. The reason for the lack of effective control can be attributed to the sporadic nature of contamination and the absence of an effective intervention step. Therefore, product testing will only provide limited confidence that a microbiological safe product is being produced.

Future directions in the vegetable sector will be likely to follow two pathways, those of prevention and intervention. Processors will continue to search for more effective decontamination methods to remove field acquired contamination. How to evaluate decontamination strategies will represent a major problem considering the presence of endophytic microflora, which would remain viable regardless of which surface decontamination treatment is applied. Using artificially inoculated vegetables to test the efficacy of decontamination treatments also has limitations considering that human pathogen populations may naturally reside within plant tissue. It is widely accepted that producing a sterile product (for example, by irradiation treatment) is not desirable as the biological buffer provided by endogenous bacteria can suppress the activity of pathogens.

In this respect future control strategies may focus on biocontrol using bacteriophage and/or antagonistic microbes. The possibility of activating plant defenses just prior to harvest selectively to inactivate internalized human pathogens is a further possibility. The second approach to improving food safety is to undertake a preventive approach. It is noteworthy that in the majority of foodborne illness cases the cause of contamination can be identified. It follows that closer monitoring of crops during cultivation will provide greater assurance in producing a safe product. Central to

achieving this will be the availability of sensors to enable the close monitoring of irrigation water and soil quality. In parallel, cost effective intervention steps for irrigation water decontamination need to be devised. As with all safety initiatives the costs incurred by implementing safety strategies need to be met by all participants in the vegetable chain.

1.8 Sources of further information and advice

1.8.1 Guidelines and reviews

'Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables.' http://www.fda.gov

'Code of Practice for the Hygienic Production of Sprouted Seeds.' http://www.inspection.gc.ca/english/plaveg/fresh/sprointe.shtml

'Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce US Food and Drug Administration.' http://www.cfsan.fda.gov/~comm/ift3-toc.html

1.8.2 Government agencies

Centers for Disease Control and Prevention: http://www.cdc.gov/

Centre of Food Safety and Applied Nutrition (cfsan): http://vm.cfsan.fda.gov/

Food Standards Agency (FSA): http://www.food.gov.uk/

European Food Safety Authority (EFSA): http://www.efsa.eu.int/

1.8.3 Producer organizations

SproutNet: http://www.sproutnet.com/SproutNet.htm

Ontario Greenhouse Vegetable Growers: http://www.ontariogreenhouse.com/onfarm.cfm

Tri-state fruit and vegetable safety consortium: http://fruitandvegetablesafety.tamu.edu/

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2

Pathogens in fruit

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2.1 Introduction

Fresh fruit and processed fruit products have generally been considered safe from pathogenic bacteria because of their high acid content. However, recent outbreaks of *E. coli* O157:H7 and *Salmonella* spp. in apple and orange juices have challenged the belief that high acid foods cannot harbor viable pathogenic bacteria. Owing to the acidic similarity of berry juice (pH 3.0–4.5), apple juice (3.0–4.0) and orange juice (3.0–4.0), there is concern that fruit juices could also act as a vector for foodborne illness. Laboratory studies have determined that berry juices and purées can support bacteria such as *E. coli* O157:H7 and *Salmonella* spp. (Fig. 2.1). These findings suggest that berries and berry products, if contaminated, might harbor pathogenic organisms long enough to cause foodborne illnesses.

Figure 2.2 illustrates the potential sources and vectors of contamination of the fruit during production and processing, as well as the potential foodborne pathogens. A major source of microbial infection is introduced through contaminated water used on fresh produce during growth, harvesting and processing. Water can be a carrier of pathogenic bacteria (e.g. *E. coli*, *Salmonella* spp., *Vibrio cholerae*, *Shigella* spp.), protozoa (e.g. *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora cayetanensis*, *Toxiplasma gondii*), mycotoxin-producing fungi and viruses (e.g. Norwalk and hepatitis A). If these organisms are accidentally consumed, even in small amounts, illness can result. Another significant source of pathogens arises from unsanitary field conditions. Cattle manure and the feces of sheep and deer may harbor *E. coli* O157:H7, resulting in contamination of fresh produce during harvesting. Improper worker hygiene may also spread *Salmonella*,

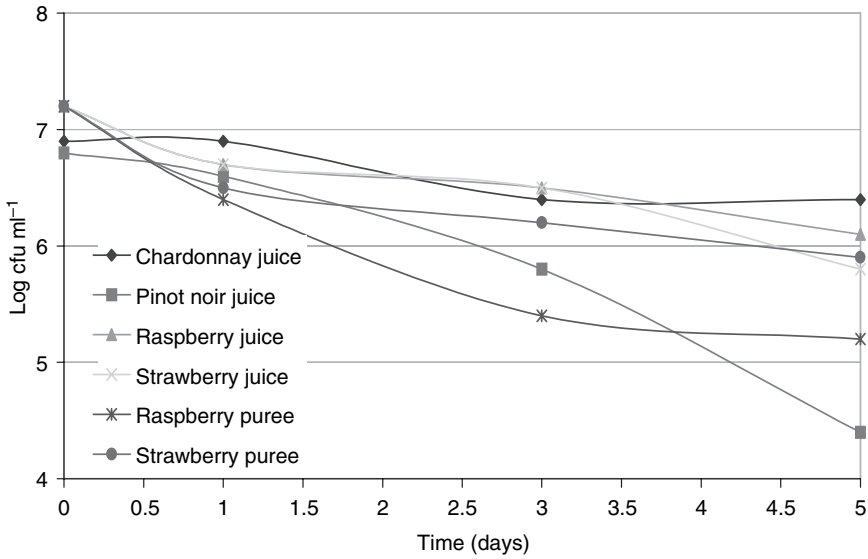

Fig. 2.1 Survival of *E. coli* in fruit juices and puree (adapted from Bower *et al.*, 2003).

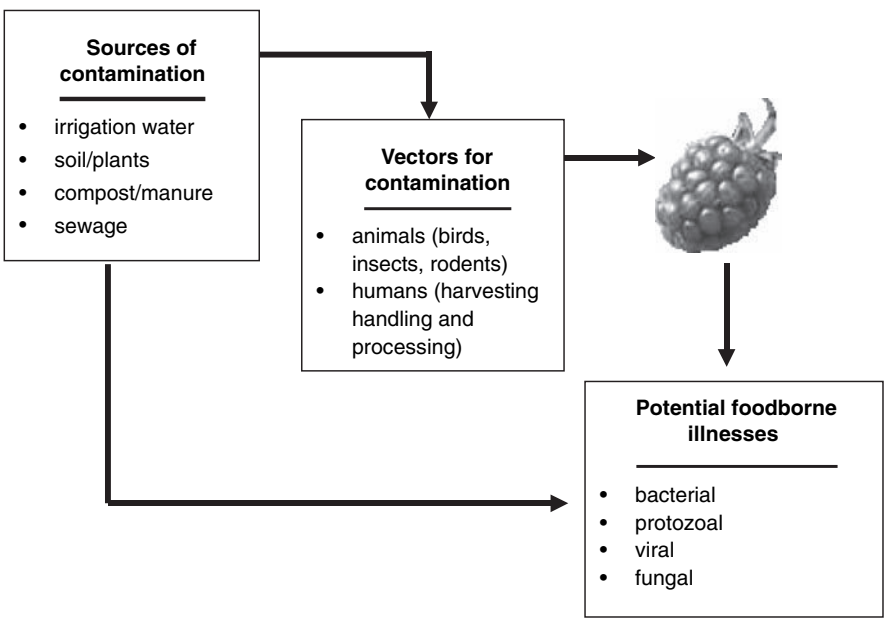

Fig. 2.2 Potential contaminations of fruit during production and processing (adapted from Bower *et al.*, 2003).

Cryptosporidium and other pathogens through human fecal matter. Some major concerns about pathogens in fruit are briefly described in the following paragraphs and summarized in Table 2.1.

2.1.1 *Escherichia coli*

Enterohemorrhagic *E. coli* O157:H7 is recognized as an important foodborne pathogen and grows rapidly in several types of raw fruits and vegetables, particularly when stored at 12 °C. The infection dose of *E. coli* O157:H7 is low and can develop acid resistance. Since cattle appear to be a primary reservoir, the vast majority of outbreaks of illness associated with *E. coli* O157:H7 have been associated with consuming undercooked beef and dairy products. However, outbreaks have also been linked to lettuce, unpasteurized apple cider, cantaloupe and sprouts. In outbreaks associated with cantaloupe and in some cases lettuce, contamination, particularly with raw beef juices, occurred during final preparation.

2.1.2 *Salmonella*

The genus *Salmonella* has over 2700 serotypes. Surveys of fresh produce have revealed the presence of several *Salmonella* serotypes capable of causing human infection. Animals and birds are the natural reservoirs. Poultry and other meat products, eggs and dairy products, are the most commonly implicated sources in salmonellosis outbreaks. Fresh fruit and vegetables are implicated less frequently, although outbreaks have been documented most notably in cantaloupe and sprouts. Several additional large outbreaks of salmonellosis have been attributed to fresh produce. Among them are three multi-state outbreaks traced to the consumption of raw tomatoes: one involved *Salmonella* Javiana in 1992, another involved *Salmonella* Montevideo in 1993 and a third in 2000 involved *Salmonella* Baildon (FDA, 2001a). Subsequent laboratory studies revealed that the pathogen can grow in damaged, chopped or sliced tomatoes (pH 4.1–4.5) stored at 20–30 °C.

2.1.3 *Listeria monocytogenes*

L. monocytogenes causes relatively mild gastroenteritis in healthy adults, but the illness can be severe in susceptible individuals including pregnant women, neonates and immune-compromised individuals. The infective dose for this organism has not been clearly established, although it is thought to be relatively low among susceptible individuals. *L. monocytogenes* is widely distributed on raw fruit and vegetables and on plant material (Beuchat, 1996). However, several studies with relatively large sample sizes failed to detect the organism. Factors affecting its presence or persistence have yet to be determined. Plants and plant parts used as salad vegetables play a role\

Table 2.1 Illness caused by the most concerned microbial infections in fresh produce

Microorganisms	Nature	Contamination	Foodborne illness
E. coli **O157:H7**	• First recognized as human pathogen in 1982 • Outbreaks often associated with undercooked ground beef • Outbreaks have involved lettuce, unpasteurized apple cider and juice, radish sprouts and alfalfa sprouts • Naturally exists in animals without symptoms – cattle, sheep, deer, dogs, cats, other animals • Can contaminate/grow on fresh produce in minimally processed cantaloupe, watermelon cubes, shredded lettuce, sliced cucumbers and mesclun lettuce	• Wild or domestic animals • Improperly composted animal manure • Fruits and vegetables dropped on ground contaminated by manure • Water may carry and spread organisms • Farm and packing house workers, any food handlers, may contaminate produce	• Severe cramps, bloody diarrhea, vomiting, dehydration • Severe complications can include kidney failure, strokes, seizures and sometimes painful death • Onset 3–9 days; lasts 2–9 days, unless there are complications
Salmonella species	• Comes from intestinal tracts of poultry, pigs, birds, and insects • Can be carried by humans • Infective dose – a few cells to millions • Isolated from many types of raw fruits and vegetables – not a frequent event • Outbreaks linked to tomatoes, bean sprouts, melons and unpasteurized orange juice and apple juice	• Similar to *E. coli*	• Nausea, vomiting, abdominal cramps, diarrhea, fever, and headache • Symptoms occur in 12–48 hours and last 2–6 days in otherwise healthy people • May last weeks in immunocompromised people • Secondary problems such as reactive arthritis or pericarditis may result in some patients

Table 2.1 *Continued*

Microorganisms	Nature	Contamination	Foodborne illness
L. monocytogenes	• Widely distributed in nature – in soil, sewage, fresh water sediments – in silage, decaying plant matter – in animal intestinal tracts • Animal carriers may not be sick • Found in raw foods – meats, unpasteurized milk – vegetables	• Common environmental contaminant • Unlike most microorganisms it grows at refrigeration temperatures • Floor drains and wheel condensate collects are frequent sources of contamination	• Flu-like symptoms in healthy people • May progress to meningitis, blood poisoning, abortion in pregnant women or death • Symptoms appear within 1 day to 3 weeks • Duration depends on treatment • High fatality rate in immune-compromised individuals
Virus – hepatitis A	• Excreted in feces by infected individuals • Can be carried by raw produce, uncooked food • Persists for weeks or months on crops or soils • Hepatitis A on lettuce, raspberries and strawberries	• Viruses can be transmitted to plants and fresh fruits and vegetables by people, tractors, equipment, clippers and insects • Viruses can cause plant and animal diseases	• Causes fever, nausea, vomiting, abdominal cramps, extreme fatigue, jaundice • Onset 15–50 days after ingestion • Lasts 1–2 weeks to months in severe cases

Source: Adapted from Bower *et al*., 2003.

in disseminating the pathogen from natural habitats to the human food supply. This role may be indirect, for example by contaminating milk via forage or silage, or direct, in the form of raw contaminated produce. *L. monocytogenes* can grow on fresh produce stored at refrigerated temperature. Growth on fresh-cut fruit as well as asparagus, broccoli, butternut squash, coleslaw and cauliflower, rutabaga stored at 4 °C, lettuce at 5 °C and chicory endive at 6.5 °C has been reported.

2.1.4 *Campylobacter* species

Campylobacter jejuni and *Campylobacter coli* are leading causes of bacterial enteritis. Consumption of contaminated food of animal origin, particularly poultry, is largely responsible for infection. However, *Campylobacter enteritis* has also been associated with lettuce or salads. Cross-contamination during food preparation was thought to be possible or probable, in one case with raw chicken juices. Cross-contamination of fresh produce with *Campylobacter* from poultry and other meats is a distinct possibility in delicatessen and other food service operations. Therefore, control should focus on reducing cross-contamination during food storage and preparation. Castillo and Escartin (1994) reported that *C. jejuni* can survive on sliced watermelon and papaya for sufficient time to be a risk to the consumer.

2.1.5 Viruses

Hepatitis A virus, calicivirus and Norwalk-like viruses have caused outbreaks associated with the consumption of some fruit. These outbreaks have been associated with frozen raspberries or frozen strawberries, melons and fresh-cut fruit. A number of these outbreaks were the result of contamination via an infected food handler during final preparation. Hepatitis A and Norwalk-like viruses are the most commonly documented viral food contaminants. Viruses can be excreted in large numbers by infected individuals and have been isolated from sewage and untreated wastewater used for crop irrigation. Although viruses cannot grow in or on foods, their presence on fresh produce, which may serve as a vehicle for infection, is of concern. Among 14 reports of viral gastroenteritis outbreaks (Hedberg and Osterholm, 1993), a food handler who was ill before or while handling the implicated food was identified as the source of infection in eight outbreaks.

2.2 Pathogens in particular types of fruit

Several types of fruit are particularly susceptible to infection by foodborne pathogens owing to their biological structure and nutritional availability. These types of fruit include, but are not limited to, fresh-cut fruit, various berries, watermelon and unpasteurized juices.

2.2.1 Fresh-cut fruit

Fresh-cut produce is prepared from a raw agricultural product produced in contact with soil, often eaten raw and with no processing kill step to ensure microbiological safety. There are particular concerns of food safety not encountered with retorted or frozen foods. Human pathogens can and do infest fresh fruit and have been responsible for foodborne illness. Processing these products can take a point source of bacterial contamination and disseminate it throughout a batch of product. Conditions inside flexible plastic packages (high humidity, low oxygen and high carbon dioxide) can encourage growth of pathogens that might not otherwise thrive on produce. If these packages then encounter temperature abuse, pathogens may grow and cause problems. While each of these may be low probability events, their possibility requires prudent processors to take them very seriously.

2.2.2 Berries

Fresh berries and processed berry products generally have been considered safe from pathogenic bacteria because of their high acid content. However, fresh fruit have occasionally been implicated in foodborne illnesses. Bacterial pathogens such as *Salmonella* and *L. monocytogenes* have been isolated from fresh strawberries and frozen blueberries, respectively. Also, recent disease outbreaks caused by *E. coli* O157:H7 and *Salmonella* spp. in apple and orange juices have challenged the belief that high-acid foods cannot harbor viable pathogenic bacteria. Owing to the acidic similarity of berry juice (pH 3.0–4.5), apple juice (3.0–4.0) and orange juice (3.0–4.0), there is concern that berry juices could carry foodborne illness (FDA, 2001b).

Raw raspberries and possibly blackberries imported from Guatemala have been associated with several large *Cyclospora cayetanensis* outbreaks (Table 2.2). The natural host for this parasite has not been identified. However, contaminated water used for pesticide application and poor harvester hygiene has been suggested as the most likely routes of contamination. Frozen raspberries or frozen strawberries have been linked to two or three outbreaks of hepatitis A, respectively (Table 2.3). Hepatitis A, a virus spread by human feces, is thought to have contaminated the berries by contact with infected harvesters or contaminated irrigation water (Table 2.3). Frozen raspberries have also been associated with illness caused by calicivirus, also spread through human feces (Table 2.3).

Raw berries destined for the fresh market are harvested by hand and field packed into retail containers without being washed. Strawberries destined for freezing are destemmed in the field, either using a metal device or a thumbnail. Berries which are to be processed are transported, usually at ambient temperature, to a processing facility where they are washed with potable water or water containing an antimicrobial (for example, chlorine),

Table 2.2 Examples of reported outbreaks of foodborne parasitic disease associated with raw berries

Pathogen	Year	Location	Produce source	Venue	Type of berry	No. of cases	No. of deaths	Isolated from produce	Comments	Reference
Cyclospora cayetanensis	1995	Florida	Guatemala likely	Two social events	Raspberries likely	87	0	No	Raspberries from both events were purchased from separate sources. Two clusters reported	Koumans *et al*., 1998
C. cayetanensis	1996	20 US states and 2 Canadian provinces	Guatemala	Various	Raspberries	1465	0	No	Possible contamination due to fruitspraying with insecticides and fungicides mixed with contaminated water	Herwaldt and Ackers, 1997; Fleming *et al*., 1998
C. cayetanensis	1997	Multistate, USA and Ontario, Canada	Guatemala	Various	Raspberries	1012	0	No	Source of contamination unknown	Herwaldt and Beach, 1999; CDC, 1997b
C. cayetanensis	1998	Ontario, Canada	Guatemala	Various	Raspberries	315	0	No	Source of contamination unknown	CDC, 1998 Herwaldt, 2000
C. cayetanensis	1999	Ontario, Canada	Guatamala likely	Banquet hall	Blackberries suspected	104	0	NR	Source of contamination unknown	Herwaldt, 2000

NR, not reported. CDC, Centers for Disease Control.
Source: adapted from FDA, 2001b.

Table 2.3 Examples of reported outbreaks of foodborne viral disease associated with contaminated frozen berries

Pathogen	Year	Location	Produce source	Venue	Type of berry	No. of cases	No. of deaths	Isolated from produce	Comments	Reference
Calicivirus	1997	Quebec, Canada	Bosnia	2 separate events	Raspberries (frozen)	>200	0	NR	Likely contamination occurred before shipping from Bosnia	Gaulin *et al*., 1999
Calicivirus	1998	Finland	Imported	Unknown	Raspberries (frozen)	>500	0	NR	Source of contamination unknown	Lund and Snowdon, 2000
Hepatitis A	1983	Scotland	Scotland	Hotel	Raspberries (frozen)	24	0	No	Suspected raspberry mousse prepared from frozen raspberries. Suggested contamination by infected picker(s)	Reid and Robinson, 1987
Hepatitis A	1988	Scotland	Scotland	Home	Raspberries (frozen)	5	0	No	Raspberries from a small farm were frozen at home. Several pickers at the farm had symptoms of Hepatitis A	Ramsay and Upton, 1989

Hepatitis A	1990	Georgia Montana	California (1988)	School Institution for disabled	Strawberries (frozen)	15 (Georgia) 13 (Missouri) +29 secondary	0	No	Frozen strawberries used to make dessert. Empty strawberry containers with same lot number obtained from both locations implicated same source. Suspected contamination by infected picker(s). Strawberries picked and stems removed in field. Fruits washed in 3 ppm chlorine prior to slicing and freezing	Niu *et al*., 1992
Hepatitis A	1997	Multistate USA	Mexico	Schools	Strawberries (frozen)	242 + 14 suspect	0	No	Frozen strawberries and strawberry shortcake were implicated in the outbreak. Possible contamination during harvesting. Handwashing in field limited. Stems removed with fingernails. Evidence suggested low levels of nonuniform contamination	Hutin *et al*., 1999; CDC, 1997a

NR, not reported. CDC, Centers for Disease Control.
Source: adapted from FDA, 2001b.

sometimes sliced and often mixed with up to 30% sucrose before freezing. The extra human handling during harvesting and co-mingling in the processing facility may explain the greater association of outbreaks with frozen berries. Also, virus and parasites may actually be preserved by the freezing step.

To date, bacterial foodborne illnesses have not been linked to consumption of berries. However, reservoirs for enteric organisms such as *Salmonella* and *E. coli* O157:H7 are similar to that of hepatitis A virus, suggesting that bacterial pathogens may also be occasional contaminants of berries. A FDA survey of imported produce found *Salmonella* in one of 143 samples of strawberries (Table 2.4).

2.2.3 Melons

Cut cantaloupe is considered a potentially hazardous food in the FDA Food Code because it is capable of supporting the growth of pathogens owing to low acidity (pH 5.2–6.7) and high water activity (0.97–0.99). The FDA investigated the frequency of *Salmonella* isolated from cantaloupe imported from Mexico (Table 2.5). In 1990, 11 of 1440 (0.76%) cantaloupes were positive for eight different *Salmonella* serotypes. In 1991, 24 of 2220 (1.08%) were positive, with 12 different *Salmonella* serotypes isolated. More recently, the FDA isolated *Salmonella* from eight (5.3%) and *Shigella* from three (2.0%) of 151 cantaloupe samples collected from nine countries exporting to the USA (FDA, 2001b). These results suggest that melons may be naturally contaminated with *Salmonella*.

Outbreaks of salmonellosis have been associated with the consumption of cut cantaloupe and watermelon (Table 2.5). At least two of these outbreaks have been relatively large and have involved multiple states and/or provinces. For most outbreaks, it has been assumed that *Salmonella* was present on the rind, presumably contaminated in the field or during washing in a packinghouse, and that the edible surface became contaminated during final preparation. Improper storage temperature combined with the favorable conditions for growth on the surface of cut melons were factors that probably contributed to the outbreak (Table 2.5). Some outbreaks associated with melons have resulted from contamination during final preparation, either through an infected food handler (with, for example, Norwalk virus) or cross-contamination from raw beef to the melon (with, for example, *E. coli* O157:H7) via knives, cutting boards or hands. *E. coli* O157:H7 and *Salmonella* can survive and grow readily on improperly stored (non-refrigerated) cut melons. When initial populations were between 2.0 and 3.0 $\log \text{cfu g}^{-1}$, final levels reached 7.0 or 8.0 $\log \text{cfu g}^{-1}$ after 24 h at 23 °C. At 5 °C, both *Salmonella* and *E. coli* O157:H7 populations did not increase.

Cut melons are subject to time/temperature requirements of the FDA model food code criteria for potentially hazardous food. Recommendations

Table 2.4 Examination of raw fruit in the presence of pathogens

Pathogen	Type	Country[a]	Place of sampling	Incidence	Percentage (%)	Comments	Reference
L. monocytogenes	Various juices	USA	Retail	2/50	0.04	Various unpasteurized fruit and vegetable juices were sampled. *L. monocytogenes* isolated from apple juice and an apple raspberry blend. Juices were also tested for *E. coli* O157:H7 and *Salmonella*. Sample tested negative for the organisms.	Sado *et al.*, 1998
Salmonella	Cantaloupe	Various	NR[b]	8/151	5.3	Produce imported into the USA. Samples were collected from 9 countries.	FDA, 2001a
Salmonella	Orange	USA	Various (orchard through juice plant)	0/375	0	Fruit surface and juice were analyzed. 1/3 oranges were graded hulls, 1/3 oranges were washed and graded, and 1/3 oranges were ungraded	Parish, personal communication

Table 2.4 *Continued*

Pathogen	Type	Country[a]	Place of sampling	Incidence	Percentage (%)	Comments	Reference
Salmonella	Orange/ tangerine	USA	Citrus packinghouses	0/336	0		Pao *et al.*, 1998
Salmonella	Strawberry	Various	NR	1/143	0.7	Produce imported into the USA. Samples were collected and analyzed from 5 countries.	FDA, 2001a
Salmonella (8 serovars)	Cantaloupe	Mexico	–	11/1440	0.76	FDA import study between March and April 1990.	Madden, 1992
Salmonella (12 serovars)	Cantaloupe	Mexico	–	24/2220	1.1	FDA import study between November 1990 through January 1991. Melons came from the same harvest area associated with 1989–90 outbreak.	Madden, 1992
Shigella	Cantaloupe	Various	NR	3/151	2.0	Produce imported into the USA. Samples were collected from 9 countries.	FDA, 2001a

[a] Country where produce samples were collected and tested.
[b] NR, not reported.
Source: adapted from FDA, 2001b.

Table 2.5 Examples of reported outbreaks of foodborne disease associated with melons

Pathogen	Year	Location	Produce source	Venue	Type of melon	No. of cases	No. of deaths	Isolated from produce	Comments	Reference
Escherichia coli O157:H7	1993	Oregon	NR	Restaurant	Cantaloupe	9	0	NR	Possible contamination of cantaloupe with organism from raw beef	Del Rosario and Beuchat, 1995; Anonymous, 1993
Norwalk virus	1987	United Kingdom	NR	NR	Melon	206	0	NR	Infected food handler	Lund and Snowdon, 2000
Salmonella chester	1989–90	Multistate, USA	Mexico and Central America	Unknown	Cantaloupe	>245 (25 000 estimated)	2	No	Cut cantaloupe from salad bars	CDC, 1991; Lund and Snowdon, 2000
S. Javiana	1991	Michigan	NA	Indoor picnic and in-school party	Watermelon	26 primary 13 secondary	0	Yes	Melon not washed prior to cutting. Suspected contamination from melon rind. Melon served over 3 h period at room temperature. Leftovers served the next day	Blostein, 1993
S. Miami	1954	Massachusetts	Florida	Supermarket	Watermelon	17	1	Yes	Laboratory demonstration of contamination of internal flesh during slicing with either contaminated melon surface or contaminated knife. Organism recovered from shelf where knife	Gayler *et al.*, 1955

Table 2.5 *Continued*

Pathogen	Year	Location	Produce source	Venue	Type of melon	No. of cases	No. of deaths	Isolated from produce	Comments	Reference
									was kept but not from knife used to cut melons. Organism was isolated from home samples but not from supermarket samples. Melons were from Florida where *S.* Maini is common	
S. Oranienburg	1979	Illinois	Illinois	Supermarket	Watermelon	18	0	No	Damaged fruits were cut, covered with plastic film and displayed, sometimes without refrigeration until sold	CDC, 1979
S. Oranienburg	1998	Ontario, Canada	USA, Mexico, or Central America	Various	Cantaloupe	22	0	No	Possible contamination with organism from surface when slicing. Cut fruit was probably left sitting at room temperature for several hours before consumption	Deeks *et al.*, 1998
S. Poona	1991	Multistate, USA and Canada	Texas or Mexico	Unknown	Cantaloupe	>400 confirmed USA, 72 Canada	0	NR	Fruit salads containing sliced cantaloupes	CDC, 1991

S. Poona	2000	Multistate, USA (8 states)	Mexico	Various	Cantaloupe				Case control study clearly implicated	Farrar, pers comm, unreferenced
S. Saphra	1997	California	Mexico	Home grocery stores, and restaurants	Cantaloupe	24	0	NR	Multiple purchase sites suggest contamination during production or harvest. Lack of refrigeration at retail may have contributed to outbreak	Mohle-Boetani and others, 1999; Farrar, pers comm, unreferenced
Salmonella	1950	Minnesota	NA	Roadside stand	Watermelon	6	0	Yes	Prepared cut melon. *S. Bareilly* isolated from melon. Melon kept at ambient temperature	Blostein, 1993
Shigella sonnei	1987	Sweden	Morocco	Dinner party	Suspect watermelon	15	0	No	Melon consumed immediately after slicing. Possible contamination of melon from injected water	Fredlund *et al.*, 1987

NR, not reported. CDC, Centers for Disease Control.
Source: adapted from FDA, 2001b.

made by the FDA to retail establishments that prepare or sell fresh cantaloupe are that melons should be washed before cutting, clean, sanitized utensils and surfaces should be used when preparing cut melons, cut melons should be kept at or below 7 °C and they should be displayed for no longer than 4 h if they are not refrigerated (Golden *et al*., 1993).

2.2.4 Unpasteurized fruit juices

Approximately 2% of all juices sold in the USA are unpasteurized. Unpasteurized fruit juices are made from fruits that are ground and/or pressed or squeezed to extract the juice. Unpasteurized juices are included here because they have not been thermally processed and an evaluation of outbreaks associated with these products might contribute to an understanding of risk factors for contamination of the raw fruit.

There have been very few surveys of retail juices for the presence of pathogens, probably because of the very low probability of finding pathogens in these products. Sado *et al*. (1998) used rapid test kits to survey retail juices for the presence of *L. monocytogenes*, *E. coli* O157:H7, *Salmonella*, coliforms and fecal coliforms. Only *L. monocytogenes* was isolated from two of 50 juices, an apple juice (pH 3.78) and an apple raspberry blend (pH 3.75) (Table 2.6). Although there is a long history of juice-related outbreaks, they have been relatively infrequent and, until 1995, were generally associated with very small commercial processors or home-prepared products (Table 2.6). While the acidity of most fruit juices prevents the multiplication of pathogens, survival is much better than has been traditionally assumed. Pathogen viability decreases with increasing temperature owing to the rapid growth of yeasts and other spoilage organisms at the higher temperatures. This also leads to a decrease in shelf life.

While pathogen contamination routes have not been definitively confirmed in any juice outbreak, the use of dropped fruit, the use of non-potable water and the presence of cattle, deer, or, in one case, amphibians, in or near the orchards or groves does appear to be a re-occurring theme. Of five documented outbreaks associated with reconstituted orange juice, three have been the result of contamination by an infected handler preparing the juice (Table 2.6). In another outbreak the water source used to reconstitute the juice was thought to be a factor.

2.3 Mechanisms of surface contamination

Microbial contamination of fruit arises during growth: from soil, organic matter, organic fertilizer, irrigation process, insects, animals, human contact and from postharvest practices, including washing, trimming and packing. This section discusses the mechanisms of surface contamination during production and post-harvest processing of fruit.

Table 2.6 Examples of reported outbreaks of foodborne disease associated with unpasteurized fruit juice

Pathogen	Year	Location	Fruit source	Type of juice	Venue	No. of cases	No. of deaths	Isolated from juice	Comments	Reference
Cryptosporidium parvum	1996	New York	New York	Apple	Small cider mill	20 confirmed, 11 suspected	0	NR	No drops used. Dairy farm across the street. *E. coli* detected in well water samples indicating fecal contamination. Apples were brushed and washed prior to pressing	CDC, 1997c
Cryptosporidium	1993	Maine	Maine	Apple	School	160 primary and 53 secondary	0	Yes	Apples shaken from trees and gathered from ground, cattle grazed on grass beneath trees, oocysts found in calf manure. Apples inadequately washed and pressed for juice at an agricultural fair	Millard *et al.*, 1994
Escherichia coli O157:H7	1991	Massachusetts	Massachusetts	Apple	Small cider mill	23 (4 HUS)	0	No	90% drops used in making juice. Apples were not washed or scrubbed. Cattle raised nearby	Besser *et al.*, 1993

Table 2.6 *Continued*

Pathogen	Year	Location	Fruit source	Type of juice	Venue	No. of cases	No. of deaths	Isolated from juice	Comments	Reference
E. coli O157:H7	1996	Connecticut	Connecticut	Apple	Small cider mill	14 (3 HUS, 1 HUS + TTP)	0	No	Some drops used in juice. Apples were brushed and washed in potable water before juiced using a wooden press. Potassium sorbate (0.1%) added as a preservative	CDC, 1997c
E. coli O157:H7	1996	Washington	Washington	Apple	Small cider mill	6	0	No	Cider was made for local church event from local orchard. Apples were washed	Farber, 2000
E. coli O157:H7	1996	British Columbia, Canada, California, Colorado and Washington	USA	Apple	Retail	70 (14 HUS)	1	Yes	Phosphoric acid wash, brushed and rinsed. Phosphoric acid based solutions may have been used incorrectly (not intended for produce/ waxed produce) or sometimes used at low concentrations. Possibly poor quality apples, some dropped apples used, apple orchard near cattle/deer.	CDC, 1996; Cody *et al.*, 1999

E. coli O157:H7	1998	Ontario, Canada	Ontario, Canada	Apple	Farm/home	14	0	No	Cattle kept in orchard prior to apple harvest. Apples collected from ground if suitable on inspection. Water supply on farm not potable. Apples used without further inspection, brushing or washing	Tamblyn *et al.*, 1999
E. coli O157:H7	1999	Oklahoma	Oklahoma	Apple	–	7	0	NR	Drop apples used. Possible contamination from wild and domestic animal manure	Farber, 2000
E. coli O157:H7 suspected	1980	Toronto, Ontario, Canada	Canada	Apple	Local market	14 HUS	1	No	Juice purchased from a local market and fair. Juice tasted 'bad' or 'different'	Steele *et al.*, 1982
Enterotoxigenic *E. coli*	1992	India	India	Orange	Roadside vendor	6	0	Yes	Two roadside vendors selling fresh squeezed juice, one was 6 m away from the garbage heap	Singh *et al.*, 1995
Salmonella Enteriditis	2000	Multistate, USA	California	Citrus	Retail and food service	14	0	No	Gallon sized containers of citrus juices were implicated in the outbreak	Butler, 2000

Table 2.6 *Continued*

Pathogen	Year	Location	Fruit source	Type of juice	Venue	No. of cases	No. of deaths	Isolated from juice	Comments	Reference
S. Gaminera, *S.* Hartford, and *S.* Rubislaw	1995	Florida	Florida	Orange	Retail	62 ill and 7 hospitalized	0	Yes	*S.* Gaminera was isolated from several containers of juice after outbreak. Numerous in-plant sanitation problems found. Surface water was used for orchard irrigation. Drops were used for juice. *Salmonella* was isolated from amphibiams and soil around the processing plant	CDC, 1995; Cook *et al.*, 1998
S. Muenchen	1999	USA and Canada	Mexico	Orange	Restaurant	207 confirmed, +91 suspected	1	Yes	Multiple strains of *Salmonella* isolated from orange juice collected from producer. Juice squeezed in Mexico and transported to Arizona in tanker trucks where it was bottled. Follow-up investigations revealed that ice	CDC, 1999

									was added illegally to juice prior to transport	
S. Typhi	1898	France	France	Apple	NR	NR	NR	NR	–	
S. Typhi	1922	France	France	Apple	NR	23	0	NR	Non-potable water was used to wash apples	Paquet, 1923
S. Typhimurium	1974	New Jersey	New Jersey	Apple	Farm and small retail outlets	296	0	Yes	A high proportion of dropped apples used to make the juice. Manure used to fertilize apple trees. Equipment rinsed with cold water, not sanitized. Six of thirty employees were *S.* Typhimurium positive	CDC, 1975
S. Typhimurium	1999	Australia	Australia	Orange	Retail	405	0	Yes	*Salmonella* was isolated from unopened cartons of orange juice	

NR, not reported.
HUS = Hemolytic uremic syndrome.
TTP = Thrombotic thrombocytopenic purpura.
Source: Adapted from FDA, 2001b.

2.3.1 Handling practices on the farms

The safety of food supply begins with grower practices on the farm. Sources of potential on-farm contamination include (Fig. 2.3):

- soil
- irrigation water, poor water quality
- animal manure
- inadequately composted manure
- wild and domestic animals
- inadequate field worker hygiene
- rainfall and temperature.

Before harvest, fruit can become contaminated with toxic chemicals (e.g. fertilizers, pesticides) and pathogenic microorganisms (bacteria, fungi, protozoa and viruses). Fecal coliforms can be spread to farmland through compost, manure fertilizers and by unclean surface water used for irrigation. These microbial contaminants can survive in the soil for three months or more. The presence of animals (both domestic and wild) can also increase the risk of field contamination. Foodborne illnesses have been linked with improper growing practices on the farm. For example, hepatitis A outbreaks associated with strawberries have been linked to infected workers who did not observe basic hygiene when harvesting, sorting and packing the berries.

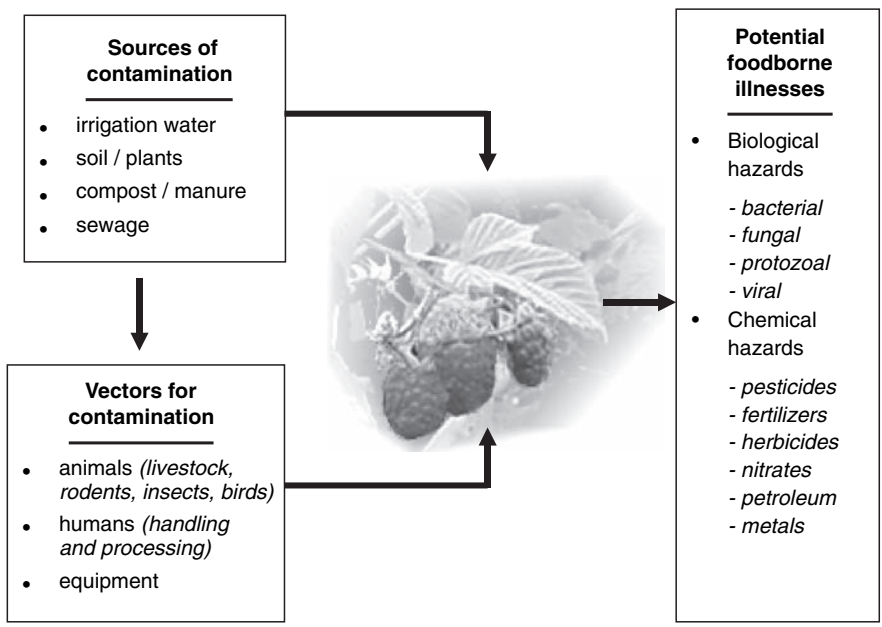

Fig. 2.3 Contamination during production of fruit (adapted from Bower *et al.*, 2003).

Cyclospora contamination of raspberries was linked to an unsanitary water source and *E. coli* O157:H7 was traced to apples collected from a field where infected livestock had been allowed to graze (Bower *et al*., 2003).

2.3.2 Conditions during processing and packaging

Fruit can be further contaminated during harvest and from post-harvest handling. Microorganisms are abundant in soil and water and can be brought into a food processing plant by insects, animals, transport containers, equipment and food handlers (Fig. 2.4). Specific sources of potential post-harvest contamination include:

- unsanitary handling during sorting and packaging, in packing facilities, in wholesale or retail operations, and at home
- equipment used to soak, pack or cut produce
- ice, cooling units (hydrocoolers)
- transport vehicles
- improper storage conditions (temperature)
- improper packaging
- cross-contamination in storage, display and preparation.

Improper hygiene practices may affect the microbial safety of fruits during harvest. Contamination of raspberries and sliced melon has been linked to

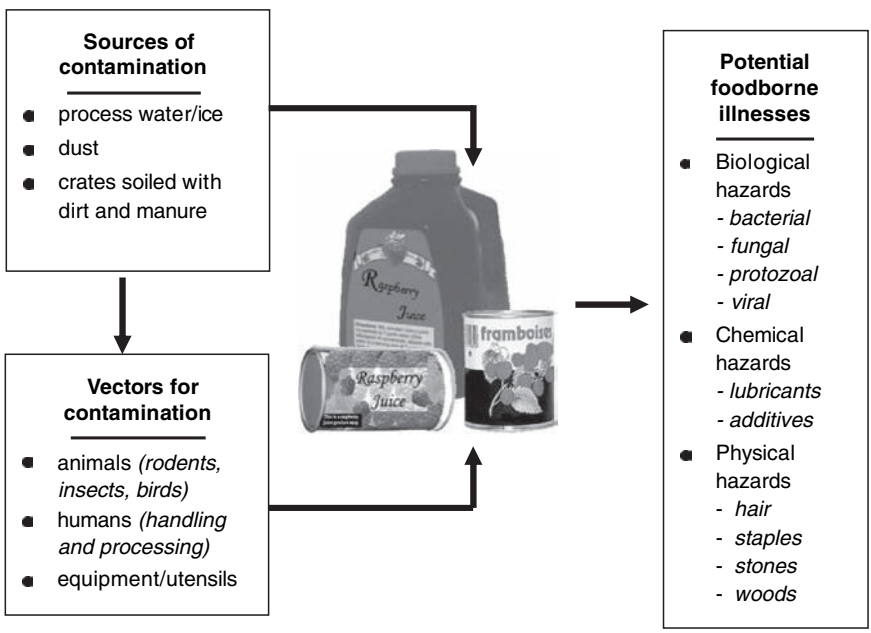

Fig. 2.4 Post-harvest contamination of fruit (adapted from Bower *et al*., 2003).

pickers (Acker *et al*., 1997; Lund and Snowdon, 2000). Cross-contamination between crops may occur during handling and harvest and post-harvest operations, and contamination with pathogens is possible from handlers' hands or from polluted wash water. Control measures, including use of clear water and sanitizer to wash fruit and work surfaces, refrigeration of pack sheds and training of workers in good manufacturing and hygiene practices, are essential.

The main sources of contamination during processing of fruit, especially fresh-cut fruit are most probably the general factory environment and processing equipment, including wash water, ice, air (dust), processing equipment and utensils, transport containers and vehicles and humans. Maintaining sanitary conditions during post-harvest processing depends on protecting the food from contaminants. A plentiful source of clean rinse water, together with workers who have received proper training, are essential to provide safe fresh fruit for the consumer.

2.4 Mechanisms of internal contamination

2.4.1 pH, water activity, nutrients and biological structure of fruit

The pH, water content, nutrients and protecting biological structures, such as skin or cuticle, are the important intrinsic properties of fruit that affect the contamination and stability of microorganisms. The conditions of temperature, pH and water activity on the growth of some pathogens are illustrated in Table 2.7.

Fruit contains organic acids in sufficient quantities to obtain a pH value of 4.6 or below. However, certain fruits, such as watermelon and bananas, have a higher pH. The low pH and type of acid itself are the major influences that select for the predominant microflora of fruit. Lowering the pH to within the range of 3.0–5.0 restricts the types of microorganisms able to grow, thus reducing the risk of spoilage or pathogenic organisms. A food may start with a pH which precludes bacterial growth, but as a result of the metabolism of other microbes (yeasts or molds), pH shifts may occur and permit bacterial growth. The pH of the fruit may also affect the antimicrobial activity of natural antimicrobial compounds of the fruit, such as phenolics and essential oils. At acid pH values, it may enhance the effects of phenolics owing to increased solubility and stability (Lopez-Malo *et al*., 2000).

Water molecules are loosely oriented in pure liquid water and can easily rearrange. When other substances (solutes) are added to water, water molecules orient themselves on the surface of the solute and the properties of the solution change dramatically. The microbial cell must compete with solute molecules for free water molecules. Except for *Staphylococcus aureus*, bacteria are rather poor competitors, whereas molds are excellent competitors.

Table 2.7 Factors affecting the growth of some foodborne pathogens

Organisms	Growth temp (°C)	Growth pH	Growth a_w
Salmonella spp.	6.5–47	4.5–?	>0.95[a]
Clostridium botulinum			
A & B	10–50	4.7–9	>0.93
Non-proteolytic B	5–?	[b]	NR[c]
E	3.3–15–30	[b]	>0.965
F	4–?	[b]	NR[c]
Staphylococcus aureus	7–45	4.2–9.3	>0.86
Campylobacter jejuni	25–42	5.5–8	NR
Yersinia enterocolitica	1–44	4.4–9	NR
Y. pseudotuberculosis	5–43	[b]	NR
Listeria monocytogenes	0–45	4.4–9.4	>0.92[d]
Vibrio cholerae O1	8–42	6–9.6	>0.95
V. cholerae non-O1	[b]	[b]	
Vibrio parahaemolyticus	12.8–40	5–9.6	>0.94
Clostridium perfringens	10–52	5.5–8	>0.93
Bacillus cereus	10–49	4.9–9.3	>0.95
Escherichia coli	2.5–45	4.6–9.5	>0.935
Shigella spp.	>8–<45	?–9–11	NR
Streptococcus pyogenes	>10–<45	4.8–<9.2	NR

[a] For a genus as large as *Salmonella*, the a_w lower limit for species growth may vary, e.g. *S.* newport = 0.941, *S.* typhimurium = 0.945.
[b] The value, though unreported, is probably close to other species of the genus.
[c] NR denotes that no reported value could be found, but for most vegetative cells, an a_w of >0.95 would be expected.
[d] Updated values from the 1996 *ICMSF Microorganisms in Foods 5*, 'Characteristics of microbial pathogens', FDA, Blackie Academic and Professional. Most values taken from *Microbial Survival in the Environment*, E. Mitscherlich and E.H. Marth (eds.), Springer-Verlag, Berlin.

The water activity of a solution may dramatically affect the ability of heat to kill a bacterium at a given temperature. For example, a population of *Salmonella typhimurium* is reduced tenfold in 0.18 min at 60 °C if the water activity of the suspending medium is 0.995. If the water activity is lowered to 0.94, 4.3 min are required at 60 °C to cause the same tenfold reduction.

A water activity value stated for a bacterium is generally the minimum water activity which supports growth. At the minimum water activity, growth is usually minimal, increasing as the water activity increases. At water activity values below the minimum for growth, bacteria do not necessarily die, although some proportion of the population does die. The bacteria may remain dormant, but infectious. Most importantly, water activity is only one factor and the other factors (e.g. pH, temperature) of the food must be considered. It is the interplay between factors that ultimately determines if a bacterium will grow or not. The water activity of a food may not be a fixed value; it may change over time, or may vary considerably between similar foods from different sources.

Fruit contains significant nutrients and are good source of vitamins (vitamin C, vitamin A, vitamin B6, thiamine, niacin), minerals and dietary fibers. These nutrients are not only important for human growth and health, but also essential to the growth of microorganisms.

For fresh-cut fruit, the skin or cuticle of the fruit is damaged and, therefore, not an obstacle for microbial growth. The physiology of various fruit is complex. The biochemical, physiological changes and interactions that occur in the fruit during processing and storage have a significant impact on the stability and growth of microorganisms.

2.4.2 Natural antimicrobial compounds from some fruit

Plants contain a large number of substances that are known to inhibit various metabolic activities of bacteria, yeasts, and molds, although many of them are yet to be completely exploited. More than 1340 plants are known to be potential sources of antimicrobial compounds (Cowan, 1999). The compounds may be lethal to microbial cells or they may simply inhibit the production of a metabolite, e.g. mycotoxin. Major components with antimicrobial activity found in plants are phenolic compounds organic acids, and flavonoids (Lopez-Malo *et al.*, 2000). Some of these natural antimicrobial systems are already employed for food preservation and many others are just being studied for use in foods.

2.5 Implications for control

2.5.1 Significance of source of contamination in raw and processed fruit

Food hazards may be microbiological, chemical and physical in nature. Microbiological safety is the major issue of concern in fresh fruit. Contamination by human pathogens of fresh fruit may occur at any stage during production, harvesting, handling, processing, storage or distribution to the consumer. Poor agronomic practices, use of contaminated water for crop irrigation, application of improperly composted animal manure as fertilizer and lack of training of field workers about good personal hygiene could contribute significantly to the contamination. Poor sanitation control during post-harvest handling activities is another mechanism for pathogen contamination to fresh fruit, which may include improperly cleaned bins, buckets and trucks used for transportation from the field to packinghouse, cross-contamination of dump tank water, poor personal hygiene among the employees and/or improperly cleaned equipment.

Microbiological, physical and chemical hazards may occur during processing of fruit. For example, the microbiological risks to fresh-cut fruit in the cutting or slicing operation. The internal tissue of fresh fruit is normally protected from microbiological invasion by waxy outer skins and peels. However, cutting circumvents this physical barrier and allows juices to leak

from inner tissues onto the surface of fruit. These juices contain nutrients for accelerating microbiological growth. Together with an increase in exposed surface area, large microbiological populations, including potentially higher human pathogens levels, may develop on cut fruits. Hurst (2002) summarized the key microbiological risks of fresh-cut produce, including: a no-kill step in the process to eliminate potential human pathogens; some pathogens, such as *L. monocytogenes* are psychrotrophic and can grow at refrigeration temperature; the longer shelf-life (10–14 days) may provide sufficient time for pathogen growth; a modified atmosphere suppresses the growth of spoilage organisms, but certain pathogens (*L. monocytogenes*) survive and may actually thrive under these conditions; and fresh-cut fruit is consumed raw.

Chemical and physical hazards may also become significant in addition to microbiological hazards. Chemical contaminants can be naturally present in foods or can be introduced during processing when compounds generally recognized as safe (GRAS) (e.g. antioxidants, sulfiting agents, preservatives) are not used according to government regulatory guidelines. It is incumbent upon the processor to ensure that chemical compounds such as sanitizers and lubricants are used with strict adherence to existing regulations and product specifications. Physical contaminants can be defined as any materials not normally found in food that can produce an injury or illness in the consumer. They can enter the food supply through contaminated raw materials, faulty processing equipment, improper packaging and poor employee hygiene practices. Examples of physical hazards that can compromise food safety include metal fragments, gravel, plastic, glass particles and jewelry. These hazards affect the product's safety. Prevention methods can rely on visual examination, frequent inspections of equipment and the use of metal and glass detectors.

2.5.2 Food safety initiatives at the farm and processing level

To minimize microbiological safety hazards during agricultural operations, the FDA Center for Food Safety and Applied Nutrition (CFSAN) developed a guide in 1998 *Guide to minimize microbial food safety hazards for fresh fruits and vegetables*. This document addresses potential food safety issues associated with farmland, irrigation water, fertilizer usage and pesticides monitoring, harvest practices and field sanitation, and work hygiene, and sets forth good agricultural practices (GAPs) for producers to implement in their farm facilities. It stresses prevention of contamination over corrective action once contamination has occurred and recommends the establishment of a format for developing a system of accountability for sanitary practices at all levels of the agricultural and packinghouse environment.

GAPs can serve as guidance for farmers throughout the growth, harvest, packing and transportation of each berry crop. However, once the fruit has

been transported from the field, other food safety protocols must be followed. Good manufacturing practices (GMPs) are described in the Code of Federal Regulations (CFR) section 21, part 110, and are required by law in the USA for all food manufacturing companies. GMPs address four main areas of food processing, including the design of buildings and facilities to protect against product contamination, sanitation of equipment and utensils to prevent contaminants from being introduced into the food, personnel hygiene to protect adulteration of foods by food handlers and process controls that ensure adequate food processing during production.

2.5.3 Intervention methods to ensure microbiological safety of fruit

Specific intervention methods can be integrated into a safe food production and processing system to control pathogens and ensure the microbiological safety of fruit. These include:

- temperature control
- use of surface disinfectants
- low dosage of irradiation
- biocontrol.

Each method has distinct advantages and disadvantages depending upon the type of fruit mitigation protocol and other variables. The best method to eliminate pathogens from produce is to prevent contamination in the first place. However, this is not always possible and the need to wash and sanitize many types of produce remains of paramount importance to prevent disease outbreaks. It should be noted that washing and sanitizing are unlikely to eliminate totally all pathogens after the produce is contaminated. Therefore, it is important to use washing and sanitizing protocols that are efficient. Another important point to consider is that some produce, such as certain berries, cannot be washed owing to their delicate structure and problems with mold proliferation. These and some other produce items are often packaged in the field with minimal post-harvest handling or washing.

The efficacy of the method used to reduce microbial populations is usually dependent upon the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces (cracks, crevices, hydrophobic tendency and texture), exposure time and concentration of cleaner/sanitizer, pH and temperature. It should be noted that the concentration/level of sanitizers or other intervention methods may be limited by an unacceptable sensory impact on the produce. Infiltration of microorganisms into points below the surface of produce is problematic. While it is known that microorganisms can infiltrate into produce under certain handling conditions, the significance of any such infiltration to public health requires further study.

Temperature control

While refrigeration temperature is critical for the quality and shelf-life of fruit, it cannot be relied upon to prevent growth of pathogenic microorganisms on produce. Populations of *L. monocytogenes* remained constant or grew on a variety of whole and cut produce stored at refrigerated temperatures (Farber *et al*., 1998). Under certain chilled storage conditions, spoilage of the product by the native microflora might not occur until after pathogen populations reach levels capable of causing disease. While growth of some pathogens may be inhibited by chilled temperatures, survival can be enhanced under certain conditions. For example, *Salmonellae* and *E. coli* O157:H7 survive for a longer time period in fruit juices under refrigeration than at room temperature (Parish, 1997; Zhao *et al*., 1993).

Surface disinfectants

The simple practice of washing raw fruit in hot water or water containing detergent removes a portion of the pathogenic and spoilage microorganisms that may be present, but studies showing the efficacy of these treatments are few. Even washing fruit in potable water, then again washing or rinsing in potable water would aide in removing microorganisms. Additional tenfold to 100-fold reductions can sometimes be achieved by treatment with disinfectants. The resistance of microorganisms to disinfectant varies greatly with the type and pH of disinfectant, contact time, temperature and the chemical and physical properties of the fruit surface. Each type of disinfectant has its own efficacy in killing microbial cells. Effectiveness depends on the nature of the cells as well as the characteristics of fruit tissues and juices. Some types of disinfectant are appropriate for use in direct contact washes, while others are suitable only for equipment of containers used to process, store or transport fruit. The mechanism of action of many disinfectants on microbial cells and the influence of factors associated with plant materials is poorly understood. The legal use of various treatments also differs from country to country.

The most commonly studied and used surface disinfectants for whole and fresh-cut fruit include chlorine, chlorine dioxide, bromine, iodine, quaternary ammonium compounds, acidic compounds with or without fatty acid surfactants, alkaline compounds, hydrogen peroxide and ozone. Their application depends on the type and nature of the fruit, application temperature, dosage, etc. The functionality, examples of application and conditions in the usage of surface disinfectants in fruit and vegetable sanitation have been discussed in great detail by Beuchat (2000) and Heard (2002).

Low dosage irradiation

Ionizing radiation from ^{60}Co, ^{137}Cs or machine-generated electron beams, alone or in combination with other treatments such as hot water, may be used as a means of extending shelf-life of produce (Diehl, 1995; Thayer *et al*., 1996). Lethality of irradiation is influenced by the target (insect or

microorganism), condition of the treated item and environmental factors. Low dose treatments (<1 kGy) inhibit sprouting of tubers, bulbs and roots, delay produce maturation, eliminate insects in grains, fruit and nuts, and kill parasites in meats. Medium dose treatments (1–10 kGy) reduce microbial populations, including pathogens, on or in foods. However, produce treated by doses above 1 kGy cannot use the term 'fresh' (21CFR101.95).

Relatively little effort has been applied to the control of foodborne pathogens on fresh foods using irradiation, however, effort was made to eliminate *E. coli* O157:H7 from apple juice, *Toxoplasma gondii* and/or *C. cayetanensis* from raspberries (Thayer and Rajkowski, 1999). Combination treatment of low dosage irradiation with other treatments was studied to prevent post-harvest spoilage of fruit. A combination of 0.75 kGy irradiation with a 10 min dip in 50 °C water provided much better control of post-harvest spoilage organisms of papayas and mangoes than either treatment alone (Brodrick and van der Linde, 1981). Neither irradiation (0.3–0.6 kGy), hot fungicide treatment, nor a combination of the two, satisfactorily prevented post-harvest spoilage of mangoes (Johnson *et al*., 1990). Higher doses of irradiation caused unacceptable peel blemishes. A combination of UV and gamma radiation was not more effective than either treatment alone at preventing storage rot of peaches (Lu *et al*., 1993). Irradiation (0.43 kGy average dose) of segments from cut and peeled citrus fruits was not as effective as chemical preservatives at preventing spoilage during chilled storage (Hagenmaier and Baker, 1998).

Biocontrol

The application of biocontrol concepts may be useful to create extra preservation hurdles for fruit, especially fresh-cut fruit to enhance microbial safety of the products. Biocontrol methods include the use of (Heard, 2002):

- antagonistic organisms to control growth of either spoilage or pathogenic species, called biopreservation
- natural antimicrobial compounds to control microbial growth
- natural plant defenses to reduce microbial attack-induced resistance.

There are few published reports on the use of biocontrol agents to prevent growth of human pathogens on fruit. Janisiewicz *et al*. (1999) reported that *Pseudomonas syringiae* prevented growth of *E. coli* O157:H7 in wounds of apples. Populations of the pathogen increased by 2 log in wounds that were not treated with the antagonist but did not increase in wounds treated with *P. syringiae*. The application of microorganisms to prevent proliferation of post-harvest spoilage organisms has been studied to a great extent (Smilanick and Denis-Arrue, 1992; Janisiewicz and Bors, 1995; Leibinger *et al*., 1997; El-Ghaouth *et al*., 2000; Usall *et al*., 2000). Studies suggest that non-pathogenic microorganisms applied to produce surfaces might out-compete pathogens for physical space and nutrients, and/or may produce antagonistic compounds that negatively affect viabil-

ity of pathogens. Research on biocontrol of human pathogens on produce is warranted.

Microorganisms such as lactic acid bacteria are used as biopreservative agents in foods to inhibit the growth of other undesirable species (Heard, 2002). Mechanisms of antagonism include competition for nutrients, binding of nutrients and production of metabolic products with antimicrobial activity. Fermentation with lactic acid bacteria is a traditional biopreservation method employed to increase the safety and quality of foods, including fruit. In recent years, lactic acid bacteria have been used as competitive biocontrol agents and antagonists in non-fermented foods (Breidt and Fleming, 1997). These organisms are often present on the surface of fruit and vegetables, and if encouraged, may reduce the growth of other indigenous spoilage organisms or foodborne pathogens. Lactic acid bacteria are known to produce antimicrobial metabolites, such as lactic and acetic acids, hydrogen peroxide and enzymes including lysozyme.

The use of national antimicrobials from plants and their possible application in minimally processed fruits and vegetables were reviewed by Lopez-Malo *et al*. (2000). Plants, herbs and spices, as well as their derived essential oils and isolated compounds, contain a large number of substances that are known to inhibit various metabolic activities of bacteria, yeast and molds, although many of them are yet incompletely exploited. Major components with antimicrobial activity found in these resources are phenolic compounds, terpenes, aliphatic alcohol, aldehydes, ketones, acids and isoflavonoids. Their effectiveness in inhibiting spoilage and pathogenic microorganisms depends on many factors, including composition of food (pH, water activity, presence of other inhibitors, interaction with food matrix, etc), initial contamination level, handling and distribution (length, temperature and packaging of storage) and possible synergistic or additive interaction effects with other antimicrobial factors. The application of natural antimicrobials in fruit requires a better understanding of the modes of action and their interactions with other preservation factors, as well as the knowledge of the interactions between the stress factors applied and the fruit matrix.

The use of bacteriophage to reduce populations of *Salmonella* on fresh-cut fruit was recently reported (Leverentz *et al*., 2001). Application of *Salmonella*-specific phages reduced populations about 3.5 log on honeydew melon slices (pH 5.8) stored at 5 or 10 °C. Salmonellae were not reduced on apple slices possibly due to the fruit's lower pH (4.2).

The concept of 'induced resistance' of plants to microorganisms that cause pathologies in plant systems has also attracted attention (Hammerschmidt, 1999). In recent years researchers have begun to focus efforts on the mechanisms and signaling pathways plants use to resist disease. Additionally, biotech companies are engineering plants to resist pests. While speculative, it is conceivable that research on biocontrol efforts through induced resistance or genetic engineering could lead to plants that resist human pathogens in addition to plant pathogens.

2.6 Future trends

The number of documented outbreaks of human infections associated with the consumption of raw fruit, vegetables and unpasteurized fruit juices has increased in recent years. According to the Center for Disease Control and Prevention, in the USA the number of reported produce-related outbreaks per year doubled between the period 1973–1987 and 1988–1992. Several reasons for the increase in produce-related human infections have been proposed. These include changes in dietary habits, including a higher per capita consumption of fresh or minimally processed fruit and vegetables, and the increased use of salad bars and meals eaten outside the home. In addition, changes in production and processing methods, sources of produce and the emergence of pathogens not previously associated with raw produce have enhanced the potential for foodborne illness outbreaks associated with raw fruit and vegetables. The end result of these changes is an increased exposure of the general public to fruit and vegetables, which has exacerbated potential problems with contamination by human pathogens (Buck *et al*., 2003). Therefore, better understanding the role of raw and minimally processed fruit and vegetables as vehicles for disease, developing strategies to prevent the contamination and eliminate pathogenic microorganisms are very important to ensure a safe supply of fruit and vegetables for consumers.

2.6.1 Sources of contamination

Determining the exact source of an outbreak is important when devising strategies and interventions to minimize risks of future outbreaks. However, identifying primary inoculum sources for contamination of fresh produce can be tremendously difficult. For example, only two of 27 outbreak investigations described in the NACMCF report on fresh produce clearly identified a point of contamination (NACMCF, 1999). Unlike other commodities such as beef and chicken, which are rigorously inspected, methods of detecting pathogens on fresh produce are less advanced, and the sporadic nature of most contamination further limits the effectiveness of testing. Bacterial pathogens may contaminate fruit and vegetables at any point throughout the production system. As discussed in previous sections, potential pre-harvest sources of contamination include soil, feces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals and human handling. To limit the introduction of pathogenic bacteria through irrigation, the origin and distribution of irrigation water, as well as the history of the land, should be known. Irrigation wells should be well maintained and all irrigation sources should be monitored for human pathogens. Manure used as fertilizer should be treated to eliminate pathogenic microorganisms (e.g. composting or aging) and animals (domestic or otherwise) should be

excluded from produce and sprout seed production fields. A maximum amount of time also should be scheduled between the final manure application and harvest. In addition, prevention of post-harvest contamination through human handling, harvesting equipment, transport containers, insects, dust, rinse water, ice, transport vehicles and processing equipment should be enforced. The GAPs and GMPs should be stringently followed to reduce the risk of microbiological contamination of produce.

2.6.2 Understanding ecological factors influencing human pathogens on fruit

Little is known about microbial ecosystems on the surface of raw fruit and vegetables. The pH of some fruit (melons and soft fruit) is 4.6 or higher, which is suitable for the growth of pathogenic bacteria. The growth and survival of human pathogens could be affected by the presence of post-harvest pathogens such as *Botrytis cinerea* or *Penicillium* spp. Growth of post-harvest fungi in subsurface tissues can alter the pH of plant tissues, allowing the growth of pathogenic bacteria. Interactions between human pathogens and the resident, non-pathogenic microflora have been studied in dairy and meat products (Nguyen and Carlin, 1994), but little is known about these interactions on fruit surfaces. Large differences in surface morphology and metabolic functions of different plant organs (e.g. fruit, flowers, leaves, roots) provide a wide range of diverse ecological niches that could be selective for specific species or communities of microorganisms. Microbial growth on raw fruit can result in the formation of biofilms by spoilage and non-spoilage microorganisms. These biofilms can provide a protective environment for pathogens and reduce the effectiveness of sanitizers and other inhibitory agents. No information is available on the behavior of pathogenic bacteria in biofilms formed by the microflora associated with raw fruit. The species composition of biofilms on various container and equipment surfaces used in the produce industry would also be predicted to differ greatly, depending on the type of produce being harvested or processed. These microflora differences could influence survival and growth characteristics of pathogenic bacteria (Buck *et al.*, 2003).

2.6.3 Development of efficiency methods to eliminate human pathogens from fresh fruit

The lack of an effective antimicrobial treatment at any step, from planting to consumption, means that pathogens introduced at any point may be present on the final food product. Washing and rinsing some types of fruit extend shelf-life by reducing the number of microorganisms on the surfaces. However, only a portion of pathogenic microorganisms may be removed by this simple treatment. Use of a disinfectant can enhance efficiency of removal up to 100-fold, but chemical treatments administered to whole and

cut produce typically will not reduce populations of pathogens by more than 2–3 log10 cfu g^{-1} (Beuchat, 2000). Pathogens also vary in their sensitivity to sanitizers. For example, *L. monocytogenes* is generally more resistant to chlorine than are *Salmonella* and *E. coli* O157:H7 (Beuchat, 2000). The general lack of efficacy of sanitizers on raw fruit can be attributed, in part, to their inaccessibility to locations within structures and tissues that harbor pathogens. Pathogenic bacteria are able to infiltrate cracks, crevices and intercellular spaces of seeds and produce. Infiltration is dependent on temperature, time and pressure, and only occurs when the water pressure on the produce surface overcomes internal gas pressure and the hydrophobic nature of the surface of the produce (Beuchat, 2002). Infiltration may also be enhanced by the presence of surfactants and when the temperature of the fruit or vegetable is higher than the temperature of a water suspension of cells. The protective mechanism of these sites is not well understood but the concept that hydrophobicity of microbial cells aids in their protection by inhibiting penetration of the disinfectants has been proposed (Buck *et al*., 2003).

2.6.4 Indicators for monitoring of pathogens

Indicators and surrogate microorganisms are used for a variety of purposes in food systems including evaluating quality or safety of raw or processed food products and validating effectiveness of microbial control measures. Although frequently used on an informal basis within a specific company, the use of indicators is highly dependent upon the microbiological criteria that are in place for the food product. All the considerations that must be addressed in establishing microbiological criteria must also be in place if indicators or surrogates are to be utilized in process verification (FDA, 2001b).

Indicators and surrogate microorganisms may be used for evaluating safety of fresh or fresh-cut fruit products by assessing or validating the effectiveness of microbial control measures. Although frequently used on an informal basis within a specific company, use of indicators is highly dependent upon microbiological criteria that are in place for the specific produce item or category. All the considerations that must be addressed in establishing microbiological criteria must also be in place if indicators are to be utilized in process verification. Sampling design, stringency and statistical significance are critical to the evaluation of indicators or surrogates in the assurance of food safety. General ideal qualities of indicators and surrogates are valuable starting points when developing a safety program. The importance of selecting the significant target pathogen for the specific product, its source, handling practices and distribution practices cannot be overemphasized. The same is true for selection of the indicator or surrogate to represent those pathogens. The extensive lists of considerations and procedures should be helpful when using indicators and surrogates with

fresh and fresh-cut produce. The use and limitations of indicators and surrogates to determine or validate treatment effectiveness have been delineated. Challenges are identified for selection of an indicator or surrogate for the specific situation and conditions of an individual produce item, including growing, harvesting, processing, handling, storage and packaging (FDA, 2001b). Future research needs in this area was covered in the FDA (2001b) document.

2.6.5 Influence of packaging technologies, especially advances in MAP

Packaging is a final stage in the production and processing, and provides protection for fresh and minimally processed fruit from damage and further contamination by microorganisms. The use of controlled or modified atmosphere packaging (MAP) provides, to some extent, a barrier against the growth of the remaining spoilage and pathogenic organisms. This technique involves either actively or passively controlling or modifying the atmosphere surrounding the product within a package made of various types and/or combinations of films and has been used broadly in fresh and minimally processed fruit to extend shelf-life.

Oxygen, CO_2 and N_2 are most often used in MAP/controlled atmosphere storage. Normally, the concentration of O_2 in a pack is kept very low (1–5%) to reduce the respiration rate of fruit (Lee *et al*., 1995), thus prolonging the shelf-life by delaying the oxidative breakdown of the complex substrates that make up the product. Also, O_2 concentrations below 8% reduce the production of ethylene, a key component of the ripening and maturation process. However, at O_2 <1%, anaerobic respiration can occur, resulting in tissue destruction and the production of substances that contribute to off-flavors and off-odors (Lee *et al*., 1995; Zagory, 1995), as well as the potential for growth of foodborne pathogens such as *Clostridium botulinum* (Austin *et al*., 1998). Therefore, the recommended percentage of O_2 in a modified atmosphere for fruit for both safety and quality is between 1 and 5%. However, it is recognized that the oxygen level will realistically reach levels below 1% in MAP produce. It is generally believed that with the use of permeable films, spoilage will occur before toxin production is an issue.

The inhibitory action of CO_2 has differential effects on microorganisms. While aerobic bacteria such as *pseudomonads* are inhibited by moderate to high levels of CO_2 (10–20%), microorganisms such as lactic acid bacteria can be stimulated by CO_2 (Amanatidou *et al*., 1999). Furthermore, pathogens such as *Clostridium perfringens*, *C. botulinum* and *L. monocytogenes* are minimally affected by CO_2 levels below 50%, and there is concern that by inhibiting spoilage microorganisms, a food product may appear edible while containing high numbers of pathogens that may have multiplied owing to a lack of indigenous competition (Zagory, 1995; Phillips, 1996). More research needs to be done on the interactions of the background microflora with foodborne pathogens in various modified

atmospheres used for produce, as well as on the effects of different gaseous environments on the survival and growth of bacterial foodborne pathogens on whole and fresh-cut fruit.

2.7 Sources of further information and advice

As consumers move towards an increased consumption of fruit and vegetables, especially partially and minimally processed items, the risk of foodborne illnesses is increasing owing to potentially improper production, processing, packaging and handling. This trend has created concern in the scientific community and at the highest levels of government. As a result of these concerns, President Clinton announced the Food Safety Initiative to improve the safety of the nation's food supply in May 1997 (the White House, 1997). In reviewing the situation, the FDA and the US Department of Agriculture (USDA) addressed specific issues for fresh and minimally processed fruit and vegetables. A warning label is required for non-pasteurized juices informing consumers of the risk of foodborne illnesses, particularly to children, the elderly and persons with weakened immune systems. Hazard analysis and critical control points (HACCP) is regulated for juice processing to achieve 5 log reduction in pathogenic microorganisms. A guidance document entitled *Guide to minimize microbial food safety hazards for fresh fruits and vegetables* was released in 1998 (FDA, 1998). In June 2004 (FDA, 2004), the FDA further proposed an action plan *Produce safety from production to consumption: a proposed action plan to minimize foodborne illness associated with fresh produce consumption* with the overarching goal of minimizing foodborne illness associated with the consumption of fresh produce. To achieve this goal, the proposed Action Plan has four general objectives: (1) to prevent contamination of fresh produce; (2) to minimize the public health impact when contamination of fresh produce occurs; (3) to improve communication with producers, preparers and consumers about fresh produce; and (4) to facilitate and support research relevant to fresh produce. For each objective, the FDA's proposed Action Plan identifies steps that could contribute to the achievement of the objective. Since it is currently not feasible to eliminate all potential hazards associated with fresh produce, fruit producers and processors must rely on risk reduction rather than risk elimination. The present regulation issues in the USA and the European Community associated with fruit production and processing are discussed in the following sections.

2.7.1 The USA

In May 1997, as part of this initiative, the Deartment of Health and Human Services (DHHS), USDA and the Environmental Protection Agency (EPA) sent the President a report identifying produce as an area of concern. On

October 2, 1997, President Clinton announced a plan entitled *Initiative to Ensure the Safety of Imported and Domestic Fruits and Vegetables* to provide further assurance that fruits and vegetables consumer by US citizens, whether grown domestically or imported, meet the highest health and safety standards. In response to this, the FDA and the USDA issued *Guidelines to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*, addressing microbial food safety hazards, GAPs and GMPs common to the growing, harvesting, washing, packing, storage and transportation of most fruits and vegetables. The most important issues that are addressed in GAPs include:

- water
- manure and municipal biosolids
- worker health and hygiene
- sanitary facilities
- field/packing facility sanitation
- transportation/distribution
- consumer packaging
- traceback.

GAPs focus on microbial hazards for fresh produce, on risk reduction, not risk elimination and provide broad, scientifically-based principles. The guide is one of the first steps under the President's produce safety initiative to improve safety of fresh produce as it moves from farm to the table.

GMPs are required by law (section 21 of CFR, part 110) and apply to all food manufacturing companies to ensure good food plant sanitation. Further processing and manufacturing into finished products in no way relieves raw materials from the requirement for cleanliness and freedom from deleterious impurities. GMPs are prescribed for four main areas of food processing:

1 personnel hygiene to prevent the spread of illness
2 adequate buildings and facilities
3 sanitary food-contact surfaces (e.g. equipment and utensils)
4 process controls to prevent cross-contamination.

Sanitation standard operating procedures (SSOPs) focus more narrowly on specific procedures that allow a fruit processing plant to achieve sanitary process control in its dairy operation. SSOPs are mandatory for all food processing plants (21 CFR 120.6) subject to HACCP. Although specific protocols may vary from facility to facility, SSOPs provide specific step-by-step procedures to ensure sanitary handling of foods. These documents describe procedures for eight sanitation conditions

1 safety of water
2 cleanliness of utensils and equipment
3 prevention of cross-contamination

4 hand washing and toilet facilities
5 protection of food from contaminants
6 labeling and storage of toxic compounds
7 monitoring employee health
8 pest control.

Specific sanitation procedures recommended for fresh-cut fruit and vegetable processing have been discussed by Hurst (2002).

A more focused approach toward controlling food safety, HACCP, was developed by the FDA to establish safety standards throughout the food industry. HACCP is a structured approach to the identification, assessment of risk and control of hazards associated with a food production process or practice. It aims to identify possible problems before they occur and establish control measures at stages in production that are critical to product safety. Design and implementation of a HACCP system involves seven basic principles or steps:

1 identify possible food safety hazards
2 determine critical control points
3 establish preventive measures
4 monitor the manufacturing process to detect hazards
5 plan corrective actions
6 prepare a method to verify that the HACCP plan is working
7 document the HACCP system by maintaining records.

HACCP is a proven, cost-effective method of maximizing food safety, because it focuses on hazard control at its source. It offers systematic control by covering all aspects of production and handling from raw materials to consumer preparation. HACCP is required for seafood and meat processing industries. In the Federal Register of January 19, 2001, the FDA published final regulations to ensure the safe and sanitary processing of fruit and vegetable juices. These regulations mandate the application of HACCP principles to the processing of juice. Even though HACCP is not mandatory, it has been embraced by the fresh-cut processing industry as a useful tool for implementing food safety practices in the production environment. HACCP is well suited to identify hazards, monitor production for adherence to operational standards and develop an effective record keeping system in a fresh-cut produce facility. With close attention to prerequisite programs, a processor can implement HACCP to round out their food safety program.

In summary, GAPs and GMPs during growing, harvesting, washing, sorting, packing and transporting fresh fruit will minimize the microbial food safety hazards. Developing specific step-by-step SSOP protocols and implementing a HACCP program will further ensure the safety of fresh and processed products, from farm to market.

2.7.2 The European Community

The European Community is also making significant strides toward ensuring food safety and solving the myriad problems associated with the production and processing of fruit and vegetables. The central goal of the European Commission's food safety policy is to ensure a high level of protection of human health and consumers' interests in relation to food, taking into account diversity, including traditional products, whilst ensuring the effective functioning of the internal market. The Commission's guiding principle, primarily set out in its White Paper on Food Safety, is to apply an integrated approach from farm to table covering all sectors of the food chain, including feed production, primary production, food processing, storage, transport and retail sale. The strategic priorities of the White Paper are:

- to create a European Food Safety Authority
- to implement consistently a farm to table approach in food legislation
- to establish the principle that feed and food operators have primary responsibility for food safety; that member states need to ensure surveillance and control of these operators; that the Commission shall test the performance of member states' control capacities and capabilities through audits and inspections

The European Food Safety Authority (EFSA), a new scientific body charged with providing independent and objective advice on food safety issues associated with the food chain, was initiated in January 28, 2002 with its primary objective to '. . . contribute to a high level of consumer health protection in the area of food safety, through which consumer confidence can be restored and maintained.' The EFSA is the keystone of European Union (EU) risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, the EFSA provides independent scientific advice and clear communication on existing and emerging risks.

Similarly to the US GAPs program, EurepGAP was started in 1997 as an initiative of retailers belonging to the Euro-Retailer Produce Working Group (EUREP). It has subsequently evolved into an equal partnership of agricultural producers and their retail customers to develop widely accepted standards and procedures for the global certification of GAPs. EurepGAP is a set of normative documents suitable for accreditation to internationally recognized certification criteria. In addition to the eight areas in the US GAPs' program, EurepGAP also addresses food safety concerns associated with:

- variety and rootstock
- soil management
- worker welfare
- crop protection

- genetically modified organisms (GMO) issues
- recycling and reuse
- environmental issues
- land stewardship

2.8 References

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3

Measuring microbiological contamination in fruit and vegetables

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3.1 Introduction

Foodborne diseases are among the most serious public health concerns all over the world and are a major cause of morbidity (Anonymous, 2001a; Wallace *et al*., 2000). This threat has been increasing with global trade and travel over the past decades, affecting both industrialised and developing countries (Käferstein *et al*., 1997). More than 200 known diseases are transmitted through food (Bryan, 1982), with symptoms ranging from mild gastroenteritis to life-threatening syndromes, with the possibility of chronic complications or disability (Mead *et al*., 1999). More than 40 different foodborne pathogens are known to cause human illness (CAST, 1994), among which over 90% of confirmed foodborne human illness cases and deaths caused by foodborne pathogens reported to the Center for Disease Control and Prevention (CDC) have been attributed to bacteria, the rest being due to fungi, parasites and viruses (Bean *et al*., 1990). In consequence, microbiological quality control programmes are being increasingly applied throughout the food production chain in order to minimise the risk of infection for the consumer. Surveillance systems that include quantification of reported foodborne illnesses and identification of emerging pathogens are needed (Blackburn and McClure, 2002).

Fresh plant products like fruit and vegetables are among the most frequently marketed perishable foods in global or local exchange markets. Vegetables include plant components as leaves, stalks, roots, tubers or bulbs.

Fruit and vegetables are rich in carbohydrates and low in proteins with pH values from 7.0 to slightly acid (especially in some fruits, owing to the presence of organic acids) and are adequate habitats for several bacteria, yeasts and moulds (Fig. 3.1).

Microbes inhabiting vegetables and fruit vary considerably, as plants harbour different microbiota in their aerial parts compared with their root system, also depending on environmental conditions (Fig. 3.2). The range of population levels in fruit and vegetables can vary from 10(3) to 10(7) cfu g^{-1}. However, since microorganisms grow faster in damaged or cut vegetables or fruits than in their intact surface, their population levels may vary according to farming, harvesting and post-harvesting conditions.

Lactic acid bacteria (e.g. *Lactobacillus*), *Pseudomonas*, *Enterobacter* (syn. *Erwinia*, *Pantoea*), *Micrococcus*, *Flavobacterium* and gram-positive spore formers (e.g. *Bacillus*, *Clostridium*) are usually dominant in fresh fruit and vegetables. Different types of moulds such as *Alternaria*, *Penicillium*, *Fusarium* and *Aspergillus* can also be found. Finally, yeasts such as *Torulopsis*, *Saccharomyces* and *Candida*, are part of the dominant microorganisms especially in fruit with high sugar contents.\

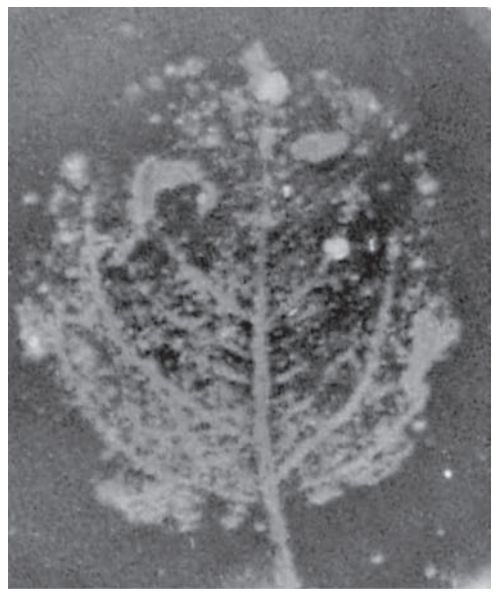

Fig. 3.1 Microbial colonies mainly composed of bacteria, fungi and yeasts grown on the surface of a nutrient agar plate printed with a healthy spinach leaf and incubated for several days.

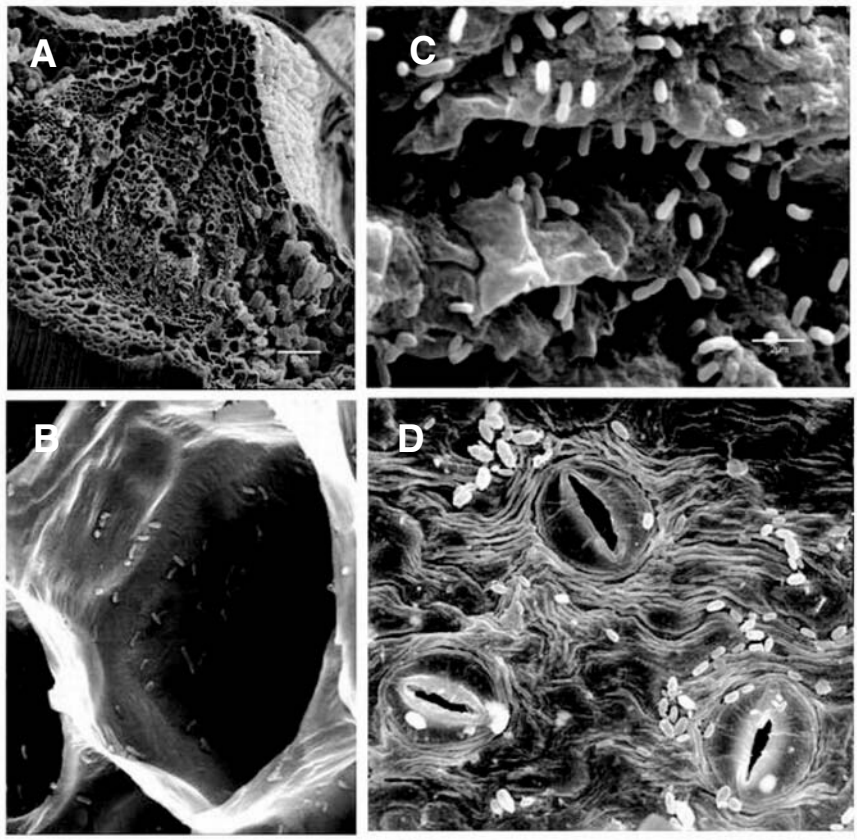

Fig. 3.2 Colonisation of different plant tissues by bacteria. Section cut from the central nerve of a leaf (A), endophytic bacterial cells colonising xylem vessels (B), apple fruit wound colonised by bacteria (C), and leaf surface with stomates and different types of microbial cells colonising the surface (D). Pictures (A) to (C) from Jordi Cabrefiga and (D) from STR-UdG-Paula Vaquero (University of Girona).

3.2 Foodborne pathogens and post-harvest microbiological spoilage of fresh fruit and vegetables

The exigency of global markets has evolved, in adapting agricultural production to the demands of the consumer, to be independent of the harvest date, forcing growers to increase the shelf-life of fruit and vegetables, whilst still maintaining the organoleptic quality during storage, commercialisation and domestic use. However, the globalisation of markets has added problems of microbial spoilage and diseases caused by foodborne pathogens such as bacteria, viruses and parasite protozoans (Table 3.1).

Table 3.1 Microbiological contamination of fresh fruit and vegetables

Microorganism	Type of microorganism	Effect
(a) Spoiling microorganisms		
Penicillium	Fungus	Blue mould rot, mycotoxins
Rhizopus	Fungus	Soft rot
Monilinia	Fungus	Brown rot
Alternaria	Fungus	Black rot, mycotoxins
Botrytis	Fungus	Grey mould rot
Pectobacterium carotovorum	Bacterium	Soft rot
Pseudomonas fluorescens	Bacterium	Soft rot
(b) Human pathogens		
Salmonella	Bacterium	Gastroenteritis
Shigella	Bacterium	Gastroenteritis and shiga toxin
E. coli O157:H7	Bacterium	ETEC, EPEC
Listeria monocytogenes	Bacterium	Gastroenteritis, septicaemia, organ invasion
Campylobacter (C. coli, C. jejuni)	Bacterium	As *Shigella*
Bacillus cereus	Bacterium	Toxin effects
Norwalk virus	Virus	Gastroenteritis
Hepatitis virus	Virus	Liver inflammation
(c) Human parasites		
Cryptosporidium parvum	Protozoan	Gastroenteritis
Giardia lamblia	Protozoan	Gastroenteritis
Cyclospora cayetanensis	Coccidian	Gastroenteritis

Average losses during post-harvest of fresh fruit and vegetables in developed countries are estimated at 5–30%. These losses are mainly due to physiological disorders and microbial spoilage caused by fungi and bacteria. Microbial spoilage of fruit and vegetables is known as rot, which consists of changes in colour (black, grey, pink), loss of texture (soft rot) and often off-odour. The main fungal rot of fruits are caused by *Penicillium expansum* and *Botrytis cinerea* in apples and pears, *P. italicum* and *P. digitatum* in citrus fruits and *Monilinia* spp. and *Rhizopus stolonifer* in peach, apricot, prune and other stone fruits (Jones and Aldwinckle, 1990; Ogawa *et al*., 1995; Snowdon, 1991). Soft rot of bacterial origin (*Pectobacterium carotovorum* (formerly *Erwinia carotovora*), *Pseudomonas fluorescens* and *Bacillus* spp.) are of main importance in fresh vegetables (Carlin *et al*., 1989; Snowdon, 1991). Most post-harvest infections are a consequence of mechanical wounds during harvest and handling of raw materials and occur upon the entrance, close-up and set-up of storage chambers (often requiring several days or weeks) and during package and commercialisation especially when the cold chain is broken.

Post-harvest fungal rot not only causes quality losses: several fungal plant pathogens (mainly various species of the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* (DeVries *et al*., 2002) produce micotoxins (Battilani and Pietri, 2002; Jackson *et al*., 2003). They are fungal secondary metabolites that can have nephrotoxic, hepatotoxic, neurotoxic, teratogenic, estrogenic or carcinogenic properties (Hayes, 1981; Sharma, 1993), thus being highly toxic to animals and humans. Being a health concern, specific regulations exist in several countries on the contents of certain micotoxins in food, especially patulin, ocratoxin A and trichotecenes.

Commercialisation of packaged fresh plant products consisting of washed, cut or germinated seeds (sprouts) ready to eat (RTE, in some countries named 4th gamma) is becoming common. Apart from losses caused by deleterious microorganisms, these products have posed public health problems owing to the uncontrolled presence of foodborne human pathogens (Doyle, 1990; Nguyen-The and Carlin, 1994). Fruit and vegetables contain nutrients that support rapid growth of foodborne human bacterial pathogens such as *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Shigella* and *Salmonella* (Beuchat, 1996; 2002). The existence of natural physical barriers like the fruit peel or epidermal cuticle in leafy vegetables, preventing contact of these pathogens with tissues and intracellular fluids, may be the cause of their low incidence in unprocessed fresh products (Lindow *et al*., 2002). This barrier is broken down in fruit and vegetables submitted to industrial processing (wounds, hits and cutting). *E. coli* O157:H7 and *L. monocytogenes* can acquire population levels of 10(7) cfu/wound in Golden apple fruits in less than two days (Berrang *et al*., 1989; Beuchat and Brackett, 1990; Conway *et al*., 2000; Heisick *et al*., 1989; Janisiewicz *et al*., 1999a, b), in salad vegetables (Abdul-Raouf *et al*., 1993) or tomatoes (Beuchat and Brackett, 1991). Foodborne intoxications or diseases associated with fresh fruit and vegetable consumption are less frequent than those associated with products of animal origin. However, an increase has been detected in the recent years (Brackett, 1999; Buck *et al*., 2003). Enterohaemoragic toxi-infections associated with the consumption of non-pasteurized fresh apple juice contaminated with *E. coli* O157:H7 (Besser *et al*., 1993) or *L. monocytogenes* (Sizmur and Walker, 1988; Heisick *et al*., 1989) and of *Salmonella* spp. (Berrang *et al*., 1989; Wells and Butterfield, 1997) in high population levels have been detected in fresh vegetables in the market, in ready to use fresh cut products under modified atmosphere package (Lin *et al*., 1996) or sprouted seeds (Ponka *et al*., 1995). Several outbreaks of *E. coli*, *L. monocytogenes*, *C. jejuni*, *Bacillus cereus*, *Shigella* and *Salmonella* spp. have been attributed to sprouted seeds in some countries (Center for Disease Control and Prevention, 2004). Also, enteric viruses like Norwalk virus and hepatitis A virus ranked after foodborne infections of bacterial origin (Rosenblum *et al*., 1990; Cliver, 1994; Mead *et al*., 1999).

Finally, some protozoan parasites like *Giardia lamblia*, *Cryptosporidium parvum* and *Cyclospora cayetanensis*, have been reported as causing \

gastrointestinal tract infections less frequently in humans upon consumption of contaminated fruit, raw vegetables or fresh juice (Rose and Slifko, 1999).

These products can be contaminated both during the production process in the field (faecal contamination of irrigation water, application of contaminated organic manure), during harvest (hand manipulation) or industrial processing (washing, cutting, etc.) or even in the market for unpackaged products owing to handling by the client (FDA, 1999; FDA-USDA-CDC, 1998; Solomon *et al*., 2002).

3.3 Methods of detection and quantification of foodborne pathogens

Methods used for detection and quantification are mainly addressed to foodborne human pathogens and micotoxigenic fungi because of the risk to human health. Detection procedures to establish the presence of a particular organism in a sample typically target microorganisms for which food safety regulations have adopted a zero tolerance level. Latterly, a less stringent criterion is being adopted for certain pathogens belonging to the former category (e.g. *Listeria monocytogenes*) for which clinical cases of disease are often associated with high loads and are difficult to eradicate from the environment of the food processing plants (Vázquez-Boland *et al*., 2001). Thus, enumeration procedures for the quantification of a particular organism in a sample are required for microorganisms for which a threshold colony forming units per gram (cfu g^{-1}) of food is considered acceptable for non-risk consumers. This would prevent the unnecessary recall and destruction of valuable food products. Rules will presumably also evolve towards the need for identification of microorganisms as there is increasing evidence indicating that the pathogenicity of many bacteria depends upon the genetic background and varies with serotype or strain. In consequence, from a clinical point of view the detection of a pathogenic strain could be more useful than the detection of a particular species itself.

3.3.1 Sample processing

The main problems of microbial analysis of fresh fruit and vegetables are derived from the fact that the pathogen often is present in much smaller population levels than the indigenous microbiota (Marchetti *et al*., 1991) and that food matrixes interfere with detection methods.

The objective of sample processing is to remove microorganisms from the fruit and vegetable matrix to generate primary microbial suspensions. Methods of removal are addressed to extract the microorganisms which in the case of bacteria can often form biofilms (epiphytic growth) or colonise internal tissues (endophytic growth) (Fig. 3.2). Most frequently used microbial removal methods consist of swabs (surface contamination), intense agi-\

tation or high pressure sprays, tissue homogenisers, ultrasonic baths and paddle or rolling blenders. However, some methods may produce adverse effects on microbial cell viability (ultrasonic waves) or produce high amounts of plant cell debris (tissue homogenisers) which can interfere during analysis. Also, mild extraction methods may not extract endophytic microorganisms adequately.

Fresh fruit and vegetable products have specific problems owing to the nature of plant cells and tissues. During maceration, plant cell vacuoles release organic acids resulting in acidification of the suspension. Also, the tissues of some plant products, when crushed, produce compounds that can inactivate microbial cells. The main problem is often caused by the activation of monooxygenases (mainly polyphenoloxidases and peroxidases) that are activated upon vacuole damage. Thus, the oxidation of some phenolic and lypidic compounds can generate antimicrobial products that affect the viability of microbial cells.

In general, the above-mentioned problems can be solved by using antioxidant buffer mixes consisting of phenolic compounds absorbers (e.g. polyvinylpolypirrolidone PVPP), antioxidants (e.g. ascorbic acid, gluthation), mild detergents (e.g. Tween 20), and buffers (e.g. buffered phosphate) (Rossen *et al.*, 1992). Also, to increase the efficiency of microbial removal, large volumes of extracting buffers are needed in relation to the amount of material processed (50–250 ml). However, the methods of analysis only permit processing small amounts of extracted material (less than 1 ml). Thus, an additional stage of removal of large particulate debris, and separation or concentration of microbial cells, is required. The removal of large materials which are generated by more vigorous extraction methods is simply accomplished by cheese-cloth filtration. Separation and concentration of microbial cells from the suspension can be performed by membrane filtration, centrifugation or immunomagnetic separation (Table 3.2).

3.3.2 Cultivation-dependent and cultivation-independent methods

As shown before, the presence of foodborne human pathogenic bacteria are of great concern in the safety of fresh plant products. Therefore, most of the methods reviewed in this chapter will be focused specifically on these microorganisms.

Classical methods for analysis of foodborne human pathogenic bacteria are those recommended by the International Organization of Standardization (ISO) technical committee. Horizontal methods of detection have been published for *Salmonella* (ISO 6579:2002) based on resuscitation, subculture on Rappaport–Vassiliadis medium and XLD agar plating (Anonymous, 2002a), for detection of *Listeria* (ISO 11290-1:1996) based on enrichment on Half-Fraser medium and isolation on Oxford and PALCAM agar (Anonymous, 1996), for *Campylobacter* (ISO 10272:1995) based on microaerobic enrichment on Preston or Park and Sanders broth followed\

Table 3.2 Sample preparation procedures used in analysis of foodborne pathogens (adapted from Rådström *et al.*, 2003)

Category	Principle	Procedure	Removal of inhibitors
Physiological	Selection	Enrichment	Low
Physical	–	Aqueous two-phase systems	Medium
		Centrifugation	Medium
		Dilution	Medium
		Filtration	High
		Heat treatment	Medium
Biochemical	Adsorption	Lectin or protein-based purification	High
	Nucleic acids extraction		High
Immunological	Adsorption	Immunomagnetic capture	Medium

by plating on Karmali agar (Anonymous, 1995) and for *E. coli* O157 with selective enrichment, immunoseparation and plating onto cefixime tellurite sorbitol MacConkey agar (Anonymous, 2001b). All these methods render with presumptive colonies that have to be confirmed by additional tests. Some of the additional tests are based on the capacity for growth in specific media (API system) or to oxidise diverse compounds (BIOLOG). However, the faster systems use serotyping and polymerase chain reaction (PCR)-based methods (see below).

Electrical methods profit from the fact that during microbial growth, catabolic processes produce CO_2 that dissolves in part in the culture medium increasing conductance. Thus, growth can be quantified directly using culture media with low basal conductivity or indirectly through special trapping systems for carbon dioxide. Thus, during population growth, the rate of conductance increase is directly related to the viable cell increase. In the analysis procedure an aliquot of the sample extract is inoculated into sterile medium and the conductance recorded with time. The time of the start of the growth curve (threshold value) is inversely related to the initial cell concentration. Several kinds of commercial equipment are available such as Bactometer, Malthus, RABIT and BacTrac. Also, a similar system based on absorbance measurements is available as Bioscreen. These apparatuses are of great utility in rapid quality control of total microbial content in raw materials and in the end product in the food industry because they permit processing of hundreds of samples in 24 h. Several specific methods have been developed for Enterobacteriaceae, coliforms and for *Salmonella*, *E. coli*, *Listeria* spp., *Clostridium* spp. and faecal streptococci, using selective culture media, and some are accredited by national or international authorities (Bolton and Gibson, 1994; Dromigny *et al.*, 1997; Edminston and Russell, 2000; Gibson, 2003; Neaves *et al.*, 1988).

One of the main concerns about classical culture-dependent methods, apart from the time-consuming nature, is the intrinsic selective properties and the limited recovery of viable cells under certain circumstances. Several foodborne human pathogenic bacteria can enter into a viable but non-cultivable (VBNC) state characterised by the transient inability to grow on routine bacteriological media, on which they normally grow and develop colonies, though still being metabolically active (Olivier, 2000). Under the proper conditions, for example animal passage or resuscitation in suitable culture media, these cells can recover the capacity for sustained cellular growth and a cultivable state (Rahman *et al*., 1996; Colwell *et al*., 1996). Obviously this phenomenon represents a public health hazard because the VBNC state may be induced by exposure to environmental stresses like temperature shifts, nutrient starvation and sub-lethal injury caused by antimicrobial compounds including disinfectants which are part of standard practices in the food industry (Chmielewski and Frank, 1995; Ekweozor *et al*., 1998).

Cultivation-independent methods are addressed to target cell components and can be divided into the function of the target molecule and the reaction used for detection of such a molecule. Main groupings consist of those based on specific enzymatic reactions, immunological methods and nucleic acid hybridisation methods (Fung, 2002).

Methods based on specific enzymatic reactions, such as ATP bioluminescence, require a specific adaptation to analyse bacterial ATP, preventing interference from plant cell ATP. Systems consist of selective lysis of somatic cells in the sample extract, concentration of bacterial cells by filtration or centrifugation, lysis of the bacterial cells and ATP measurement using light emission luciferin–luciferase assay. The system has been adapted for food products based on fruit juice, poultry and meat carcasses, but can be used in fresh fruit and vegetable matrixes (Griffiths and Brovko, 2003). Several commercial systems are available using bioluminometers (Biotrace Inc), image analysis of a filter adapted bioluminiscent device (Millopore Corp) and a swab-based system (Promega Corp). These systems are developed for total bacterial ATP but several methods based on bioluminescent reporter bacteriophages have been developed specifically for *Salmonella* and *Listeria* (Chen and Griffiths, 1996).

Immunological methods have the advantage of specificity, but to increase sensitivity, sample extracts are processed in order to enrich target antigens by either immunocapture or selective culture enrichment. Immunocapture can be performed using magnetic beads coated with specific antibodies (immunomagnetic separation) or the antibodies can be adsorbed to a membrane. The advantage of enrichment is that the number of target cells increases several orders of magnitude but the pathogen can be overcompeted by other fast growing saprophytes unless using selective growth media. The most popular immunological methods of analysis rely on immunochromatography (lateral flow devices) and on enzyme-linked-

immunosorbent-assay (ELISA). Lateral flow devices (LFD) use a selective capture (usually a binding protein or antibody) and subsequent detection of the target antigen into a membrane by immunoprecipitation (Feldsine *et al*., 1997a, b). Several commercial kits are available from BioControl Inc, Neogen Corp, Merk KgaA, Oxoid, Binax Inc, Celsis Ltd and Meridian Diagnostics for *Salmonella*, *Listeria*, *E. coli* O157:H7 and *Campylobacter*. ELISA tests use microtitre plates and are generally accepted methods for detection of pathogens in foods. In ELISA methods, capture antibody binds antigen and antigen reacts with a second antibody that is detected by a third antibody conjugated to an enzyme. Several commercial systems are available for *Salmonella*, *Campylobacter*, *Listeria*, *E. coli* O157:H7 and staphylococcal enterotoxins developed as TECRA UNIQUE, SALMONELLA-TEK and EIAFOSS (Baylis, 2003). Former ELISA systems rely on chromogenic substrates for detection (e.g. alkaline phosphatase) but new developments are based on fluorogenic substrates such as 4-methylumbelliferyl phosphate (MUP). Automation of the method has been provided by the Vitek Immuno Diagnostic Assay System (VIDAS) developed by Biomérieux.

Direct microscopic techniques (DMT) and flow cytometry (FC) are based on microscopic principles and direct analysis of individual cells. In both types of methods, stains have been developed with fluorochromes based on non-specific stains (acridine orange, redox indicators, fluorescein isothiocyanate-FITC), or specific stains coupled to fluorescent antibodies or oligonucleotide probes which have been developed for *Salmonella*, *E. coli* O157:H7, *Listeria* and total bacteria in food products (Donnelly and Baigent, 1996; McClelland and Pinder, 1994; Tortorello *et al*., 1998; Wang and Slavik, 1999).

DMT is used with conventional microscopy, confocal laser scanning microscopy (CLSM) or electron microscopy. However, CLSM permits direct observation of thick specimens within a certain depth across the internal structure without thin sectioning or complicated sample processing, because the specimen is scanned by a light beam in a given plane of focus and cells emit fluorescence upon a specific stain with fluorochrome chemical compounds (Takeuchi and Frank, 2001). Flow cytometry (Fc) consists of hydrodynamically focusing a narrow sample stream under laminar flow containing cells or particles and moving it through a laser beam and detector system located at the focal point of the beam (Givan, 2001). An analysis based on cell-to-cell measurements is performed according to forward (FSC) and side (SSC) scattered light. FSC is an indication of the cell size, whereas SSC reflects internal or surface structure. Thus, FC analyses populations of cells and the computered output permits multiple parameter plots (FSC versus SSC) which can be used to obtain information about cell concentration, cell size and the 'cell state' (e.g. viability, certain metabolic states, cell components). However, for bacterial analysis a high resolution (less than 0.2 μm) is needed, which is not always possible owing

to the electronic noise in some commercial equipment (Shapiro, 2001; Steen 2000). For food analysis FC is applied to sample extracts (suspensions) and offers advantages mainly because of the high sample throughput and quantitativeness (Raybourne and Tortorello, 2003).

Nucleic acids-based methods mostly use molecular hybridisation properties, which involve complementary sequence recognition between a nucleic acid probe and a target. Since nucleic acids have very distinct recognition patterns, they can be optimised to provide taxonomic information at the level of genera, species, serotype or strain; or at the level of specific characters of pathogenicity, especially genes coding for toxins, virulence factors or major antigens. They are able to achieve a high degree of specificity and sensitivity, frequently without the need for previous cultivation and additional confirmation steps, thus permitting the detection of microorganisms within hours, instead of the days required with the traditional methods (Hill, 1996; Lantz *et al*., 2000; Olsen *et al*., 1995; Scheu *et al*., 1998). The most frequently used nucleic acids-based methods are the polymerase chain reaction (PCR) for the analysis of a single or a few microorganisms. New approaches address the specific detection of viable organisms and the simultaneous detection of multiple targets.

3.3.3 Nucleic acids-based methods

The exponentially increasing sequence databases, including the recent sequencing of a number of microbial genomes and the development of new analytical tools has led to improved methods for detection and quantification of microorganisms. Here we describe the most commonly used. Fluorescence in situ hybridisation (FISH) consists of the hybridisation of DNA probes to (mostly) specific regions of bacterial ribosomal RNA and subsequent microscopic analysis (Amann *et al*., 1990). FISH is theoretically capable of detecting single cells, although detection levels of 10^3 cells/ml are achieved in practice for the detection of bacteria in plants (e.g. Wullings *et al*., 1998).

PCR is the specific exponential *in vitro* amplification of a DNA fragment (usually below 2 kbp) by a thermostable DNA polymerase (initially Taq, purified from *Thermus aquaticus*) and a pair of oligonucleotides to prime the enzymatic activity. The DNA template is denatured by heat; the primers are allowed to hybridise with their complementary sequences in the single-stranded DNA template and each annealed primer is then elongated by the polymerase activity. This cycle is subsequently repeated normally 35 to 45 times, leading to exponential amplification of the target DNA fragment (i.e. limited by the two oligonucleotide sequences) up to around 10^6-fold. The PCR products are usually resolved by gel electrophoresis and visualised by staining. A large number of publications report on the development of PCR-based diagnostic tools for the detection of foodborne pathogens (Rijpens and Hermann, 2002; Scheu *et al*., 1998), several of which have

been validated through collaborative trials and proposed as a standard to international standardisation bodies (http://www.pcr.dk). PCR techniques are basically easy to handle and highly applicable to routine diagnostics. Additionally, as prices of instruments have impressively decreased, many laboratories are implementing PCR as a diagnostic tool. Routine reference methods are predicted to include PCR-based ones alongside traditional detection techniques within the next ten years (Hoorfar and Cook, 2003).

Similar approaches include methods based on nested PCR, i.e. two consecutive rounds of PCR using two different primer sets, the second one targeting internal sequences of the first amplicon. This allows the achievement of greater sensitivity and specificity, but the risk of contamination is very high unless a single-tube approach is used (Llop *et al.*, 2000; Olmos *et al.*, 2002).

A development of PCR, namely real-time (RTi-)PCR allows accurate quantification of the bacterial target DNA (which is directly related to the size of the bacterial population present in the sample). In addition, it is quick and simple, highly sensitive and specific, and the closed-tube format avoids risks of carryover contaminations and permits high throughput and automation (Klein, 2002). Quantification is achieved by monitoring the synthesis of new amplicon molecules along the reaction through emission of a fluorescent signal as the PCR progresses (Fig. 3.3). Various chemistries have been developed to produce such fluorescent signal. Sequence-specific chemistries rely on the use of probe oligonucleotides complementary to an

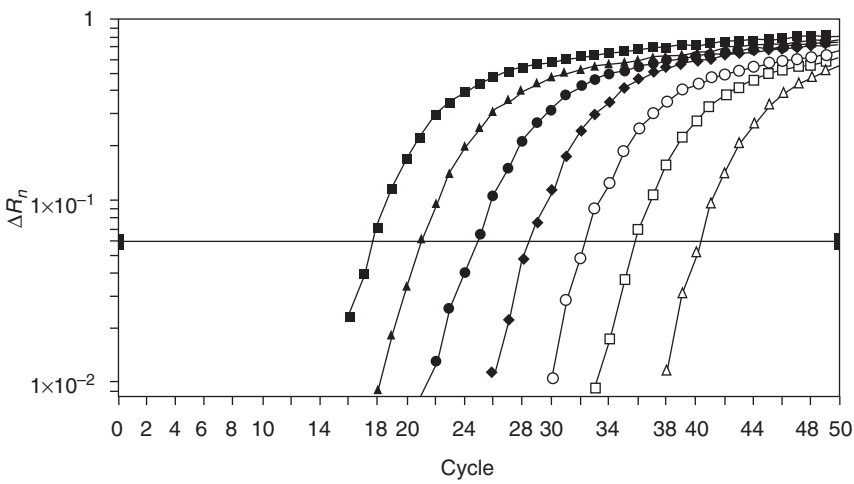

Fig. 3.3 Real-time PCR detection and amplification of the sequences from the *23S-rDNA* gene in the *Listeria monocytogenes* strain ATCC 5577, Representative amplification plots are shown with genomic DNA corresponding to 3×10^5 (■), 3×10^4 (▲), 3×10^3 (●), 3×10^2 (◆), 30 (○), 15 (□) and 3 (△) target molecules per reaction.

internal portion of the amplicon. Hybridisation probes are based on the fluorescence resonance energy transfer (FRET) from a donor to an acceptor fluorophore, which are strongly dependent upon their distance (e.g. Molecular Beacons, Tyagi and Kramer, 1996; FRET probes, Bernard and Wittwer, 2000; Scorpion primers, Whitcombe *et al*., 1999). Hydrolysis probes are double-labelled with a reporter and a quencher dye, they anneal with the single-stranded target at the same time as the primers do, and are hydrolysed by the polymerase upon synthesis of each new DNA molecule, allowing the emission of the reporter dye to be measured (e.g. TaqMan® probes, Heid *et al*., 1996). Alternative chemistries exist which are not sequence-dependent, such as those based on fluorescent dyes, where fluorescence emission depends upon binding to double-stranded DNA (e.g. SYBR-Green I, Wittwer *et al*., 1997), or sunrise primers (Nazarenko *et al.*, 1997), which are more versatile but also less specific than the chemistries relying on specific probes. An increasing number of RTi-PCR assays have been developed for food microbiology diagnostics. Table 3.3 summarises those published for detection of the five most representative foodborne pathogenic bacteria in fresh fruit and vegetables or in pure cultures. Even if the cellular genome content varies with the growth phase of the microorganisms, for many of these RTi-PCR-based methods good correlation between gene quantification and bacterial growth has been reported (e.g. Ludwig and Schleifer, 2000; Rodriguez-Lázaro *et al*., 2004a; 2004g).

Nucleic acid sequence-based amplification (NASBA) is an isothermal amplification technique performed at low temperature (41°C) involving three enzymes (reverse transcriptase, ribonuclease and RNA polymerase), permitting the selective amplification of RNA sequences (Compton, 1991). The reaction uses two primers complementary to the RNA region of interest, one of which includes a promoter sequence to drive RNA polymerase activity. The predominant product of NASBA is a RNA molecule that can be detected by different methods including agarose gel and ethidium bromide staining, electrochemiluminiscence (ECL, e.g. Cook *et al*., 2002; Rodríguez-Lázaro *et al*., 2004d), enzyme-linked gel assay (ELGA, Uyttendaele *et al*., 1995) or molecular beacons, the latter allowing real-time detection of the product (e.g. Leone *et al*., 1998; Rodríguez-Lázaro *et al*., 2004c) (Fig. 3.4). NASBA is especially attractive for viability purposes (see later), although its level of development and validation is still well behind that of PCR (Cook, 2003) (Table 3.4).

Multiplex amplification assays allow simultaneous detection (and quantification) of multiple DNA or RNA targets in a single reaction. There are several examples in which a small number of microorganisms (usually not more than three) can be assayed by multiplex PCR (e.g. Jofré *et al*., 2005; Rodríguez-Lázaro *et al*., 2004b) and this requires a very accurate design of the primers. DNA microarrays offer the possibility of simultaneously analysing a high number of target nucleic acids. Multiple capture probes (usually up to 30 000 per cm^2) are linked on a surface and allowed to hybridise

Table 3.3 RTi-PCR-based methods published for detection of the five most representative foodborne pathogenic bacteria in fresh fruit and vegetables or in pure cultures

Target	Sequence	System	Matrix	Detection limit	Reference
E. coli 0157		BAX	Alfalfa sprouts and mushrooms	10 cfu ml^{-1}	Strapp *et al.*, 2003
	uidA	TaqMan MGB	Culture	–	Yoshitomi *et al.,* 2003
	uidA	TaqMan	Culture	–	Jinneman *et al.*, 2003
		BAX	Fresh vegetables	1–10 cfu ml^{-1}	Bhagwat, 2003
	stx1, *stx2*	TaqMan	Commercially bagged salad Greens, and salad dressing	5340 cfu g^{-1}	Heller *et al.*, 2003
	stx1, stx2 and *eae*	TaqMan	Water	10 cfu g^{-1}	Ibekwe *et al.*, 2002
	stx1 and *stx2*	SYBR Green	Culture broth	10^3 cfu ml^{-1}	Jothikumar and Griffiths, 2002
	rfbE	Molecular Beacons	Apple juice	1 cfu ml^{-1}	Fortin *et al.,* 2001
		TaqMan *E. coli* O157:H7 Kit	Water	–	Fratamico and Bagi, 2001
		BAX	Fresh fruit and vegetables	1 cfu/ 25 g	Shearer *et al.*, 2001
	eaeA	TaqMan	Culture medium	10^2 cfu ml^{-1}	Oberst *et al.*, 1998
L. monocytogenes	*hly, iap*	TaqMan, Ampli Fluor	Culture broth	1 cell/reaction	Rodríguez-Lázaro *et al.,* 2004a
		BAX	Fresh vegetables	1–10 cfu ml^{-1}	Bhagwat, 2003
	hly	SYBR Green	Culture broth	2.5 cells /reaction	Jothikumar *et al.*, 2003
	hly	TaqMan	Cabbage	9 cfu/reaction	Hough *et al.*, 2002
		BAX	Several	–	Hochberg *et al.*, 2001
	hly	TaqMan	Pure cultures, water	6 cfu/reaction	Nogva *et al.*, 2000b
		BAX	Culture	10^5–10^6 cfu ml^{-1}	Stewart and Gendel, 1998

Salmonella spp.	*fimI*	SYBR Green	Culture broth	2.5 cells /reaction	Jothikumar *et al.*, 2003
		BAX	Orange juice	–	Fukushima *et al.*, 2003
	invA	TaqMan	Agar plate	–	Rodríguez-Lázaro *et al.*, 2003
		BAX	Fresh vegetables	1–10 cfu ml^{-1}	Bhagwat, 2003
		BAX	Alfalfa sprouts and mushrooms	10 cfu ml^{-1}	Strapp *et al.*, 2003
		BAX	Fresh fruit and vegetables	1 cfu/ 25 g	Shearer *et al.*, 2001
	himA	Molecular Beacons	Culture broth	2 cfu/reaction	Chen *et al.*, 2000
		Applied Biosystems (TaqMan) Kit	Culture broth	25 cfu/reaction	Nogva and Lillehaug, 1999
		BAX			Bennett *et al.*, 1998
Salmonella enterica	*invA*	TaqMan	Culture broth	–	Knutsson *et al.*, 2002b
	invA	TaqMan	Agar plate	–	Hoorfar *et al.*, 2000
Y. enterocolytica	16S rDNA	SYBR Green	Culture	10^1 cfu ml^{-1}	Knutsson *et al.*, 2002a
	ERIC Fingerprint Fragment	SYBR Green	Culture	–	Aarts *et al.*, 2001
	16S rDNA	Light-up			Wolffs *et al.*, 2001

Table 3.4 NASBA-based methods reported for detection of pathogenic microorganisms in fresh fruit and vegetables, and environmental samples (adapted from Cook, 2003)

Target	Sequence	Matrix	Detection system	Detection limit	Reference
Escherichia coli	*clpB*	Water	ECL	40 cfu ml^{-1}	Min and Baeumner, 2002
E. coli	*clpB*	Water	RNA biosensor	40 cfu ml^{-1}	Baeumner *et al.*, 2003
Salmonella enteritidis	*dna*K	Ready-to-eat salads	ECL	$<10^1$–10^2 cfu/25 g	d'Souza and Jaykus, 2003
Listeria monocytogenes	16S rRNA	Vegetables, dairy products	ELGA	<10 cfu/25 g	Uyttendaele *et al.*, 1995
Mycobacterium avium subsp. *paratuberculosis*	*dnaA*	Water	Molecular Beacons	10^3 cfu/20 ml	Rodríguez-Lázaro *et al.*, 2004c
Cryptosporidium parvum	*hsp70*	Water	ECL	50 oocysts/1001	Baeumner *et al.*, 2001
Hepatitis A virus	VP2	Blueberries, lettuce, wastewater, sewage	Probe hybridisation/ dot-blot		Jean *et al.*, 2001
Hepatitis A virus	VP2	–	Microtitre plate hybridisation	400 PDU ml^{-1}	Jean *et al.*, 2002a
Rotavirus	gene 9	Sewage	ELISA	10^4 PDU ml^{-1}	Jean *et al.*, 2002b
Rotavirus	gene 9	–	Microtitre plate hybridisation	40 PDU ml^{-1}	Jean *et al.*, 2002b

ECL, ElectroChemiLuminescence (electrochemiluminescent labelled probes); ELGA, Enzyme Linked Gel Assay; PDU, polymerase chain reaction detectable unit.

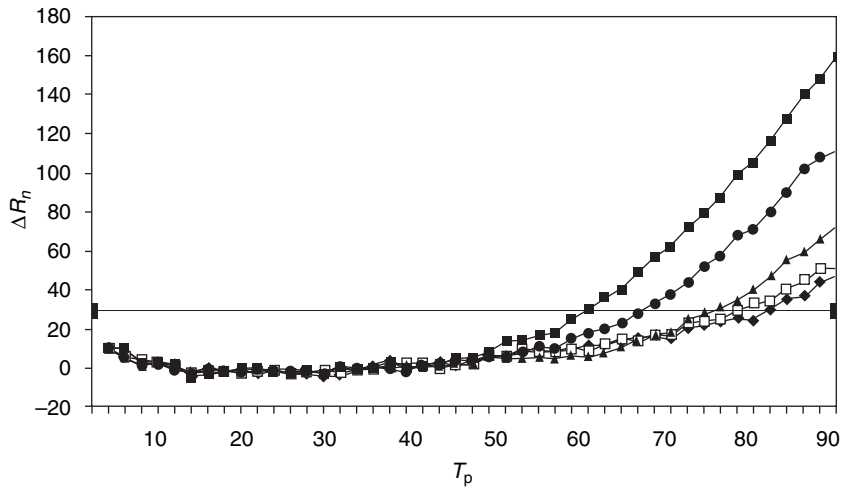

Fig. 3.4 Real-time NASBA detection and amplification of the sequences from the *dnaA* gene in the *Mycobacterium avium* subsp. *paratuberculosis* strain ATCC 19698 (type strain). Representative amplification plots are shown with nucleic acids corresponding to 10^5 (■), 10^4 (●), 10^3 (▲), 10^2 (□) and 10^1 (◆) target cells per reaction. *Y*-axis, increment of fluorescence; *X*-axis, time to positivity.

with labelled DNA or RNA from the sample. The detection systems are usually based on fluorescence and laser technology, but optical systems are also reported. Microarrays can generate fast results for several pathogens but their cost is very high and their interpretation is difficult (Lucchini *et al*., 2001). At present, this technology is mostly at the research level, however significant advances point to its use as a diagnostic tool in the future.

3.3.4 Key points in the development of molecular methods

Prior to implementation as a diagnostic tool in food microbiology, several aspects of molecular methods must be carefully considered such as the preparation of the sample, the need to assess viable cells, the compatibility with ISO methods and the need for optimisation of the conditions for each particular food matrix.

Preparation of the sample

It is well known that components of food samples, growth media and nucleic acids extraction reagents can reduce or even block amplification reactions. They are generally known as amplification inhibitors and they may cause a dramatic decrease in sensitivity of these reactions compared to pure solution of nucleic acids (Hill, 1996; Lantz *et al*., 1994, 2000; Powell *et al*., 1994; Rossen *et al*., 1992; Scheu *et al*., 1998). Consequently, sample preparation prior to the

amplification reaction is crucial for the robustness and performance of amplification-based methods. Such sample preparation should not only increase the concentration of the target organism to the practical operating range of a given assay, but in particular it should reduce or exclude amplification-inhibitory substances. Amplification inhibitors may interfere with the cell lysis, degrade or capture nucleic acids and/or inhibit the amplification reaction (Wilson, 1997). As plant and fruit samples vary in homogeneity, consistency, composition and accompanying microbiota, pre-amplification procedures should be adapted to each food matrix. A large range of pre-amplification procedures have been developed, many of them being laborious, expensive and time-consuming (Jaffe *et al*., 2001). They can either be biochemical, immunological, physical or physiological procedures (Rådstöm *et al*., 2003) (Table 3.2); or a combination of them, e.g. a pre-step with a biochemical nucleic acid extraction protocol (Chen *et al*., 1997) or with a physical pre-amplification procedure (Lantz *et al*., 1998). Of especial importance are the controls to assess the correct performance of the reaction, for example the positive internal amplification control (IAC) (Hoorfar *et al*., 2003; Rodríguez-Lázaro *et al*., 2004f) and the lack of cross-contamination (e.g. non-template control, negative sample, environment control).

Viability issue

The implementation of molecular methods is currently hampered by its still incomplete ability to distinguish between viable and dead cells, since establishing bacterial viability is an essential issue for appropriate risk management. Microbiological methods for the detection and enumeration of pathogens in food products are based on the equivalence of 'viability' and 'cultivability'; and the relationship between cells and viable counts depends on the methods used to determine cultivability. Viable cells determined by ISO techniques such as plate counting or most probable number (MPN) correspond to colony forming units, which bring about several problems, for example cells that have undergone division but not completed fission, or aggregated cells, will be considered as a unique cell. Conversely, viable but non-cultivable (VNC) cells will not be detected, which poses a serious problem especially for pathogens since loss of cultivability does not guarantee loss of pathogenicity.

The ability to detect DNA correlates well with the presence of cultivable bacteria in some situations, for example the clinical (Van der Heijden *et al*., 1999) or animal model (Wicher *et al*., 1998). However, positive amplification products only signal that the target nucleic acid is present in the sample and does not imply that the target organisms were viable. PCR has been demonstrated to detect non-viable cells (Josephson *et al*., 1993), with only the requirement that target DNA be available. DNA molecules typically have long half-lives even in dead bacterial cells (Lindahl, 1993), a property that may vary greatly and is highly dependent on the environmental conditions, although DNA can be stable even under the extreme conditions

used, for example during processing of some food products (Wiseman, 2002). Moreover PCR and especially RTi-PCR target very short amplicons (typically below 100 bp for RTi-PCR), which are more likely to persist in dead cells. Therefore, care must be taken in the interpretation of PCR data with respect to viability of microorganisms.

Physical differences, such as the integrity of cell walls and membranes, have been exploited to distinguish between living and dead cells. Nucleic acids from living cells are protected by these barriers, whereas in dead cells they are damaged and the nucleic acids are thus exposed to the environment. The differential exposure of DNA in dead cells may be utilised to degrade or inactivate the exposed nucleic acids, while the nucleic acids within living cells are protected from the treatment by the cell membrane and wall. Efforts have been made to reduce amplification of DNA from dead cells through selective degradation by treatment with externally added DNases. As an example, relatively good discrimination between viable and dead *Campylobacter jejuni* cells was achieved (Nogva *et al.*, 2000a). Recent reports focus on the use of nucleic acid-intercalating dyes such as ethidium monoazide selectively to enter dead bacteria, be covalently linked to DNA and inhibit its subsequent PCR amplification (Nogva *et al.*, 2003). This is a promising method for DNA-based differentiation between viable and dead bacteria.

RNA molecules have in average shorter half-lives than DNA. Therefore, RNA seems to be a target analyte more suitable than DNA for viability assays (Bej *et al.*, 1991). Two different types of RNA amplification techniques have been used for the detection of viable microorganisms, i.e. reverse-transcription (RT) coupled to PCR (Sheridan *et al.*, 1998; Szabo and Mackey, 1999) and nucleic acid sequence-based amplification (NASBA) (Compton, 1991) (see Table 3.4). They are becoming widely used for the detection of microorganisms in various sample types including environmental and food (Chan and Fox, 1999; Cook, 2003).

Although RNA has been considered a good indicator of cell viability (Bej *et al.*, 1991; Klein and Kuneja, 1997; Lleo *et al.*, 2000), not all RNA species are suitable for unambiguous detection of viable bacteria. Bacterial ribosomal RNA –rRNA- and transfer RNA –tRNA- have strong secondary structures and are much more stable than bacterial messenger RNA –mRNA-, which in general has a short half-life within viable bacterial cells and is rapidly degraded by specific enzymes (RNases) (Nierlich and Murakawa, 1996; Rauhut and Klug, 1999; Sela *et al.*, 1957). However, mRNAs' half-lives in *E. coli* range from 30 s to 8 min for 80% of mRNAs (Bernstein *et al.*, 2002), although certain mRNA sequences have been detected up to 30 h (Birch *et al.*, 2001) or 24 h (Min and Baeumner, 2002) post-heat killing. mRNA stability depends upon the sequence itself, to the physiological state of the bacteria prior to death (Barer and Harewood, 1999) and to the cause of death. The selection of an mRNA target should consider its pattern of expression (Barer and Harewood, 1999) and of

degradation (Alifano *et al*., 1994). Genes constitutively expressed even in stress conditions can be desired (Deiman *et al*., 2002), but also genes whose expression can be induced as a first step in the analytical protocol to ensure the detection of metabolically active bacteria (e.g. heat inducible genes, Simpkins *et al*., 2000). Moreover, the precise location of the primers within a given mRNA (e.g. the *L. monocytogenes hlyA* transcript) can have an effect on the correlation of the results of the assay and CFU: primers located internal on the amplicon, but not those located at the 3′ end of the transcript which have been shown to overestimate the cell viability as measured by CFU determination or staining with 5-sulphofluorescein diacetate (Norton and Batt, 1999).

It is of great importance in RNA detection assays to avoid genomic DNA being amplified instead, thereby producing false positive results. RT-PCR assays require the digestion of DNA by DNase treatment (Cook, 2003; Klein and Kuneja, 1997; Nogva and Lillehaug, 1999) or alternatively when possible (e.g. fungus) the primers need to be located in intron-flanking sequences. NASBA is a very promising alternative method since it has been shown to amplify mRNA selectively even in the presence of genomic DNA (Simpkins *et al*., 2000). This is due to the fact that DNA strands are not melted out at the low temperature of operation and therefore they should not become a substrate for amplification. However, an exception to this generally accepted rule has been recently reported (Rodríguez-Lázaro *et al*., 2004d). NASBA signals obtained from *Salmonella enterica* serotype t*yphimurium* and *Mycobacterium avium* subsp. *paratuberculosis* nucleic acids extracts were proven to originate from RNA and DNA, respectively, which indicates that in the latter bacterium, NASBA can detect DNA. Voisset *et al*. (2000) had previously shown that plasmid DNA sequences could also be amplified by NASBA. Therefore, the nucleic acid origin of the NASBA signal must be carefully evaluated if detection of RNA is the objective.

Compatibility of molecular and ISO methods

The routine use of molecular methods as diagnostic tools lacks appropriate validation and acceptance by international standardisation bodies such as CEN (Comité Européen de Normalisation). Work is in progress in that direction especially regarding PCR-based methods (e.g. Anonymous, 2002b); however, considerable further effort is required. Compatibility of such new tools with the existing standard methods currently implemented in routine laboratories may presumably facilitate its implementation. A general scheme can be envisaged in which standard methods are a backbone and molecular techniques that allow rapid and simple identification and/or quantification of microorganisms can be placed at different levels acting as a complementary diagnostic tool. The application of molecular assays prior to enrichment steps is the quickest method and has the potential of quantification (by means of RTi-PCR), although the sensitivity is

limited. As an example, considering standard amounts of food sample and volumes of reagents, no losses during manipulation and sensitivity of the molecular assay of one target cell, then the theoretical limit of detection would be around 1000 cells/g and the limit of quantification around 10^4 cells/g.

The use of an enrichment step prior to amplification allows the detection of lower concentrations of target microorganisms that have the capacity to grow in the standard culture conditions used (normally those recommended by ISO). Owing to the sensitivity of the molecular methods, enrichment times shorter than the ISO have been reported in some cases (Malorny *et al*., 2003). Dilution of the food matrix usually avoids inhibition of the reaction by food components, but even if some formulations have been shown not to inhibit subsequent amplification reactions (e.g. Rodríguez-Lázaro *et al*., 2004e), many broths common for plant samples are very rich in amplification inhibitors and thus nucleic acids must be extracted prior to the analysis. An additional advantage of enrichment is that it can avoid detecting dead cells (Candrian, 1995). But the accuracy of such an approach will depend upon the number of dead cells in the sample (Cook, 2003); roughly, above 1000 dead cells per gram could produce false positive results. A very interesting approach is the combination of enrichment steps with the MPN strategy, in which the most diluted cultures giving positive growth would be confirmed by molecular assays, resulting in the enumeration of the target microorganism (Jofré *et al*., 2005).

Optimisation of molecular methods: parameters to consider prior to implementation

Specificity (ISO 16140:2003) (Anonymous, 2003) is often substituted by selectivity in the context of foodborne pathogen analyses by molecular methods. A new method should be selective, that is, fully capable of detecting the target microorganism, without detection of the non-target microorganism, or inclusive and exclusive, respectively (Hoorfar and Cook, 2003). Most attention has been devoted to exclusivity, but inclusivity has often been treated only at the level of end-point amplification. This raises a potential problem that can seriously compromise the applicability of the real-time amplification techniques especially for quantification purposes, that is, the existence of inter-strain variability in the target nucleic acid sequences. While certain genes are relatively well conserved in all strains of the same species, others are not. Although sequences exhibiting a certain degree of divergence can still be end-point-detected, primers and probes anneal less efficiently for non-identical target sequences, resulting in weak signals and in consequence a decrease in the limit of detection and an underestimation of the amount of target in the sample (Rodríguez-Lázaro *et al*., 2004a). This highlights the need to assess primers and probes carefully and exhaustively, using a large and comprehensive collection of isolates representative of the biodiversity within the target species, before adopting an amplification-

based method for routine testing. It also shows that target sequences prone to genetic variability must be avoided. The frequency of spontaneous mutations in prokaryotes, aggravated by the relative abundance of hypermutator phenotypes among certain pathogenic bacteria (Bayliss and Moxon, 2002), appears therefore to be a potential limitation of RTi-PCR-based methods for the quantitative detection of bacterial pathogens in natural samples.

Perhaps one of the most challenging aspects when designing methods for the detection of foodborne pathogens is to achieve a low detection limit (LOD). This is of particular interest for species such as *L. monocytogenes* as it is often present in low numbers in food products (Donnelly, 1999; Farber and Peterkin, 1991). The LOD is expressed as the minimum level at which the target analyte can reliably be detected, with a probability that varies from 95% (ISO 16140:2003) to 99% (Knutsson *et al.*, 2002b). Therefore, ten-fold lower amounts of target genomes can be detected in around 10% of the replicates. Most published RTi-PCR methods have LOD below 10 copies of the target (Nogva *et al.*, 2000a, b; Rodríguez-Lázaro *et al.*, 2004a, b, d, g), with conventional amplification-based methods presenting in general slightly higher LOD.

The capacity of a real-time PCR method for quantification depends upon its performance and linearity (ISO 16140:2003), which can be determined by the calculation of a regression line (RTi-PCR results plotted against known initial amounts of target) using an adequate statistical method. The regression coefficient (R^2) is generally used to measure the linearity of the system. Values above 0.90–0.95 can be considered adequate and many examples of RTi-PCR systems have been published with R^2 values above 0.95 (Hough *et al.*, 2002; Nogva *et al.*, 2000a, b; Rodríguez Lázaro *et al.*, 2004a, b, g). The RTi-PCR efficiency (E) can be calculated from the same standard curve, according to the following equation $E = 10^{-1/s} - 1$ (s, slope, Klein *et al.*, 1999). In optimal conditions, E equals 1 ($s - 3.3219$). For diagnostic purposes, the practical operating range of the assay is generally considered between E values of 0.78 and 1.15. In general, RTi-PCR assays achieve quantification ranges of around 5 logarithmic units down to limits of quantification (LOQ, ISO 16140:2003) close to 100 or 10 target molecules per reaction with 95% probability. The theoretical LOQ (calculated using simulation analyses such as MonteCarlo, and considering the error associated with the particular experimental design such as volumes, pipetting, etc.) has been reported for some RTi-PCRs to match experimental LOQ (Rodríguez-Lázaro *et al.*, 2004a).

The international guideline for the validation of a microbiological alternative method (ISO 16140:2003) considers the assessment of the relative accuracy a fundamental parameter, especially for indirect assays such as those based on nucleic acids amplification. Although it has not been addressed in most RTi-PCR methods reported to date, its determination should become a mandatory prerequisite for publication in scientific jour-

nals. It is defined as the degree of correspondence between the response obtained by the reference method (usually established by ISO) and the response obtained by the alternative method on identical samples (ISO 16140:2003; Hoorfar and Cook, 2003). Relative accuracy values between 70% and 130% can be considered adequate and examples exist of RTi-PCR assays fulfilling this requirement (Hough *et al*., 2002; Nogva *et al*., 2000b; Rodríguez-Lázaro *et al*., 2004a, b, g).

The choice of the standards is crucial for accurate quantification. The development of new RTi-PCR methods requires extensive optimisation using purified target genomic DNA. Not only for Gram negative, but also for several Gram positive bacteria (e.g. Hein *et al*., 2001; Hough *et al*., 2002; Nogva *et al*., 2000b; Rodríguez-Lázaro *et al*., 2004a, b, g), various RTi-PCR systems have been shown to perform equally well when using broth- or agar-grown bacterial cultures directly as a template, facilitating its use under routine laboratory conditions. For the quantitative analysis of food samples, the ideal standard curve should be built using the same type of samples artificially contaminated with known concentrations of the target microorganism, and the same pre-PCR treatment should be applied to both, the standard curve and unknown samples. If a different standard curve is to be used (e.g. purified genomic DNA), its suitability must be demonstrated experimentally for this particular food matrix.

3.4 Traceability and subtyping of foodborne pathogens

In tracing foodborne diseases back to their sources, subtyping methods for pathogens are necessary to detect and identify routes of transmission, potential reservoirs and new or emergent strains that cause disease in humans.

The ability to discriminate or subtype pathogens below the level of species requires reliable, sensitive and informative subtyping methods. The best subtyping method should accomplish a high typability and reproducibibity (intra- and inter-laboratories), high discrimination power, be easy to perform and interpret, and have an acceptable cost. The methods available can be differentiated between those attending phenotypic or genotypic characteristics.

The most important phenotypic subtyping methods (Gargan *et al*., 1982) are biotyping, serotyping, phage typing and multilocus enzyme electrophoresis (MEE) which have been classical tools used for typing foodborne pathogens like *Salmonella*, *E. coli*, *Campylobacter*, *Shigella* and *L. monocytogenes* (Table 3.5). However, these methods are not universal and have to be adapted specifically to each pathogen. Problems caused by atypical strains that complicate the use of biotyping have been described (Sadowska *et al*., 2003). Serotyping is also complicated by the large number of antigenic types, effect of culture history and cross reactions. For example

Table 3.5 Phenotypic subtyping methods used in bacterial foodborne pathogens

Pathogen	Method	Details	Reference
E. coli	Biotyping	Vitek and API20E systems	Buckwold *et al.*, 1979
	MEE	10 polymorphic enzymnes	Pupo *et al.*, 1997
	Serotyping	O, K and H antigens	Penteado *et al.*, 2002
	Phage typing	16 bacteriophages	Grif *et al.*, 1998
Salmonella	Biotyping	Four carbon sources	Old *et al.*, 1999, 2000; Purushothaman *et al.*, 1996
	MEE	20 polymorphic enzymes	Cox *et al.*, 1996
	Serotyping	O, H, and Vi antigens	Gruenewald *et al.*, 1990
	Phage typing	10 bacteriophages	Liebana *et al.*, 2001; Liesegang *et al.*, 2002; Demczuk *et al.*, 2003; Maré *et al.*, 2001; Ward *et al.*, 1987
Shigella sonnei	Biotyping	API20E and six additional substrates	Nastasi *et al.*, 1993
	Serotyping	Several antisera	Coimbra *et al.*, 1999
	Phage typing		Bentley *et al.*, 1996
Campylobacter	Biotyping	5 test substrates	Kapperud *et al.*, 1984; Moore and Madden, 2003
	Serotyping	O antigen	Mills *et al.*, 1991
	MEE	11 polymorphic enzymes	Sails *et al.*, 2003
	Phage typing	24 phages	Patton *et al.*, 1991
L. monocytogenes	Biotyping	EN ISO11920 method	Dauphin *et al.*, 2001
	Phage typing	33 phages	McLauchlin *et al.*, 1996; Capita *et al.*, 2002
	Serotyping	O and H antigens	Schönberg *et al.*, 1996
	MEE	11 polymorphic enzymes	Harvey and Gilmour, 1994; Caugant *et al.*, 1996

in *E. coli*, 167 somatic (O antigen), 80 membrane surface (K antigen), and 56 flagellar type (H antigen) antigens have been described. Thus, careful typing may involve hundreds of antisera types in each laboratory. Phage typing also has problems of reproducibility for some pathogens owing to the loss of some receptors upon cultivation and needs careful standardisation among laboratories (McLauchlin *et al*., 1996).

The limitations of classical phenotyping methods are solved by many of the new techniques based on molecular biology methods which exhibit high

sensitivity and reproducibility, are rapid, easily performed and automated, and are able to be analysed by computer-based methods which facilitate the exchange of information.

Genotypic subtyping methods can be addressed to the whole genome or to a limited part (e.g. specific genes involved in pathogenicity). Also the methods may consist of direct analysis of these sequences or be mediated by specific amplification using PCR methods.

Among the molecular methods using the whole genome directly, restriction endonuclease analysis (REA) uses enzymes recognising high-frequency sequences (4 bp target) in total genomic DNA and subsequent separation of products by agarose gel electrophoresis. However, this method often gives a high number of bands which are complicated to analyse when working with a high number of strains. A variation of REA is macro-restriction fragment length polymorphism (MRFLP) which uses digestion of intact total DNA with restriction endonucleases of a low-frequency target (8 bp) and results in larger fragment size. Separation of fragments is performed by pulsed field electrophoresis (PFGE) in agarose gels that permit large fragment separation (often between 10 to 1000 Kbp) (Fig. 3.5). PFGE was first described by Schwartz and Cantor (1984) and a system known as contour clamped homogeneous electric field (CHEF) (Chu *et al*., 1986) led to a straight migration of fragments within the gel. MRFLP-PFGE has been used as a powerful subtyping method in molecular epidemiology of several foodborne pathogens.

Methods using the whole genome but coupled to PCR amplification are random amplified polymorphic DNA (RAPD), arbitrarily primed polymerase chain reaction (AP-PCR), analysis of repetitive DNA sequences (rep-PCR) and amplified fragment length polymorphisms (AFLP).

RAPD and AP-PCR utilise the PCR principle with a single primer (10-mer en RAPD and longer primers for AP-PCR). Owing to the lack of specificity for a given target sequence, hybridisation is performed under low stringency conditions (low temperature) (Welsh and McClelland, 1990; Williams *et al*., 1990). The method is simple, rapid and with high discrimination power, but the main problem is the low reproducibility because it is affected by DNA extraction methods and the type of equipment used for PCR. Also, fragment patterns are often complex and the intensity of bands is variable.

Rep-PCR use primers for repetitive sequences within the genome such as the enterobacterial repetitive intergenic consensus (ERIC, 38 bp), repetitive extragenic palindromic (REP, 126 bp) and box sequences (BOX, 154 pb) (Stern *et al*., 1984). In the case of ERIC and REP, two primers are used, whereas in BOX only one primer is used. Since amplified fragments comprise primer hybridisation sites distributed across the genome, a highly reproducible fingerprinting pattern is obtained. Rep-PCR is performed under higher stringency conditions than RAPD, thus the patterns generated are more reproducible.

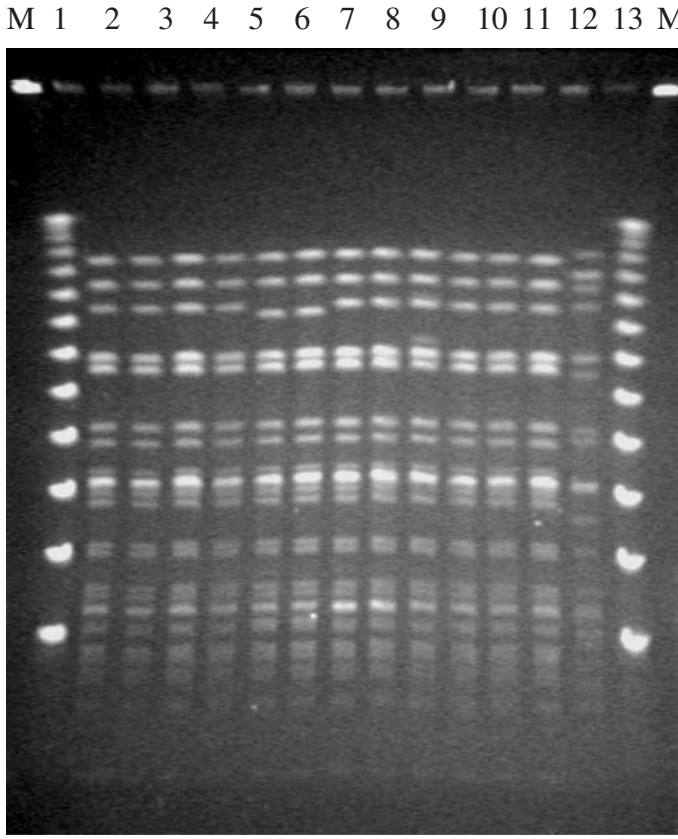

Fig. 3.5 Molecular fingerprinting of *Shigella sonnei* isolates from a diarrhoea outbreak using *Xba* I digested DNA and PFGE. Lanes: M, lambda concatamers size marker; 1–12, strains isolated from samples and diseased patients; 13, reference clinical isolate. Data from S. Méndez-Álvarez, Hospital Universitario Ntra. Sra. De Candelaria, Sta. Cruz de Tenerife, Spain.

AFLP consist of digestion of DNA by two restriction enzymes, ligation of the fragments obtained to oligonucleotide adaptors (18–22 mer) and subsequent amplification of the resulting fragments with primers specific for the adaptors. The system was first used for genomic analysis of plants but was rapidly adopted in bacterial subtyping (Janssen *et al*., 1996). The high number and complex pattern of bands is resolved by polyacrilamide gel electrophoresis. In the case of subtyping bacteria, primers are enlarged by the addition of a base to decrease the number of resulting fragments, and primers are also tagged with fluorochromes which facilitate analysis with a DNA sequencer. A variant of the technique is the ISR-PCR (infrequent restriction PCR) which uses an additional primer of 7 bp and the number

of types of fragments is considerably reduced resulting in an easier interpretation (Garaizar *et al*., 2000),

Several examples of genotypic subtyping methods using the whole genome of foodborne pathogenic bacteria are given in Table 3.6.

Genotypic subtyping methods using part of the genome include plasmid profile analysis (PPA) and hybridisation methods based on rybotyping. Plasmid profile analysis was the first molecular method used for subtyping. The method requires extraction of plasmid DNA and electrophoretic separation which can be combined with restriction analysis.

Table 3.6 Genotypic subtyping methods using the whole genome

Pathogen	Method	Details	Reference
E. coli	PFGE		Hahm *et al.*, 2003; Tutenel *et al*., 2003; Avery *et al.*, 2002; Boop *et al.*, 2002
	RAPD		Kaerkkaeinen *et al*., 1996; Tutenel *et al.*, 2003
	Rep-PCR		Hahm *et al.*, 2003
	AFLP		Hahm *et al.*, 2003
Salmonella	PFGE		Old *et al.*, 1999; Wonderling *et al.*, 2003; Fernandez *et al.*, 2003; Liebana *et al.*, 2001; Gudmundsdottir *et al.*, 2003; Biendo *et al.*, 2003
	RAPD		Soto *et al.*, 1999; Maré *et al.*, 2001; Biendo *et al.*, 2003; Shangkuan and Lin, 1998
	Rep-PCR	ERIC, BOX	Johnson and Clabots, 2000; Johnson *et al.*, 2001
	AFLP		Aarts *et al.*, 1998; Lan *et al.*, 2003; Hu *et al.*, 2002
Shigella sonnei	PFGE		Liu *et al.*, 1995; Navia *et al.*, 1999; DeLappe *et al.*, 2003
	Rep-PCR	ERIC, REP	Liu *et al*., 1995; Navia *et al.*, 1999
L. monocytogenes	PFGE		La-Scola *et al.*, 1997; Giovannacci *et al.*, 1999; Dauphin *et al.*, 2001; Lukinmaa *et al.*, 2003; Wagner and Allenberg, 2003
	RAPD	5 different primers	Kerr *et al.*, 1995; Aguado *et al.*, 2001; Giovannacci *et al.*, 1999
	Rep-PCR	ERIC, REP	Jersek *et al*., 1999
	AFLP		Guerra *et al.*, 2002
Campylobacter	PFGE		Duim *et al.*, 2003
	RAPD		Carvalho *et al.*, 2001
	AFLP		Moreno *et al.*, 2002; Duim *et al.*, 1999, 2003

Thus, the presence or absence of a given plasmid or plasmid modifications can be studied.

Hybridisation techniques are based in a first step in REA, followed by a second stage of hybridisation with known probes (Southern blot). The most popular technique consists of rybotyping which uses the genes for rRNA synthesis as a target. A large number of copies of rDNA operons exist in bacteria that are highly conserved. Thus, to detect variability after REA analysis, fragment bands are transferred to a membrane and hybridised with specific probes for the rDNA genes (16 and 23 S rDNA). Other probes can be used, such as for the insertion sequences IS2000, which have been used for *Salmonella* or specific probes for toxins in *E. coli*.

Genotypic subtyping methods using part of the genome with PCR-based techniques consist of analysis of polymorphic genes (PCR-RFLP), single strand conformation polymorphism typing (SSCP), comparative DNA sequencing-based subtyping (CSBS), multiplex-PCR, multilocus sequence typing (MLST) and multilocus variable number tandem repeat analysis (MLVA).

PCR-RFLP consists of amplification of the target gene sequence, digestion with restriction enzymes and separation by agarose gel electrophoresis. However, for this type of subtyping it is necessary to know, at least partially, the genome of the bacteria, because the genes should be genetically stable (low frequency of recombination, deletion, mutation). The results depend on the gene selected and the restriction enzymes used. To increase information obtained, several genes and restriction enzymes can be used individually and the information analysed globally, thus increasing the capacity for discrimination.

SSCP consists of analysing the polymorphisms in the conformation of single strand DNA fragments obtained upon PCR amplification, denaturation and separation by polyacrilamide gel electrophoresis. The single strand fragment DNA mobility will depend upon nucleotide changes and conformation. The advantage of the method is that it permits detection of variability at the nucleotide level within the whole fragment amplified, whereas in RFLP analysis differences are detected only at the target site of the restriction enzyme. In the above mentioned methods, the objective is to detect changes of nucleotides in the genes analysed. However, owing to the development of automatic sequencers, comparative DNA sequencing-based subtyping can be easily performed.

Multiplex-PCR permits the comparison of DNA sequences from variable regions in several genes and is often used in molecular epidemiology. Virulence genes have been the main targets in *Listeria*, *Salmonella* and *E. coli*. The simultaneous amplification of several genes, generally associated with the virulence of the pathogen or related to proteins involved in antigens used for serotyping, is known as multiplex-PCR. This method has given good results for subtyping *E. coli* and *Listeria*. Recently, this technique advanced greatly with the development of DNA microarrays (Borucki

et al., 2003; Borucki and Call, 2003; Call *et al*., 2003). The specific design of microarrays, thanks to the knowledge of new virulence genes, is of great interest for the study of emerging pathotypes (Bekal *et al*., 2003). A variant of the multiplex-PCR is multilocus sequence typing (MLST) (Enright and Spratt, 1999), which is analogous to the MLEE, but instead of using the electrophoretic mobility of the gene products (proteins), is based on the sequencing of seven housekeeping genes. Owing to the increasing number of bacterial genomes sequenced, several new repeated sequences within the genome have been described that can be used. These sequences are the basis of multilocus variable number tandem repeat analysis (MLVA) (Noller *et al*., 2003a, b; Schouls *et al*., 2003).

Several examples of genotypic subtyping methods using part of the genome of foodborne pathogenic bacteria are given in Table 3.7.

In spite of the great number of methods available, the main problem of subtyping is the comparison of results among different laboratories. Thus, validation and interlaboratory trials are necessary for the standardisation of the methodology, before the recognition of new emergent subtypes at regional or world level.

Several efforts have been addressed with this objective and one of the most intensive collaborative studies deals with evaluation of subtyping using phenotypic and genotypic approaches for *L. monocytogenes* (Bille and Rocout, 1996).

In the case of MRFLP-PFGE, one of the methods most widely employed because of its high reproducibility and discrimination power, the National Molecular Subtyping Network for foodborne diseases surveillance (PulseNet) was established in 1996 in the USA. PulseNet (www.cdc.gov/pulsenet) uses MRFLP-PFGR for subtyping of *Salmonella, L. monocytogenes, E. coli* O157:H7, *Shigella* and other foodborne pathogens, and is a platform for interchange of DNA fingerprintings between laboratories.

Another approach used, thanks to the development of sequencing methods, is MLST. A laboratory network has been created that permits online introduction and interchange of sequences (www.mst.net).

Another key problem in strain subtyping for molecular epidemiology is interpretation of results. Because of the high resolution of the technique used, small changes can be detected that can be caused either by changes in the pathogen strain during the outbreak development itself or by strain subcultivation changes in the laboratory. For example, in spite of the general stability of MRFLP-PFGE patterns, in some cases random genetic events (insertions, deletions, mutations) can alter patterns in one or more bands within strains isolated from the same outbreak (Fitzgerald and Swaminathan, 2003). Thus, selection of molecular methods for traceability studies should be performed upon accurate analysis of the rate of change of the 'molecular clock' selected. Also, owing to the well-established and abundant knowledge of strain serotyping in many foodborne pathogens, in

Table 3.7 Genotypic subtyping methods using part of the genome

Pathogen	Method	Details	Reference
E. coli	Plasmid profiling		Abimbola *et al.*, 1993
	Ribotyping		Machado *et al.*, 1998; Hahm *et al.*, 2003; Avery *et al.*, 2002
	RFLP-genes	*rfb*, *fli*C	Coimbra *et al.*, 2000; Machado *et al.*, 2000; Prager *et al.*, 2003
	PCR multiplex	*ef*, *saa*, *fyu*A, *iha*, *esp*I, *btu*B, *stx*, *eae*, *irp2*, *stx2*, *stx1*, *hly*	Friedrich *et al.*, 2003; Hahm *et al.*, 2003; Penteado *et al.*, 2002; Normanno *et al.*, 2004
	MLST	7 housekeeping genes, 2 membrane protein genes	Noller *et al.*, 2003b
Salmonella	Plasmid profiling		Purushothaman *et al.*, 1996; Maré *et al.*, 2001; Liebana *et al.*, 2001
	Ribotyping	Standar, IS2000	Esteban *et al.*, 1993; Bailey *et al.*, 2002; Oscar 1998; Old *et al.*, 1999; Clark *et al.*, 2003; Old *et al.*, 2000; Chichton *et al.*, 1998; Old *et al.*, 1999 Ezquerra *et al.*, 1993
	RFLP-genes	ITS *r*DNA	Jensen and Hubner, 1996
	MLST	16S *r*DNA, *pdu*F, *man*B	Kotetishvili *et al.*, 2002
	SSCP		Nair *et al.*, 2002
L. monocytogenes	Ribotyping	*gro*EL	Nadon *et al.*, 2001
	RFLP-genes	*inl*A, *inl*B	Giovannacci *et al.*, 1999
	PCR multiplex	Division specific multiplex primers	Borucki and Call, 2003
	MLST		Ericsson *et al.*, 2000; Cai *et al.*, 2002; Revazishvili *et al.*, 2004
Shigella sonnei	Plasmid profiling		Liu *et al.*, 1995; Navia *et al.*, 1999
	Ribotyping		Nastasi *et al.*, 1993; Liu *et al.*, 1995
	RFLP-genes	*rfb*	Coimbra *et al.*, 1999
Campylobacter	Ribotyping		Duim *et al.*, 2003
	RFLP-genes	23S rDNA, *iam*, *wla*	Payne *et al.*, 1999; Moreno *et al.*, 2002; Carvalho *et al.*, 2001; Shi *et al.*, 2002
	SSCP	*fla*	Duim *et al.*, 2003; Hein *et al.*, 2003
	MLST		Duim *et al.*, 2003; Schouls *et al.*, 2003

order to be validated molecular methods have to be correlated with serotyping.

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4

Pesticide residues in fruit and vegetables

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4.1 Introduction

Pesticides are used extensively throughout the world to improve agricultural production by reducing pest populations such as insects, weeds and plant diseases. While the inherent toxicological properties of pesticides allow them to control pests in agriculture, there is significant concern about the potential risks posed by pesticides to the environment, non-target organisms and to humans who may be inadvertently exposed to them.

A significant public debate concerning pesticide residues in foods emerged in the late 20th century and continues today. The debate has been sparked by several widely publicized events that have focused consumer, regulatory and media attention upon the issue. Illegal applications of a highly toxic insecticide to California watermelons resulted in more than 1000 cases of probable or possible human poisoning throughout the western United States and Canada in 1985 (Goldman *et al.*, 1990). A landmark publication of the US National Research Council (NRC) followed in 1987; this report presented exaggerated estimates of potential human cancer risks from pesticides in the diet based upon unreasonable assumptions of human exposure (NRC, 1987). A 1989 report from an environmental advocacy organization claimed that children face 'intolerable' risks from exposure to residues of neurotoxic and cancer-causing pesticide residues in foods (Natural Resources Defense Council, 1989). A subsequent NRC report concluded that the US pesticide regulatory system did not adequately address the potential differences in susceptibility or in exposure to pesticides of infants and children relative to adults (NRC, 1993). This report recommended significant changes in pesticide regulation and risk assessment

practices; most of its recommendations were adopted following the passage of the Food Quality Protection Act (FQPA) by the US Congress in 1996.

Consumer concern regarding pesticide residues in foods has been responsible, in part, for the increased demand in foods certified to be 'organic.' US national standards for organic foods were developed in 2000 and have ensured greater uniformity among organic food production practices. The standards prohibit the use of most synthetic pesticides although natural pesticides derived from sources such as minerals, plants and microbes are permitted.

While pesticides may leave residues in a variety of foods including meats, poultry, dairy products and grains, this review focuses primarily upon pesticide residues in fruit and vegetables. This review will consider the types and amounts of pesticides used, pesticide regulation, residue monitoring and risk assessment.

4.2 Pesticide use

The term 'pesticide' is used to describe any chemical agent that controls pests. The major classes of pesticides used in fruit and vegetable production are insecticides, herbicides and fungicides, although other types of pesticides such as molluscicides, bacteriocides, nematicides, acaricides, plant growth regulators and pheromones are also frequently used.

Estimates of world and US pesticide expenditures in the years 2000 and 2001 are provided in Table 4.1 (Kiely *et al*., 2004). Total world expenditures were US$32.5 billion in 2000 and nearly $32.0 billion in 2001 while US expenditures were US$11.2 billion (34% of world expenditures) in 2000 and US$11.1 billion (35% of world expenditures) in 2001. Herbicides accounted for the highest percentages of worldwide (44%) and US pesticide expenditures (57–58%), followed by insecticides (28% worldwide and 28% in the USA) and fungicides (19% worldwide, 8% in the USA).

Pesticides are frequently used in industrial, commercial, government, and home and garden settings in addition to their use in agriculture. Table 4.2 shows US expenditure among various market sectors for the years 2000 and 2001. The agricultural use of herbicides and plant growth regulators represented 79% of their use in 2000 and 78% in 2001. Insecticides and miticides used in agriculture constituted 45% and 42%, respectively, of total expenditure in 2000 and 2001. The agricultural use of fungicides represented 75% of total expenditure in 2000 and 74% in 2001.

The amounts of the various types of pesticides used in US agriculture in 2000 and in 2001 are shown in Figs 4.1 and 4.2. A total of 722 million pounds (lb) (328 million kg) of pesticide active ingredients was estimated to be used in 2000, including 432 million lb (196 million kg) of herbicides and plant growth regulators, 90 million lb (41 million kg) of insecticides and miticides and 44 million lb (20 million kg) of fungicides. Estimated pesticide us

Table 4.1 World and US pesticide expenditure at user level by pesticide type, 2000 and 2001 estimates

Year Type	World market		US market		US percentage of world market
	US$ (million)	%	US$ (million)	%	
2000					
Herbicides[1]	14319	44	6365	57	44
Insecticides[2]	9102	28	3129	28	34
Fungicides[2]	6384	19	860	8	13
Other[3]	2964	9	811	7	27
Total	32769	100	11165	100	34
2001					
Herbicides[1]	14118	44	6410	58	45
Insecticides[2]	8763	28	3124	28	36
Fungicides[2]	6027	19	835	8	14
Other[3]	2848	9	721	7	25
Total	31756	100	11090	100	35

Note: Totals may not add up owing to rounding. Table does not cover wood preservatives, speciality biocides and chlorine/hypochlorites.
Source: EPA estimates based on Cropfile America annual surveys. Cropnosis Limited data and EPA proprietary data.
[1] Herbicides include herbicides and plant growth regulators.
[2] Insecticides and fungicides exclude sulfur and petroleum oil.
[3] Other includes nematicides, fumigants, rodenticides, molluscicides, aquatic and fish/bird pesticides, other miscellaneous conventional pesticides, plus other chemicals used as pesticides (e.g. sulfur and petroleum oil).
Source: Kiely *et al*., 2004.

declined in 2001 to 675 million lb (306 million kg), including 433 million lb (197 million kg) of herbicides and plant growth regulators, 73 million lb (33 million kg) of insecticides and miticides and 42 million lb (19 million kg) of fungicides. Another major class of pesticides, nematicides and fumigants, accounted for an estimated 131 million lb (60 million kg) of active ingredient used in 2000 and 102 million lb (46 million kg) used in 2001.

4.3 Pesticide residue regulation

4.3.1 US regulation

While it is clear that many types of pesticides are frequently used in agriculture, the use of a pesticide does not automatically result in a food residue. Pesticides such as fumigants and nematicides are commonly volatile chemicals injected into the soil prior to planting of a food crop to sterilize the soil and disappear from the site of injection long before the food is produced. Many herbicides are used on a 'pre-plant' basis to inhibit

Table 4.2 User expenditure on pesticides in the USA by pesticide type and market sector, 2000 and 2001 estimates

Year Market sector	Herbicides / plant growth regulators		Insecticides / miticides		Fungicides		Other[a]		Total	
	US$ (million)	%	US$ (million)	%	US$ (million)	%	US$ (million)	%	US$ (million)	%
2000										
Agriculture	5007	79	1411	45	647	75	547	67	7612	68
Ind/comm/gov	762	12	468	15	172	20	83	10	1485	13
Home & garden	596	9	1250	40	41	5	181	22	2068	19
Total	6365	100	3129	100	860	100	811	100	11165	100
2001										
Agriculture	4987	78	1326	42	615	74	476	66	7404	67
Ind/comm/gov	792	12	510	16	172	21	61	8	1535	14
Home & garden	631	10	1288	41	48	6	184	26	2151	19
Total	6410	100	3124	100	835	100	721	100	11090	100

Note: Totals may not add up owing to rounding. Table does not cover industrial wood preservatives, speciality biocides and chlorine/hypochlorites.
Source: EPA estimates based on Croplife America annual surveys and EPA proprietary data.
[a] Other includes nematicides, fumigants, rodenticides, molluscicides, aquatic and fish/bird pesticides, other miscellaneous conventional pesticides, plus other chemicals used as pesticides (e.g. sulfur and petroleum oil).
Source: Kiely *et al*., 2004.

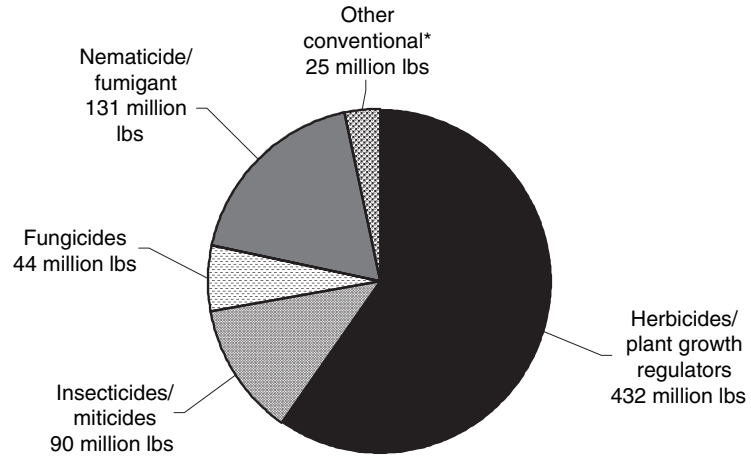

Fig. 4.1 US agricultural use of pesticides, 2000. (*Source*: Kiely *et al*., 2004.)

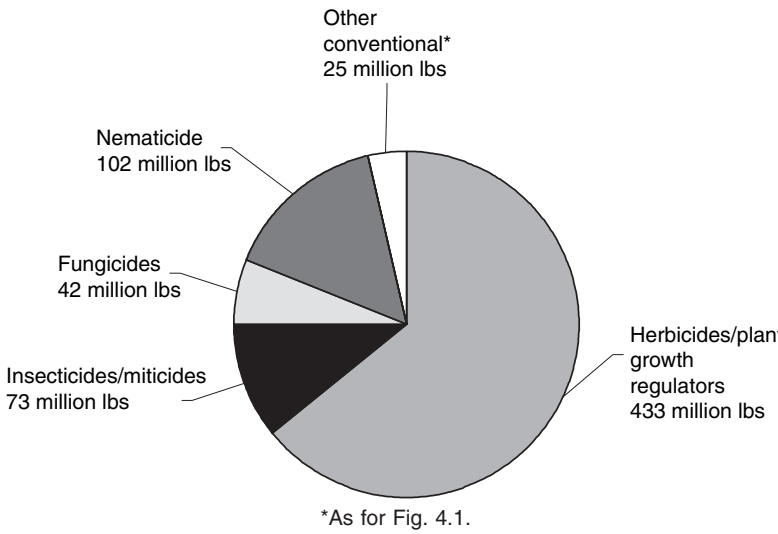

Fig. 4.2 US agricultural use of pesticides, 2001. (*Source*: Kiely *et al*., 2004.)

germination of weed seeds before crops are planted while others are contact herbicides that might kill the food crop if the herbicide were inadvertently applied to the food. In many other cases, pesticides may chemically degrade, volatilize, and/or run off in water before the food is harvested, thus eliminating the potential to leave a residue on the food.

In cases where the normal use of a pesticide on a food crop poses the potential to leave a food residue, the US Environmental Protection Agency

(EPA) will typically establish a tolerance, representing the maximum permitted residue level (Winter, 1992a). US tolerances are pesticide and commodity specific; the same pesticide may have different tolerances established on different commodities while the same commodity may permit different levels of specific pesticides. The practices used by EPA to establish pesticide tolerances are confusing and frequently misunderstood. Readers interested in a more detailed discussion of the topic are urged to consult a comprehensive summary (Winter, 1992a).

Briefly, tolerances exist as enforcement tools to ensure that pesticide applications are made in accordance with regulations. Contrary to popular belief, they are not specifically considered to be indicators of safety. Tolerances represent the maximum expected levels of pesticides on food crops resulting from the legal application of a pesticide. The maximum expected levels are based upon the findings from controlled field studies performed by the pesticide's manufacturer under conditions chosen to produce the highest legal residue levels. Such conditions include applying the pesticide at the maximum allowable rate, harvesting the commodity at the shortest interval following application and making the maximum number of applications during the growing season.

Once the highest expected residues are determined, the manufacturer petitions the EPA to set a tolerance at, or slightly higher than, this level. As a result, it is anticipated that pesticide applications made legally should not result in residues approaching the tolerance level and that only when pesticides are misused would tolerance levels be exceeded. Pesticide residues are considered to be illegal in cases where the tolerance level is exceeded and also when pesticides are detected on food crops for which they do not have tolerances established owing to pesticide application to the wrong crop, drift from adjacent crops or uptake from contaminated soil.

Before granting a pesticide tolerance, the EPA will consider the potential consumer risks from exposure to the pesticide. If the EPA determines that it cannot demonstrate a 'reasonable certainty of no harm' from all registered and proposed uses of the pesticide, according to provisions of FQPA, it will not approve a tolerance at any level. If the risk assessment concludes that there is a 'reasonable certainty of no harm', the EPA will approve the tolerance as described above.

Prior to the passage of FQPA in 1996, the EPA allowed tolerances to be established on a chemical-by-chemical basis and considered only consumer exposure from pesticide residues in food. The FQPA broadened the scope of the EPA's risk assessment practices by requiring the EPA to consider the aggregate exposure from a pesticide in food, drinking water and residential settings. Additionally, the EPA must also consider the cumulative risks from families of toxicologically related pesticides rather than the risks from individual pesticides within a pesticide family.

An important provision of FQPA is the requirement that the EPA consider the potential increased susceptibility of infants and children to pesti-

cides. In cases where toxicological data comparing the sensitivity of infants and children to adults are absent, the EPA may determine that infants and children are more sensitive and establish a lower acceptable level of exposure for infants and children. This level may be as much as ten times lower than a comparable acceptable exposure level for adults.

4.3.2 International regulation

All world nations possess the sovereign right to establish their own acceptable levels for pesticide residues in foods. Because many nations lack the resources to develop their own pesticide regulatory programs, the majority of the world's countries rely upon a set of international standards developed by the Codex Alimentarius Commission, commonly referred to as Codex. Codex 'maximum residue levels' (MRLs) are analogous to the US 'tolerances' and represent the maximum legal pesticide residues permitted on specific commodities. Codex MRLs, like US tolerances, primarily serve as enforcement tools to determine whether pesticide applications are made according to established directions.

Codex MRLs and US tolerances are similar in many cases but differ in others. In cases where the US tolerances and Codex MRLs can be directly compared, 47% are equivalent, 34% of Codex MRLs were lower (more restrictive) than the US tolerances and 19% of Codex MRLs exceeded US tolerances (General Accounting Office, 1991). Some of the differences may be traced to the use of different data sets and/or different methods to regulate pesticide breakdown productions by US and Codex officials. Agricultural production and pest management practices may also differ, leading to differences in the maximum expected residues following pesticide application.

4.4 Pesticide residue monitoring in fruit and vegetables

While the EPA is responsible for registering pesticides, determining acceptable application practices and establishing tolerances, it is not directly involved in the monitoring of pesticide residues. At the federal level, the two primary US agencies involved in pesticide residue monitoring are the US Food and Drug Administration (FDA) and the US Department of Agriculture (USDA). Several states are also involved in pesticide residue monitoring; those with the largest programs are California, Texas and Florida.

4.4.1 FDA pesticide residue monitoring

FDA regulatory monitoring program

The FDA conducts its regulatory monitoring program to enforce the tolerances established by the EPA. Sampling includes imported and domestic

foods. Imported foods are sampled at the point of entry into US commerce, while domestic samples are collected near the point of production in the distribution system. Sampling primarily involves raw agricultural products that are analyzed without washing or peeling although some processed foods are also included (FDA, 2004).

Illegal residues result when pesticides are detected at levels that exceed the established tolerance for the pesticide on the specific commodity and in cases where the pesticide is detected on a commodity for which a tolerance has not been established. For domestic samples containing illegal residues, the FDA can invoke sanctions such as seizure or injunction. For imported samples that violate the prescribed tolerance ('violative'), shipments may be stopped at the port of entry.

Sampling in the FDA's regulatory monitoring program is not devised to be representative of the food supply in general but is designed to maximize the chances of identifying illegal residues. The FDA considers factors such as state and FDA generated residue data, regional intelligence on pesticide use, dietary importance of the food, information on the amount of domestic and imported food that enters interstate commerce, chemical characteristics and toxicity of the pesticide, and production volume/pesticide usage patterns in planning the types and numbers of samples to collect.

The FDA uses multiresidue analytical methods capable of simultaneously determining about half of the approximately 400 pesticides with EPA tolerances, as well as other pesticides with no tolerances, and many metabolites, impurities and alteration products of pesticides. In some cases, the FDA will use single or selective analytical methods to look specifically for an individual pesticide or a small number of chemically related pesticides that may not normally be detected using multiresidue methods.

The FDA typically attains detection limits well below tolerance levels, which generally range from 0.1–50 ppm (parts per million). Methods are usually capable of measuring residues at the 0.01 ppm level with a range of 0.005–1 ppm.

In 2002, the FDA analyzed 6766 samples in its regulatory monitoring program. Samples included 2122 domestic samples (31.3% of the total samples) and 4644 imported samples (68.7% of the total samples). Sampling categories included grains and grain products, milk/dairy products/eggs, fish/shellfish/other aquatic products, fruit, vegetables and 'other'.

The FDA analyzed 725 domestic and 1408 imported fruit samples in 2002. More than two-thirds (67.1%) of the imported fruit samples showed no detectable pesticide residues, while nearly half (49.5%) of the domestic fruit samples showed no detectable residues. Imported fruit samples showed a higher rate of 'violative' residues (3.2%) than did domestic samples (0.7%). For imported fruit, 46 samples were found to be in violation including two samples for which the tolerance was exceeded and the remaining 44 contained residues on crops for which a tolerance was not

established. Five domestic fruit samples were determined to be 'violative'; in all five cases, the violation resulted from the detection of a pesticide not registered for use on the commodity on which it was detected.

The FDA analyzed 895 domestic and 2546 imported vegetable samples in 2002. In contrast with the fruit samples, imported vegetable samples had a lower incidence of no residues detected (66.9%) than did the domestic samples (72.5%). 'Violative' residues were higher in the imported vegetable samples (5.4%) than in the domestic vegetable samples (0.8%). A total of 138 'violative' vegetable samples were found, including 10 for which the tolerance was exceeded and 128 where residues were detected without established tolerances. Domestically, 16 vegetable samples were 'violative' and all resulted from the finding of pesticides that did not have tolerances established on the vegetables.

Commodity specific findings for FDA regulatory monitoring of fruit are provided in Table 4.3 while findings for vegetables are given in Table 4.4.

FDA total diet study

In addition to its regulatory monitoring program, the FDA also annually conducts the Total Diet Study. In this study, FDA inspectors purchase market baskets of more than 250 food items. Foods are prepared in ready-to-eat form and subsequently analyzed for residues. The data generated in the Total Diet Study is used to develop crude estimates of human dietary exposure to pesticides and other contaminants. By multiplying residue levels by estimates of consumption for each food item, it is possible to develop daily dietary exposure levels. Unfortunately, the FDA no longer provides its dietary exposure estimates when reporting Total Diet Study findings. In 1991 (the last year in which such dietary exposure estimates were reported), exposure to most pesticides from all foods (including fruits and vegetables) typically represented less than 1% of the amounts the EPA deemed acceptable on a daily basis (FDA, 1992).

The most commonly identified pesticides in the 2002 Total Diet Study are provided in Table 4.5. Several of these pesticides, including DDT, dieldrin and toxaphene, are no longer allowed for use in the USA but probably result owing to their significant environmental persistence that makes them available for uptake into fruits, vegetables and animal feeds.

4.4.2 USDA pesticide data program

Since 1991, the USDA has operated the Pesticide Data Program (PDP) to collect data on pesticide residues in foods. In contrast to the FDA's regulatory monitoring program, which is designed primarily to enforce pesticide tolerances, the PDP's primary focus has been to provide the EPA with data that can be used to assist in the determination of human exposures and risks from pesticide residues (USDA, 2004).

Table 4.3 Results of FDA's 2002 regulatory monitoring program – fruit

	Domestic					Imported				
	Total samples	Samples without residues (%)	Samples violative[a] (%)	# Over tolerance	# No tolerance	Total samples	Samples without residues (%)	Samples violative[a] (%)	# Over tolerance	# No tolerance
Blackberries	4	75.0	0.0			27	51.9	0.0		
Blueberries	27	88.9	0.0			16	68.8	0.0		
Cranberries	5	60.0	0.0			4	100.0	0.0		
Grapes, raisins	20	55.0	0.0			80	48.8	2.5		2
Raspberries	15	13.3	0.0			39	46.1	2.6		1
Strawberries	55	43.6	0.0			122	32.0	9.0		11
Other berries	12	16.7	0.0			2	100.0	0.0		
Clementines	–	–	–			16	0.0	0.0		
Grapefruit	10	60.0	0.0			1	100.0	0.0		
Lemons	8	75.0	0.0			10	70.0	0.0		
Limes	–	–	–			11	100.0	0.0		
Oranges	65	66.2	0.0			61	47.5	1.6		1
Other citrus fruit	6	50.0	0.0			4	100.0	0.0		
Apples	167	32.3	0.0			38	63.2	2.6		1
Pears	40	60.0	0.0			45	68.9	4.4		2
Other pome fruit	2	50.0	0.0			2	50.0	50.0		1
Apricots	8	62.5	0.0			1	100.0	0.0		
Avocados	3	100.0	0.0			35	100.0	0.0		
Cherries	32	21.9	6.2		2	13	53.9	7.7		1
Dates	–	–	–			6	83.3	16.7		1
Nectarines	12	58.3	8.3		1	7	28.6	0.0		
Olives	–	–	–			45	75.6	4.4		2

Peaches	96	34.4	2.1		2	43	62.8	2.3	1	
Plums	10	40.0	0.0			10	70.0	0.0		
Other pit fruit	2	100.0	0.0			4	100.0	0.0		
Bananas, plantains	–	–	–			93	48.4	0.0		
Guava	–	–	–			4	100.0	0.0		
Kiwi fruit	–	–	–			18	77.8	5.6	1	
Mangoes	2	100.0	0.0			50	92.0	2.0		2
Papaya	–	–	–			73	76.7	5.5		4
Pineapple	2	100.0	0.0			59	83.0	0.0		
Other sub-tropical fruit	1	100.0	0.0			50	80.0	12.0		6
Bitter melon	–	–	–			11	45.5	0.0		
Cantaloupe	22	77.3	0.0			49	38.8	0.0		
Honeydew	2	50.0	0.0			32	46.9	0.0		
Watermelon	23	78.3	0.0			21	61.9	9.5		2
Other melons	11	81.8	0.0			2	100.0	0.0		
Other fruits	–	–	–			6	100.0	0.0		
Apple juice	27	81.5	0.0			65	92.3	0.0		
Citrus juice	4	100.0	0.0			8	100.0	0.0		
Other fruit juices	12	91.7	0.0			77	97.4	1.3		1
Fruit jams/jellies/ pastes/toppings	20	25.0	0.0			148	88.5	4.0		3
Totals	725	49.5	0.7	0	5	1408	67.1	3.2	2	41

[a] Includes samples with residues over tolerance or action level and samples with residues with no tolerance.
Adapted from FDA (2004).

Table 4.4 Results of FDA's 2002 regulatory monitoring program – vegetables

	DOMESTIC					IMPORTED				
	Total samples	Samples without residues (%)	Samples violative[a] (%)	# Over tolerance	# No tolerance	Total samples	Samples without residues (%)	Samples violative[a] (%)	# Over tolerance	# No tolerance
Corn	30	93.3	0.0		4	36	97.2	0.0		
Mung beans & bean sprouts	–	–	–			12	83.3	0.0		
Peas (green/snow/sugar/sweet)	34	94.1	5.9		3	69	58.0	18.8	1	16
String beans (green/snap/pole/long)	82	59.8	1.2		1	78	61.5	9.0	1	6
Other bean & peas & products	51	90.2	2.0			138	75.4	3.6		
Cucumbers	33	75.8	0.0			97	37.1	1.0	1	
Eggplant	3	100.0	0.0			35	80.0	0.0		
Okra	2	100.0	0.0			19	94.7	5.3		1
Peppers, hot	1	0.0	0.0			291	45.0	9.3	2	26
Peppers, sweet	14	78.6	0.0			294	57.8	7.1	2	18
Squash/pumpkins	40	85.0	0.0			195	40.0	5.1		10
Tomatoes	84	70.2	0.0			229	66.4	3.9		9
Other fruiting vegetables	2	100.0	0.0			51	78.4	2.0		1
Artichokes	–	–	–			13	100.0	0.0		
Asparagus	18	100.0	0.0			77	89.6	0.0		
Bamboo shoots	–	–	–			14	92.9	7.1		1
Bok choy & Chinese cabbage	3	100.0	0.0			9	77.8	0.0		
Broccoli	28	92.9	0.0			52	80.8	0.0		
Cabbage	40	80.0	0.0			22	72.7	0.0		
Cauliflower	5	80.0	0.0			12	100.0	0.0		
Celery	9	22.2	22.2		2	13	38.5	7.7	1	

Collards	5	80.0	0.0		1	0.0	0.0		
Endive	2	0.0	50.0	1	11	100.0	0.0		
Kale	3	66.7	0.0		13	30.8	7.7		1
Lettuce, head	19	57.9	0.0		27	66.7	3.7	1	
Lettuce, leaf	19	79.0	0.0		13	76.9	0.0		
Mustard greens	–	–	–		2	100.0	0.0		
Radicchio	–	–	–		6	83.3	0.0		
Spinach	12	41.7	0.0		24	58.3	12.5		3
Other leaf & stem vegetables	6	83.3	0.0		123	72.4	16.3		21
Mushrooms & truffles	3	100.0	0.0		43	93.0	4.7		2
Carrots	49	67.3	0.0		48	75.0	2.1		1
Cassava	–	–	–		16	100.0	0.0		
Onions/leeks/ scallions/shallots	65	96.9	0.0		175	78.9	1.1		2
Potatoes	124	37.9	0.0		33	87.9	0.0		
Radishes	6	50.0	0.0		37	91.9	2.7		1
Red beets	6	83.3	0.0		9	88.9	11.1		1
Sweet potatoes	24	91.7	0.0		24	91.7	4.2		1
Turnips	7	85.7	0.0		6	83.3	0.0		
Water chestnuts	–	–	–		16	100.0	0.0		
Other root & tuber vegetables	10	60.0	0.0		24	91.7	8.3		2
Vegetables with sauce	–	–	–		3	100.0	0.0		
Vegetables, dried or paste	27	88.9	0.0		85	83.5	7.1	1	5
Other vegetables/ vegetable products	29	65.5	0.0		51	86.3	0.0		
Totals	895	72.5	0.8	11	2546	66.9	5.4	10	128

[a] Includes samples with residues over tolerance or action level and samples with residues with no tolerance.
Source: Adapted from FDA (2004).

Table 4.5 Frequency of occurrence of pesticide residues found in Total Diet Study foods in 2002[a]

Pesticide[b]	Total no. of findings	Occurrence (%)	Range (ppm)
DDT	212	21	0.0001–0.025
Chlorpyrifos-methyl	175	17	0.0002–0.059
Malathion	156	15	0.0007–0.071
Endosulfan	142	14	0.0001–0.166
Dieldrin	115	11	0.0001–0.010
Chlorpropham	62	6	0.0007–1.278
Chlorpyrifos	49	5	0.0001–0.105
Permethrin	43	4	0.0004–1.680
Carbaryl[c]	42	4	0.001–2.040
Dicloran	33	3	0.0002–0.263
Thiabendazole[d]	31	3	0.013–0.991
Lindane	20	2	0.0001–0.002
Methamidophos	19	2	0.001–0.345
Hexachlorobenzene	19	2	0.0001–0.002
Dicofol	19	2	0.002–0.538
Pirimiphos-methyl	17	2	0.001–0.024
Quintozene	17	2	0.0001–0.0424
Toxaphene	17	2	0.002–0.028
Acephate	16	2	0.002–0.350
Ethion	16	2	0.0003–0.007

[a] Based on four market baskets analyzed consisting of 1030 total items.
[b] Isomers, metabolites and related compounds are included with the 'parent' pesticide from which they arise.
[c] Reflects overall incidence; however, only 93 selected foods per market basket (i.e. 372 items total) were analyzed for *N*-methylcarbamates.
[d] Reflects overall incidence; however, only 67 selected foods per market basket (i.e. 268 items total) were analyzed for the benzimidazole fungicides.
Source: FDA, 2004.

The USDA does not participate in the collection or analysis of the residue samples but works closely with participating states that perform these functions. In 2002, participating states included California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington and Wisconsin. Sampling in the PDP program is designed to allow residue data to be reliable for use in pesticide residue exposure assessments. The choice of pesticides and commodities sampled each year varies and is governed by EPA data needs and on information about the types and amounts of food consumed by infants and children.

In 2002, the PDP program was responsible for the analysis of 12 899 food samples, including 10 056 fresh and processed fruit and vegetable samples. Samples included domestic and imported foods. Fruit and vegetable samples analyzed in this program in 2002 were apple juice, apple sauce, canned and frozen sweet peas, sweet corn, fresh apples, asparagus, bananas,

broccoli, carrots, celery, cucumbers, mushrooms, onions, peaches, pineapples, potatoes, spinach and sweet bell peppers.

Results from the PDP's 2002 monitoring of pesticide residues in fruit and vegetables are provided in Table 4.6. Less than half of the samples (46.9%) contained detectable levels of residues but the detection rates were greatly dependent upon the commodity analyzed. Residues were detected in 90% or more of all of the apple, celery, peach and potato samples while only 11% of pineapple samples, 10% of asparagus samples, 9% of processed sweet pea samples, 4% of processed sweet corn samples and less than 1% of onion samples contained detectable residues.

A total of 33 fruit and vegetable samples (0.33%) showed residues that exceeded established tolerance levels. These included one apple sample, nine asparagus samples, two banana samples, one celery sample, one

Table 4.6 Results of pesticide residue sampling of fruits and vegetables, USDA Pesticide Data Program, 2002

	Total samples analyzed	Samples with residues detected	Samples with detections (%)	Different pesticides detected
Fresh fruit and vegetables				
Apples	556	508	91	19
Asparagus	708	72	10	16
Bananas	727	280	39	7
Broccoli	737	224	30	12
Carrots	554	472	85	22
Celery	737	694	94	26
Cucumbers	183	126	69	21
Mushrooms	728	449	62	17
Onion	741	1	0	1
Peaches	563	552	98	34
Pineapples	360	39	11	6
Potatoes	370	333	90	15
Spinach	363	267	74	22
Sweet bell peppers	186	139	75	30
Total fresh	7513	4156	55	
Processed fruit and vegetables				
Apple juice	729	289	40	8
Apple sauce	358	173	48	7
Sweet corn	727	29	4	2
Sweet peas	729	69	9	6
Total processed	2543	560	22	

Fruit and vegetables: number of samples analyzed = 10056; number of samples with residues detected = 4716; residue detections = 46.9%; total number of different pesticides detected = 78.
Adapted from USDA, 2004.

cucumber sample, 15 peach samples and four spinach samples. Additionally, 333 fruit and vegetable samples (3.31%) had residues for which no tolerance was established. Of these, 314 samples contained one residue for which no tolerance was established, 17 samples contained two residues for which no tolerance was established and two samples contained three residues for which no tolerance was established.

The PDP program also reported on the incidence of multiple pesticide residues detected on individual samples. In 2002, 20.2% of the fruit and vegetable samples contained a single pesticide residue. Samples containing multiple residues included 11.0% with two residues, 6.5% with three residues, 3.4% with four residues, 2.7% with five residues, 1.5% with six residues, 0.8% with seven residues, 0.3% with eight residues, 0.1% with nine residues and a single sample (0.01%) of celery that contained residues of 11 different pesticides. Most multiple residue detections resulted from the application of more than one pesticide to a crop during the growing season, but some may have resulted from degradation of the parent pesticide to one or more breakdown products, spray drift, transfer through crop rotation, cross-contamination at packing facilities, or uptake of persistent environmental residues. Most multiple pesticide residue detections resulted from the analysis of composite food samples each weighing 3–5 lb. As a result, the number of residues detected in a particular composite sample does not necessarily reflect the number of residues in an individual sample or in a single serving of a commodity.

4.4.3 California department of pesticide regulation residue monitoring program

Individual states are also involved in monitoring fruit and vegetables for pesticide residues. The largest state residue monitoring program belongs to California, which analyzed 3424 samples of fresh produce in 2003. Sampling included 72 different commodities of domestic and imported origin, and analysis involved multiresidue methods capable of detecting more than 200 individual pesticides and breakdown products (California Department of Pesticide Regulation, 2004). Sampling in the program is not random and is skewed to maximize the opportunity to identify 'violative' residues. Factors considered in determining sampling strategies include a history of violation, a significant percentage of detectable residues in previous years and the dietary significance of the food, based upon consumption frequencies and amounts consumed.

In 2003, 68.3% of samples analyzed in the California Department of Pesticide Regulation Residue Monitoring Program contained no detectable residues. Residues within tolerances were detected in 30.8% of the samples. The majority of samples containing detectable residues had residue levels less than 10% of the established tolerances. 'Violative' residues were detected in 0.88% of the samples, including 0.06% of the samples where

residues were detected in excess of tolerances and 0.82% of the samples where pesticides were detected on commodities for which no tolerance was established.

4.5 Risk assessment

The presence of pesticide residues in foods has stimulated a significant debate, both in the USA and worldwide, concerning the potential risks posed by pesticides in the diet. Pesticides are, by their nature, toxic chemicals and it is clear that consumers frequently ingest pesticide residues in their diets. The most common approach used to address the question of the pesticide residue risks is to present the results of the various monitoring programs as described previously. The percentages of violations are frequently used to guide arguments about how safe the food supply is.

Unfortunately, since pesticide tolerances are not developed to serve as safety standards and are more suitable as enforcement tools to determine if pesticide applications are made in accordance with directions, violation rates are basically inconsequential as indicators of health risks. In the cases where pesticide residues exceed tolerances, exposures to the pesticides are rarely of health significance (Winter, 1992a). Most pesticide residue violations, as discussed previously, occur when residues of pesticides are detected on commodities for which no tolerance has been established; most of the offending pesticides are legally allowed at much higher levels on other commodities. In simplest terms, dietary pesticide risk assessment requires knowledge of (1) the toxicity of the pesticide in question and (2) the amount of exposure to the pesticide (Winter, 1992b; Winter and Francis, 1997; Winter, 2003).

Animal toxicology tests are used to determine the maximum level of exposure to a pesticide that an animal species (typically rats or mice) can experience before exhibiting any signs of toxicity. This level is often referred to as the no observed adverse effect level, or NOAEL, and may be calculated from both acute (brief periods) and chronic (continuous, long-term) exposures to a pesticide. To account for potential differences in the extrapolations from animal to human populations and from typical to sensitive human populations, uncertainty factors of 10 are typically used in each case and are multiplied together. The EPA uses the term reference dose (RfD) to represent the typical daily exposure of a pesticide that is not expected to result in harm. The RfD is calculated by dividing the NOAEL found in the most sensitive animal study by the uncertainty factors (often 100) and yields a value that is typically expressed as the amount of pesticide that can be consumed per day relative to the body weight of the consumer.

Historically, before the EPA would approve a pesticide tolerance, it would develop an estimate of the daily exposure to the pesticide from its existing and proposed uses and compare that level with the RfD. If the

estimated daily exposure were below the RfD, the pesticide tolerance would be established. Estimates of exposure frequently involved a deterministic model in which average food consumption levels were used and residues were considered all to be present at the tolerance level. While this technique often exaggerated exposure levels by factors of 10 000 times or more (Archibald and Winter, 1989, Winter 1992a), resulting exposure estimates were still often below the RfD. Refinements in exposure estimation procedures, such as using realistic residue levels, considering the actual extent of pesticide use and/or accounting for post-harvest reductions in residue levels from processes such as washing, cooking and peeling, could also be used to develop exposure estimates that could be compared with RfDs for the purpose of approving tolerances.

Such an approach is still useful today to assess the risks posed by chronic exposure to pesticides in the diet. In the case of short-term, acute exposure to pesticides in the diet, this deterministic approach to risk assessment has been often replaced by probabilistic methods that consider both pesticide residue levels and food consumption estimates as probabilities rather than as point estimates.

One of the key provisions of FQPA is to ensure that pesticides demonstrate a 'reasonable certainty of no harm'. With respect to chronic risks, the traditional deterministic approach that compares exposure estimates with the RfD (or, in the case of potentially carcinogenic pesticides, determines if exposure estimates exceed a one in a million excess cancer risk) is usually appropriate to allow regulators to make that determination. For acute risks, however, approaches are more complicated and require greater computational capabilities as well as greater regulatory judgment.

Since both pesticide residue levels and human food consumption patterns are subject to considerable variability, appropriate dietary pesticide exposure models need to be developed to represent this variability adequately. Using probabilistic modeling approaches, it is possible to develop a distribution of daily dietary pesticide exposure by multiplying random data obtained from pesticide residue studies with random data for food consumption of particular food items (Winter, 2003). Exposure can then be calculated at levels representing various percentiles such as the 50th percentile (median level) as well as the 99th percentile or the 99.9th percentile. The EPA has frequently considered acute exposure to pesticides to meet the 'reasonable certainty of no harm' criterion in cases where daily exposure at the upper 99.9th percentile is below the acute RfD or, in the case of exposure of infants and children, is below the daily level determined to be protective of this population subgroup.

Other provisions of FQPA have also complicated the practice of pesticide residue risk assessment. The aggregate exposure provision requires that exposure is considered, not just in terms of food residues of pesticides but also exposure from drinking water and from residential exposure

to pesticides. The cumulative exposure provision requires consideration of the exposure of all members of a family to toxicologically related pesticides rather than simply the exposure of the individual members of the family.

A variety of software programs have been developed to allow such estimates of exposure to be determined. The most prominent software developed is provided by The LifeLine Group, a non-profit US organization with the goal of using state-of-the-art risk assessment approaches that are both transparent and publicly available (Price *et al.*, 2001b). Their software accesses a large number of databases and allows determination of daily estimates of pesticide exposure for thousands of simulated individuals throughout their lifetimes. Recent developments by The LifeLine Group directors include the development of physiologically based pharmacokinetic modeling techniques that allow more realistic modeling of inter-individual variation in internal human doses of chemicals, including pesticides (Price *et al.*, 2001a).

4.6 Future trends

Following the passage of the FQPA in 1996, efforts to improve the methods used to determine risks from pesticides in food, water and in residential environments have been considerable and it is likely that similar improvements will continue in the near future. Improved risk assessment methodologies and software developed for pesticides should also be applicable for the estimation of risks from other types of chemicals such as environmental contaminants and consumer products.

Regulatory monitoring programs to analyze pesticide residues from fruit and vegetable samples play an important enforcement role that provide economic disincentives for food producers to ignore pesticide regulations. It is likely that such programs will continue in the future. Since these programs are not primarily designed to provide public health protection, it is possible that advances in the risk assessment process may allow regulators better to identify situations in which pesticide residues may pose health risks and to increase sampling and enforcement efforts.

Agricultural production practices for fruit and vegetables may change as a result of improvements in non-pesticide control measures, increased stringency in pesticide regulation and public demand for foods containing lower residue levels. The continued development of genetically modified fruit and vegetables may also reduce pesticide use owing to the engineering of pest resistance directly into the food crops. Environmental and occupational risks from pesticide use might also be reduced if such genetically engineered food crops become widely accepted in agriculture.

4.7 Sources of further information and advice

Many agencies are involved in the regulation and monitoring of pesticides in fruits and vegetables. Addresses for the agencies include:

US Environmental Protection Agency – http://www.epa.gov

US Food and Drug Administration – http://www.fda.gov

US Department of Agriculture – http://www.usda.gov

California Department of Pesticide Regulation – http://www.cdpr.ca.gov

The LifeLine Group is involved in the development of state-of-the-art risk assessment software that is transparent and publicly available. More information about The LifeLine Group is available at http://www.thelifelinegroup.org

The University of California has developed several websites dealing with fruit and vegetable quality and safety. They include:

The Fruit and Vegetable Processing Webpage – http://www.fruitandvegetable.ucdavis.edu/homepage.html

University of California FoodSafe Program – http://foodsafe.ucdavis.edu

Food Safety Music – http://foodsafe.ucdavis.edu/music.html

Vegetable Research and Information Center – http://vric.ucdavis.edu

University of California Postharvest Research and Information Center – http://postharvest.ucdavis.edu

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5

The rapid detection of pesticide residues

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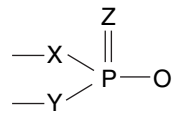
5.1 Introduction

In today's world many different chemicals are used to protect food and our environment from spoilage by a range of pests such as rodents, weeds, insects and fungi. This has a great positive economic value by increasing the yield in the food supply chain. Despite their having great benefit to society, the very nature of their use means that pesticides are highly toxic to humans and measures must be taken to prevent accidental exposure, whether from occupational exposure or more covertly via the food supply chain itself.

A wide range of compounds are used as pesticides such as the chlorinated hydrocarbons, which have been shown to be highly toxic and may have long-lasting effects on the environment. Research has shown that dichlorodiphenyltrichloroethane (DDT) has had a devastating effect on parts of the food chain. Another important group of pesticides is the organophosphate compounds, which are safer than chlorinated hydrocarbons but are still highly toxic. It is thought that the safest pesticides are those derived from plants, such as pyrethrum, but a disadvantage in using these compounds is that they require more frequent application. For the purpose of this chapter, further discussion will be focused on the detection and measurement of organophosphate compounds, although much of the discussion could be applied to other types of pesticides such as those mentioned above.

Organophosphates (OPs) are small molecules derived from phosphoric acid with the oxygen atoms being either replaced by other atoms, for example sulphur, and/or linked to aliphatic, aromatic, anhydrides or heterocyclic groups. Table 5.1 lists the more important categories of OP

Table 5.1 Main side groups on different classes of organophosphate compounds



Class of organophosphorus	X	Y	Z
Phosphate	—O	—O	O
Phosphorothionate	—O	—O	S
Phosphorothiolate	—S	—O	O
Phosphorodithionothiolate	—S	—O	S
Phosphorodithiolate	—S	—S	O
Phosphoramidate	═N	—O	O
Phosphordiamidate	═N	═N	O
Phosphoramidothionate	═N	—O	S
Phosphoramidothiolate	═N	—S	O
Phosphonate	≡C	—O	O
Phosphonothionate	≡C	—O	S
Phosphonothionothiolate	≡C	—S	S

compounds with their particular side chains and Fig. 5.1 shows the structure of three common OPs.

For pesticide applications the sulphur-containing compounds are more widely used than the other derivatives. However, all types of OPs are highly toxic to mammals, to differing extents; some are considered 'relatively' safe, such as malathion and dimethoate. OP compounds exert their toxic effects by their propensity to inhibit a number of important enzymes, in particular the enzyme acetylcholine esterase. This enzyme is important in the inactivation of the fast-acting neurotransmitter acetylcholine found in the nerve synapses of the neuromuscular junction and brain nicotinic junctions. The inherent toxicity of OP compounds has been exploited by various nations in the production of chemical warfare nerve gas agents, such as sarin and tabun. Accidental occupational exposure to agricultural OP results in symptoms that are similar to those experienced by being exposed to OP nerve gas agents. Symptoms include nausea, vomiting, cramps, headache, dizziness, blurred vision, muscle twitches, difficulty in breathing, convulsions, respiratory paralysis and death.

The widest application for OPs has been their use as insecticides, although they are also used as nematocides, helminthicides and have fungicidal and herbicidal properties. Owing to the inherent toxicity of organophosphates, there is strict control over their use, particularly in their application to foodstuffs, which is supported by legislation. In many countries, the use of the most harmful compounds is banned but illegal application can still be a problem. From the results of a number of studies it has

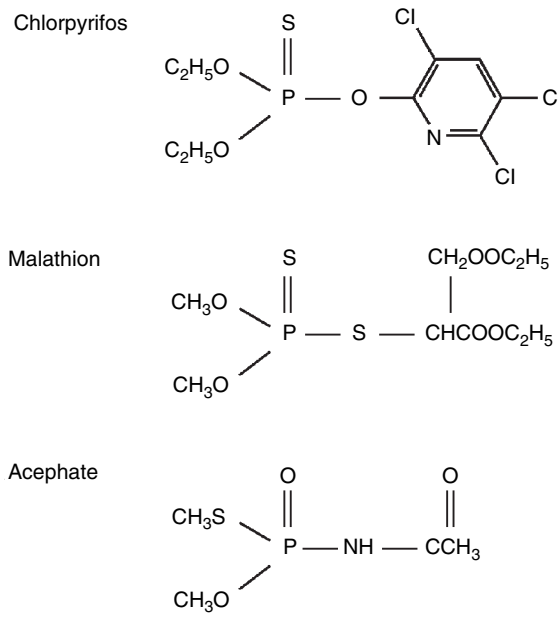


Fig. 5.1 Three examples of organophosphates.

been estimated that, worldwide, pesticides are responsible for 10 000 deaths a year. The problems are associated with over-application to crops and spray drift with subsequent contamination of surrounding areas. To prevent harmful effects to the population in general, the use of agricultural pesticides is strictly regulated and tables have been produced detailing the maximum permissible level of OP residue, known as the maximum residue limit (MRL) measured in ppm. MRL levels are set for different pesticides and different crops and additional variation is also seen between the different regulating authorities. In addition, only certain OPs are licensed, with many OPs being banned. Table 5.2 gives examples of MRLs for three different OP compounds and three different foods. Tenfold differences, or more, in the MRLs for particular crops are not uncommon.

For health and litigation considerations it is necessary to monitor the use of pesticides applied to crops, as the pesticide residues may find their way into the food chain. The technology available for measuring pesticide residues is becoming much more sophisticated and sensitive and consequently MRL levels are now being set at much lower levels. The sophisticated analytical techniques used within laboratories tend to be expensive, relatively time consuming and require a sample of the foodstuff that is then transported to the laboratory where skilled personnel perform the analysis. Increasingly, there is a need for inexpensive, rapid tests to detect and measure levels of pesticides at, and below, ever-reducing MRLs on raw

Table 5.2 Maximum residue limits for three different crops and three different OP compounds

Organophosphate compound	Crop	Maximum residual limit (ppm)
Chlorpyrifos	Apples	0.50
Chlorpyrifos	Bananas	3.00
Chlorpyrifos	Cucumbers	0.05
Chlorpyrifos-methyl	Apples	0.50
Chlorpyrifos-methyl	Bananas	0.05
Chlorpyrifos-methyl	Cucumbers	0.05
Dichlorvos	Apples	0.10
Dichlorvos	Bananas	0.10
Dichlorvos	Cucumbers	0.50

food, which can be used on site by unskilled operatives. These new rapid tests may act as a preliminary screen giving assurance that there is no pesticide residue present on the food being tested, with a positive test being verified by traditional analytical techniques. In the near future, as new rapid tests become more reliable and are validated against 'gold standard' methods, the rapid test could replace expensive analytical technology.

5.2 Detecting pesticides: physicochemical methods

Traditionally, OPs have been measured by exploiting their chemical and physicochemical properties using a separation technique such as chromatography or electrophoresis. The spectral characteristics of pesticide residues have also been used in NMR techniques and mass spectroscopy to aid identification and measurement. In recent years, other approaches using the biochemical and immunological properties of pesticide residues have been developed and are now widely used; these are the immunoassay and enzyme inhibition techniques. In order to detect and measure pesticide residues at and below current MRL levels any method of analysis should demonstrate appropriate selectivity and sensitivity. For example, many chemical reactions are only specific for groups of compounds and do not show selectivity, but when combined with separation techniques, individual compounds can be identified; an example of this approach is thin layer chromatography.

Conventional analysis of pesticide compounds is dominated by techniques employing a separation stage. This group of methods achieves selectivity by separating a mixture into individual components that are then identified by comparing the separation to pure standards. These approaches have the advantage that they can measure more than one compound simultaneously. Thin layer chromatography (TLC) and high performance thin

layer chromatography (HPTLC) separate compounds according to their polarity and differential adsorption on silica gel. Visualisation of the separated OP compounds can be with direct ultraviolet irradiation or a chemical reaction to produce a coloured spot. These methods, although allowing a number of samples to be analysed simultaneously, are simple and relatively quick; however, they are only qualitative and involve the use of solvents. Recent studies have shown that quantitation can be achieved by measuring the density of the spots with detection limits being recorded from 0.05 μg to 1.0 μg of pesticide residue applied. These constraints limit the use of these methods to an analytical laboratory where skilled personnel perform them.

Quantification is more traditionally achieved using gas chromatography (GC) or high performance liquid chromatography (HPLC). In GC, the sample is heated to volatilise the OPs which are carried through a column by a flowing inert gas and separated by differential adsorption to a solid phase in the column. Newer instruments use a capillary column where adsorption takes place on the capillary wall rather than packing in the column, which leads to a faster separation and greater sensitivity. In some instances, called gas liquid chromatography (GLC), the solid phase may be covered with 'waxy' liquid to promote greater separation. OPs are measured as they come off the column by a thermionic emission or alkali-flame detector. Some OPs decompose at elevated temperatures resulting in misleading results. The technique is sensitive and relatively quick but uses expensive equipment that must have a gas supply, so is limited to laboratory use.

HPLC does not have the disadvantage of thermal degradation of the sample and is perhaps a preferred method for OP analysis. In this technique the sample is injected into a flowing solvent and is carried through a column containing a solid phase. Again, separation is due to differential adsorption on to the solid phase and is determined to some extent by differing polarities of the OP compound in the sample. Detection is by UV absorption or refractive index change. As with the previous techniques described, there are a number of limitations on its application; the equipment is expensive, uses solvents and requires trained operators and as such is limited to laboratory use.

Where identification is required, this can be achieved using spectral methods such as NMR and mass spectroscopy. NMR allows identification of a single pesticide residue whereas mass spectroscopy, when interfaced with either GC or HPLC, can identify a number of different pesticide residues. GC-MS is considered to be the gold standard for pesticide measurement and identification. Extremely low detection limits can be reached using tandem-mass spectroscopy with examples of 1300 ppt for dichlorvos and 0.1 ppt for trifluralin being quoted. These techniques are highly specialised, expensive and limited to laboratories where trained personnel perform the analysis.

As indicated in the above discussion, separation techniques do not lend themselves to the rapid analysis times that are required for use in the field. Generally they rely on the use of expensive instrumentation, skilled personnel and are not easily transported.

5.3 Detecting pesticides: biological methods

These methods differ from the techniques described in the previous section as they depend on the interaction between a biological molecule and the pesticide residue. This interaction may be specific for a particular pesticide as in the interaction with an antibody, or non-specific as in the way a number of different pesticides interfere with an enzyme reaction.

5.3.1 Antibody methods

Antibodies are biological molecules formed as a part of a host response to foreign substances or microorganisms, for example toxins, viruses or bacteria. The substance to which an antibody is formed is called an antigen. Antibodies bind very specifically to the antigen they are directed against. Thus, methods that use antibodies are generally very specific for a particular pesticide but similar molecules may show some cross-reactivity. Antibodies are produced from animal cells in either a live animal or a cell culture. In both cases an immune response has to be initiated to start cells of the immune system synthesising specific antibody. In order to trigger the immune system to produce an antibody the antigen involved must be a large, complex molecule. Small molecules such as OP compounds do not generally trigger antibody production on their own. In order for small molecules to be recognised by the immune system and start antibody production, they have to be conjugated to a larger molecule such as a protein.

Antibodies are used in a group of techniques collectively called immunoassays. Here the antibody binds to a specific pesticide which it has been designed to recognise and forms an immune complex consisting of the antibody molecule binding with the pesticide residue. The higher the concentration of pesticide in the sample the more immune-complex formed. The immunoassay measures the amount of immune-complex formed and relates this to pesticide concentration.

As pesticides are small molecules, the immunoassay is designed to be a competitive technique where the pesticide in the sample is mixed with a fixed amount of labelled pesticide and then competes with it for a limited number of antibody binding sites. After an incubation period, the antibody has reacted to both the sample pesticide and the labelled pesticide. In order to make the measurement the unreacted label has to be removed leaving only the label associated with the antibody. In this competitive system, as the sample concentration increases the greater numbers of pesticide

molecules from the sample will occupy more and more binding sites on the antibody. As a result there is less labelled pesticide in the antibody-binding sites. This gives rise to an inverse dose response curve with a high signal being seen with a low concentration of pesticide. Commonly the labels used in an immunoassay are enzyme labels, a fluorescent molecule, or sometimes a radioactive label. Enzyme labels can be used to generate a coloured product; a fluorescent product or an electroactive compound. The range of different end points of the immunoassay gives rise to a number of different measurement technologies that can be employed to detect the immune reaction.

Immunoassays are often performed in test tubes, 96 well plates and more recently by using lateral flow devices such as those used in pregnancy tests. These lateral flow devices give a rapid answer, are convenient and can be easily used by non-skilled personnel in the field, but are only semi-quantitative and limited in sensitivity, thus only useful as a screening test. A positive result is seen as the absence or presence of a coloured line, depending on how the test has been devised. Recently equipment has been developed to measure the intensity of the coloured line making the test more quantifiable.

5.3.2 Enzyme methods

These methods rely on the fact that OP compounds inhibit the biological activity of particular enzymes preventing them forming their products from given substrates, in other words the enzyme is poisoned. As different OPs will inhibit the enzymes, these methods are not specific for a particular OP as are the antibody methods, but give an indication of total OP concentration. The most commonly used enzyme in these methods is acetylcholine esterase (AChE) although butyrylcholine esterase, organophosphorus hydrolase and ascorbate oxidase have been used.

The principle behind enzyme methods is that the organophosphate enters the active site of the enzyme and binds to the protein structure through a serinehydroxyl group. This organophosphate binds strongly and is not released from the active site for many hours, in effect inactivating the enzyme. The natural substrate, acetylcholine, binds through the same serine-hydroxyl group. The natural substrate is cleaved by the enzyme, releasing choline and at the same time acetylating the serine-hydroxyl group. After only a few milliseconds the acetyl group is released returning the enzyme to its native state.

The amount of organophosphate that is required to inhibit enzyme activity by 50% is called the IC_{50} (inhibitory concentration-50%). It should be noted that different organophosphates have different IC_{50} values depending on both the particular organophosphate and the source of AChE. This is due to the particular side groups on the organophosphate causing steric hindrance and preventing the molecule entering the active site fully or at

all. Secondly, AChE from different sources has an active site of differing sizes. Those enzymes possessing a small active site are not inhibited by larger organophosphates but only by smaller organophosphates. Conversely enzymes with large active sites are also inhibited by larger organophosphates. For example frogs, which tend to be resistant to acute organophosphate poisoning, have an AChE that has a smaller active site and shows greater enzyme activity with acetylcholine compared with propionylcholine, a larger molecule. Conversely, in chickens, which are sensitive to acute organophosphate poisoning, AChE has a larger active site, and shows greater activity for propionylcholine compared to acetylcholine.

In addition to the size of the active site, susceptibility of a particular AChE to poisoning by organophosphate also depends on the hydrophobicity and electrophilicity of that organophosphate and the nucleophilic strength of the serine residue within the active site. For example, trout AChE shows greater inhibition of enzyme activity as the acidity of the phosphorus atom increases. In other types of AChE, such as from monkeys or rats, it is the nucleophilic strength of the active site that is more important in determining susceptibility of that enzyme to the organophosphate.

The enzyme assay depends on measuring the activity of the enzyme in the absence and presence of the sample. If organophosphate residues are present then there will be a decrease in enzyme activity noted. Enzyme activity is measured by monitoring the disappearance of substrate or the accumulation of product. This can be linked to a chemical reaction that produces a colour and the change in colour is monitored.

In developing new and rapid detection methods for the detection and measurement of pesticides it is the biological technologies that have been exploited. In particular it has been the development of biosensor technology where the greatest advances have been made. Very sensitive instruments can be constructed to be light, portable, easy-to-use, inexpensive and can be operated by untrained personnel.

5.4 The principles of biosensors

Biosensors are analytical devices that use a biological molecule to interact with the analyte in question to produce a measurable output. Figure 5.2 shows a schematic of a biosensor device. The discussion below examines the parts of the biosensor that form the sensing element and briefly reviews the approaches that have been used in developing biosensors for pesticide analysis.

The unique feature of a biosensor is the biological layer, which is integral to the device and interacts with the analyte. The biological molecule is important for giving the device specificity and selectivity. Many different types of biological molecules exhibit selective or specific binding as part of their biological function. These include antibody molecules, enzymes,

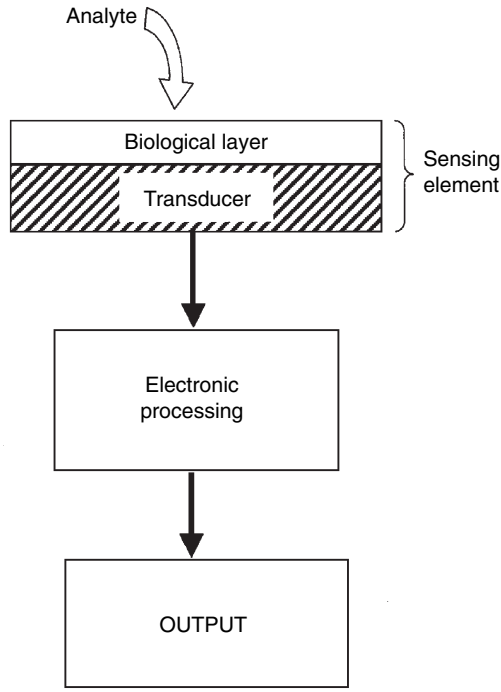


Fig. 5.2 Schematic diagram of a biosensor.

receptor molecules and lectins. In addition to these protein molecules, specific binding is also seen between complementary strands of nucleic acids. Nucleic acids on the sense surface are used to capture DNA from microbiological samples for the detection of bacteria or viruses. The great majority of biosensors for other analytes, including pesticides, use a protein molecule in the sensing element.

The biological molecules employed in the sensing element are immobilised on the surface of the transducer to form the sensing surface of the biosensor. Many different approaches have been employed to capture and hold biological molecules depending on the nature of the transducer surface and the biological molecule. These methods fall into three categories:

- adsorption-type methods
- chemical coupling
- biological coupling.

The simplest of these is adsorption of the biological molecule on to the transducer surface through the formation of non-covalent chemical bonds, such as electrostatic and hydrophobic bonds. Electrostatic bonds can be formed between charges on the transducer surface and charged groups on

the protein whereas hydrophobic bonds are formed between hydrophobic surfaces and hydrophobic domains of proteins. This type of immobilisation is simple and does not require any chemical reactions but has the disadvantage that the biological molecules are randomly orientated on the transducer surface. A proportion of the molecules will have parts of the molecule containing the reactive site for the analyte forming non-covalent bonds with the transducer surface and the reactive site will not be available to the analyte, leading to a loss of sensitivity; this is particularly true of antibodies. The other potentially major drawback of this type of immobilisation is that biological molecules can be lost from the surface during incubation and wash stages of the assay, again leading to loss of sensitivity.

Covalent coupling is achieved through chemical reactions between reactive groups on the surface of the transducer and the protein molecule. The principal groups used to cross-link proteins to a surface are amine, carboxyl and sulphydryl groups. A wide range of coupling chemistries using cross-linking agents is available for use with different reactive groups. The coupling or cross-linking agents can be broadly divided into those with homofunctional or heterofunctional activity. Homofunctional agents have the same reactive group at either end of the molecule and react with the same type of group on the transducer surface and protein molecule, such as an amino group. Glutaraldehyde is a good example of a homofunctional cross-linking agent. Heterofunctional agents have a different reactive site at either end of the cross-linking molecule and therefore can react with different reactive groups on the transducer surface and the protein, for example an amine group being coupled with a hydroxyl group. The advantage of chemically coupled biological molecules is the fact that they are not lost from the transducer surface during the assay and are necessary if a reusable biosensor is being developed. A potential disadvantage of chemical coupling is that the chemistry could inactivate a percentage of the biological molecules thus reducing sensitivity. This depends somewhat on the harshness of the chemical reaction used. The advantage of covalent cross-linking is that biological molecules can be orientated on the transducer surface to present the reactive part of the molecule to the analyte, allowing greater sensitivity. This is particularly important in the orientation of antibodies on the transducer surface, where to gain maximum sensitivity the antigen-binding site should be orientated towards the sample.

As with covalent cross-linking, biological coupling also ensures that the correct orientation of the biological molecule interacts with the analyte. These methods are usually employed with antibody-coated biosensors and use another protein that binds to an antibody by the non-specific Fc portion of the antibody. This leaves the antigen specific, antigen binding sites in the correct orientation to interact with the antigen.

The interaction between the biosensor and the analyte can be broadly grouped into three modes of action. Figure 5.3 shows these different modes of action. In the first mode of action, the direct mode (Fig. 5.3(a)), the

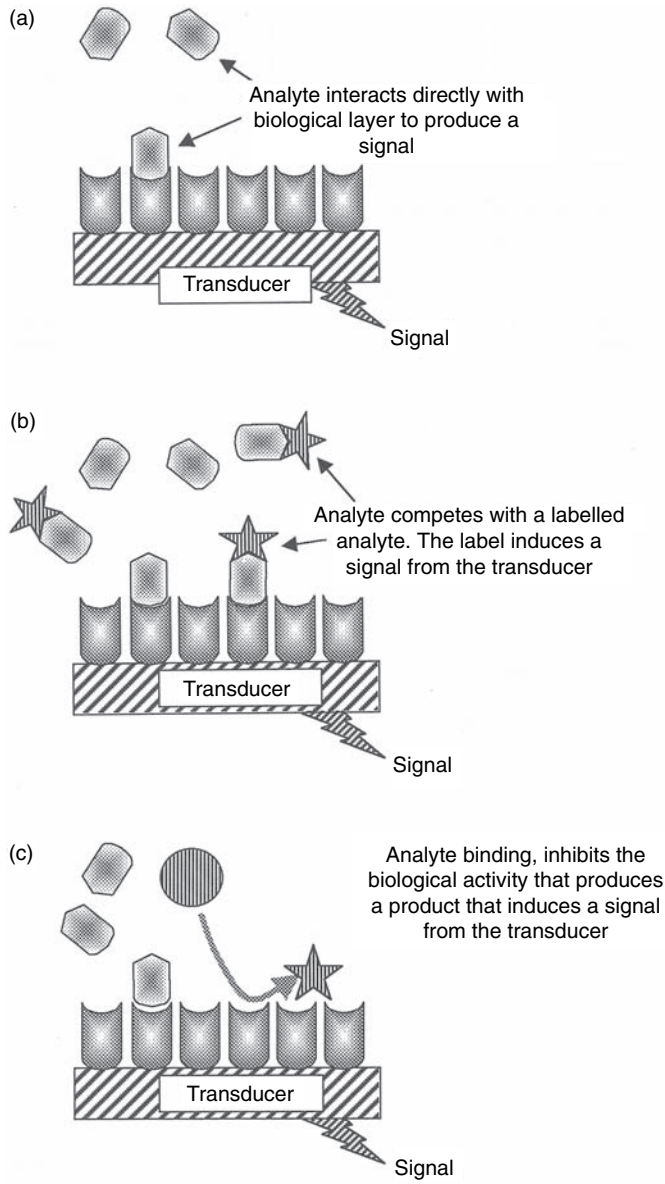


Fig. 5.3 Three modes of action for a biosensor determining pesticides: (a) direct mode, (b) competitive mode, (c) functional mode.

analyte interacts directly with the biological layer on the surface of the transducer to produce a signal. Here, it is the analyte itself interacting with the biological layer that generates the change in signal measured by the transducer. The second mode (Fig. 5.3(b)) of action involves competition

between analyte and a labelled species for binding sites on the transducer surface. It is the label that is detected by the transducer. This competitive mode is a form of indirect detection and commonly involves a fluorescent label or an enzyme label that produces the fluorescent or electroactive product. A third type of interaction is where the analyte binds to the biological layer on the transducer surface and causes a change in the biological activity or function (Fig. 5.3(c)). A good example of this is seen where enzyme is immobilised on the transducer surface, the reaction of pesticide to the biosensor inactivates the enzyme changing its biological activity.

The role of the transducer in a biosensor is to generate a measurable signal when the analyte interacts with the biological molecule associated with the transducer surface. The two common forms of transducers used for pesticide analysis are optical transducers and electrochemical transducers. Optical transducers generate a signal measured as a light intensity proportional to the concentration of pesticide in the sample; this may be an inverse relationship. Electrochemical transducers generate a current or voltage in proportion to the pesticide being measured; again this may be an inverse relationship.

5.4.1 Optical biosensors

Optical transducers used in biosensors utilise the evanescent wave effect. The evanescent wave may interact directly with molecules on the surface of the transducer bringing about a change in signal, this is the principle behind surface plasmon resonance technologies. These devices measure a very small change in the refractive index at the surface of the transducer. As antigen binds to antibody immobilised on the transducer surface there is a mass change which in turn brings about a change in the refractive index measured. The greater the mass of the component binding to the immobilised biological layer the greater the signal generated. This has important implications when trying to detect small molecules using an antibody immobilised on the surface of the transducer. When small molecules bind there is only a small change in the mass on the surface, generating only a small signal. So for the detection and measurement of small molecules, such as pesticides, surface plasmon resonance-type technologies can suffer from a lack of sensitivity. Typical detection limits quoted in the literature range from 0.05 to 5.0 $\mu l l^{-1}$, two specific examples for pesticide residues are atrazine and simazine, which have detection limits of 1.0 $\mu l l^{-1}$ and 0.1 $\mu l l^{-1}$, respectively. Instrumentation used for surface plasmon measurement is often large and not particularly portable and can be very expensive. New developments in this technology have seen surface plasmon resonance devices that utilise a capillary or fibre-optic rod that can be dipped manually into the sample. Using this type of technology, a rapid handheld device is easily constructed, but is still expensive. Optical sensors suffer from the problem of non-specific binding; any interaction on the surface results in a

change in the measured signal, so there is an issue of specificity. With high affinity antibodies immobilised on the transducer surface and for use of good blocking chemistry, non-specific interaction should be minimised.

In another type of optical transducer, the evanescent wave interacts with a fluorescent marker or label mixed with the sample as seen in Fig. 5.3(b). A fluorescently labelled antigen competes with antigen from the sample for antibody binding sites at the surface of the transducer. The evanescent wave penetrates into the sample interacting with the fluorescent label that absorbs light and emits its fluorescent signal, which enters the wave guide and is measured. When high concentrations of antigen in the sample are found, only small amounts of labelled antigen can bind to the antibody, generating a small signal. Conversely, with a low concentration of antigen in the sample, greater numbers of antibody binding sides are occupied with fluorescently labelled antigen, giving rise to a larger signal.

5.4.2 Electrochemical biosensors

In recent years, there has been increasing interest in the construction and operation of organophosphate pesticide biosensors based on electrochemical transducers. One of the most common approaches has involved the use of acetylcholinesterase (AChE) as the biological recognition element, which has been integrated with a variety of carbon electrodes as transducers. Hart and co-workers have been investigating OP biosensors based on screen-printed carbon electrodes (SPCEs) which contain cobalt phthalocyanine (CoPC) as an electrocatalyst. In one approach, AChE (from electric eel) was immobilised onto the CoPC-SPCE by simply drop coating a solution of this enzyme onto its surface, followed by a solution containing the cross-linking agent glutaraldehyde. Figure 5.4 shows a schematic diagram of the biosensor and the various reactions taking place during its operation.

Studies by Hart and co-workers have focused on optimisation of the OP biosensor for operation in several different modes. The first mode involved amperometry in stirred solution and two approaches were investigated. Initial studies were performed by transferring an aliquot of phosphate buffer at pH 7.4 into a voltammetric cell at 37 °C; the biosensor was then immersed in the solution and the potential applied. After nine minutes, 50 µl of acetylcholine was added to initiate the enzymatic reaction, giving a final concentration of 0.5 mM. When a steady state signal was achieved, the reaction was allowed to proceed for a further nine minutes before the addition of pesticides. Initial rates of decrease in current were measured and these data were used in the construction of calibration plots. It was found that plots of initial rates of current decrease vs log concentration of paraoxon were linear between 3.24×10^{-7} M and 3.24×10^{-6} M, the former representing the detection limit. Similarly for dichlorvos, the linear range was from 1.7×10^{-6} M to 1.4×10^{-5} M, the former representing the detection limit.

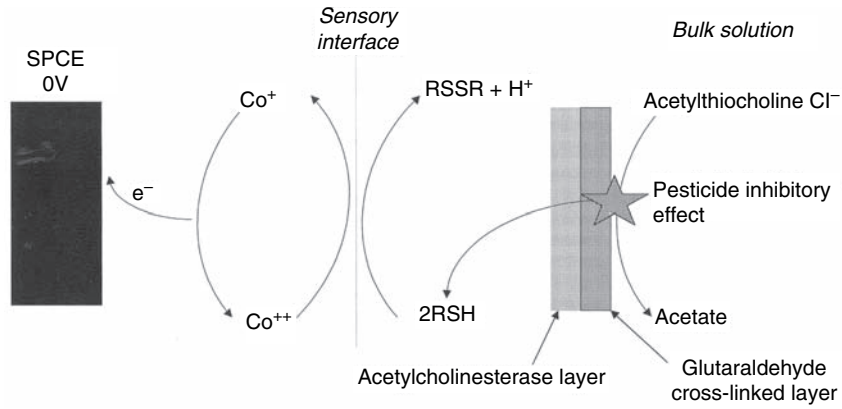


Fig. 5.4 Diagram to show the reactions taking place on the surface of a screen-printed carbon electrode (SPCE) biosensor using acetylcholinesterase as the biological layer. In the absence of an OP, acetylthiocholine is enzymatically converted to thiocholine as it passes through the AChE layer; this species then chemically reduces the central cobalt ion, which is in the +2 state, to the +1 state. This latter ion is re-oxidised at the SPCE back to the +2 state by loss of an electron, and this current constitutes the analytical response. In the presence of an OP, the enzymatic conversion of acetylthiocholine to thiocholine is inhibited, which results in less thiocholine being produced. Consequently, the current is attenuated and this decrease is proportional to the OP concentration.

The second method utilising amperometry in stirred solution was performed by placing an OP biosensor into a solution containing buffer only, switching the cell on, then adding pesticide after three minutes; after a further ten minutes, acetylthiocholine was added and the resulting currents allowed to reach steady state (i_{ss}). The inhibition was calculated by measuring the difference between the i_{ss} values in the absence, and presence, of pesticide and representing this difference as a percentage of the former value. Calibration plots were constructed by plotting percentage inhibition vs log pesticide concentration; in the case of dichlorvos, the plot was linear from 7.1×10^{-7} to 5.6×10^{-6} M, the former representing the limit of detection. It should be mentioned that the sensitivity of this second approach could be improved by simply increasing the incubation time with pesticide before addition of the substrate.

The second mode of operation of the OP biosensor, investigated by Hart and co-workers, involved flow-injection analysis with amperometric detection. The biosensor was incorporated into a thin-layer flow cell and the mobile phase allowed to flow over the surface at a rate of 1 ml min^{-1}. Pesticide determinations were carried out in three stages. First the amperometric response was recorded when a 20 µl aliquot of 1 mM acetylthiocholine was injected into the system. Next, the sample stream containing an OP was directed through the flow cell; thirdly, the flow was switched back to buffer

only and the current measured after making an injection of 20 µl of substrate. The concentration of pesticide was determined from any decrease in the biosensor response. The detection limits obtained with an enzyme loading of 1.0 U per sensor were 6×10^{-9} M and 7×10^{-11} M for dichlorvos and paraoxon, respectively; with an enzyme loading of 0.05 U per sensor, a detection limit of 4.0×10^{-11} M was achieved for paraoxon. The use of a flow cell, in conjunction with amperometry, does seem to offer certain advantages, perhaps the most important being the possibility of producing a fully automated system. Further research is under way to develop an array of biosensors based on this technology for the identification and quantification of multiple OPs in a single food sample. In this case, mutations of AChE from drosophila are being investigated as the biorecognition elements of the proposed array; these are immobilised onto the SPCE array and interrogated using chronoamperometry. The goal of this research is to develop a fully automated system to determine OPs in a variety of raw food produce.

An alternative pesticide biorecognition system, for use with electrochemical transducers, has been developed by Wang and co-workers. The enzyme organophosphorus hydrolase (OPH) is reported to have broad substrate specificity and is able to hydrolyse a number of pesticides including parathion, methyl parathion, fenitrothion and paraoxon. In these cases, the enzyme catalyses hydrolysis of the OP compounds to generate *p*-nitrophenol, which is electroactive. Consequently biosensors could be constructed which were based on the direct oxidation of *p*-nitrophenol, and the magnitude of the response is directly proportional to the concentration of the pesticide. These workers constructed a remote OP biosensor by incorporating the device into a PVC housing tube attached to a 16 m long shielded cable via three-pin environmentally sealed rubber connections; a Ag/AgCl reference electrode and platinum counter electrode completed the cell system. The biosensor was operated in the chronoamperometric mode by stepping from open circuit to +0.85 V vs Ag/AgCl. The response was found to be linear in the range 4.6–46 µM for paraoxon and up to 5 µM for methyl parathion; the limits of detection for these two pesticides were 0.9 µM and 0.4 µM, respectively. It was reported that an advantage of this system is that the biosensors are reusable. However, they do not yet appear to possess the sensitivity achieved with the AChE-based systems.

5.5 Developing low-cost biosensors

Biosensors are the ideal technology for developing rapid low-cost devices for measuring pesticides at the site of food production or food intake, alleviating the need to send a sample to a specialist laboratory. The sensor elements on which the biological layer is incorporated are small and can be built into a robust housing incorporated into the portable device. Ideally

the measurement time will only be a few minutes, the incorporated electronics take the signal from the transducer, process the signal and present a result to the operator. The device should be easy to use, enabling unskilled operators to make measurements. But the biggest factor in determining whether any such device will be a commercial success is the cost of the analysis.

Factors that influence the cost of analysis include choice of sensor, the nature of the biological material immobilised on the sensor surface and the number of units to be manufactured. For example, optical and surface plasmon resonance sensors are more expensive than screen-printed electrodes. The biological layer on the biosensor surface has to be from a reliable source ensuring consistency of purity and reactivity. The source of the biological material may have a significant impact on the price of the sensor particularly if genetically modified biological molecules are used. This in turn will influence the way the biosensor is used in practice.

There are two fundamentally different ways in which low-cost biosensors have been employed for pesticide analysis. The first approach is to use a reusable sensor where a number of different samples are applied to the same instrument, where the same sensor surface is regenerated between samples and can give a number of sequential readings. Obviously, this will reduce the cost of each analysis, recycling expensive biological molecules. The exact number of measurements that can be made from a single biosensor depends on the immobilisation chemistries, the nature of the biological molecule being used in the biosensor, the nature of the analyte being detected and other physical parameters such as the temperature. Typically, between 5 and 100 measurements have been described, but as the biosensor ages, the sensitivity decreases. The second approach is to develop a sensor using a disposable chip, in this case a new sensor is used for each sample, with the loss of the biological material. The manufacturing process controls the reproducibility of these systems and the operator does not have to worry about the biosensor's performance slowly becoming degraded.

Whether using a reusable biosensor or a single-shot biosensor, sample presentation is a critical factor in the design of an instrument for the measurement of pesticides. Again there are two fundamentally different approaches to this. First, a system of fluidics or microfluidics can be used to present the sample to the sensor surface thereby necessitating the use of a sample entry port. The fluidics system delivers sample, wash buffer and regeneration solutions in turn. Ideally, as the sensor surface is regenerated there is no change in the activity and density of the biological layer so each subsequent reaction will occur under identical conditions. In practice, some of the biological layer is lost as a result of inactivation or being washed from the sensor surface during regeneration. The advantage of this type of system is that it can be self-contained with minimal user interaction. The second method of sample presentation is to design the biosensor in such a way as

to enable it to be dipped into the sample. This has the advantage of not requiring any fluidics and keeping the device simple to operate and minimising costs. The big disadvantage of a dipping system is the potential problem of damage to the sensor surface.

Cost notwithstanding, the reliability and reproducibility of any biosensor device is vitally important for a commercial biosensor designed for unskilled use, whether it has a reusable sensor or a disposable sensor. Although still in its infancy, biosensor systems designed for pesticide analysis more commonly employ a fluidics or microfluidics system that allows the reaction and the biosensor surface to be carefully controlled ensuring greater reproducibility for use by semi- or unskilled personnel at the point of sampling.

5.6 Using biosensors: pesticide residues in grain, fruit and vegetables

The detection and measurement of pesticide residues in water presents little problem in terms of sample presentation to the biosensor. On the other hand the analysis of foodstuffs such as grain, fruit and vegetables presents other problems. Pesticide residues have to be extracted from the food sample and then presented to the biosensor for the analytical measurement. In terms of developing a commercialised system for the detection and measurement of pesticide residues, the extraction and interfacing with the analytical module is a serious concern. Traditional extraction techniques are not applicable to portable devices; the use of solvents is incompatible with the technology and the environment in which the measurements are being made.

Pesticide residues are extracted from food samples and have been ground up in the case of grain, or mechanically homogenised in the case of fruit and vegetables. Solvent is added to extract the pesticide, the solid material has to be removed and the extract presented to the biosensor. Organic solvents are incompatible with the biological layer and thus have to be removed and the extracted pesticide redissolved in a solvent compatible with the biological layer of the biosensor.

Newer techniques such as supercritical fluid extraction (SFE) have been used to extract pesticide residue from food samples. Gas such as carbon dioxide is in a supercritical state when the pressure and temperature equal or exceed the critical point (31 °C and 73 atm for carbon dioxide). Supercritical fluids have been known for about 100 years and have both gas-like and liquid-like properties, with high solvation power making them ideal for rapid extractions with high recoveries. This also gives supercritical fluids lower viscosity and higher diffusivity than other liquid solvents, allowing them to penetrate into the sample more efficiently. By controlling the pressure or temperature the density and solvation power can be controlled thus

simulating traditional organic solvents, for example for chloroform or hexane. By adjusting the solvation power targeted compounds can be preferentially extracted. Carbon dioxide has been greatly used in supercritical fluid extraction systems, as it is non-toxic, inexpensive and can be obtained at high purity. As the extraction process is usually carried out at a low temperature this reduces decomposition of organic compounds and prevents other reactions. Supercritical carbon dioxide is very good for extracting hydrocarbons and non-polar compounds, but in order to extract polar compounds a modifier can be added to the supercritical carbon dioxide. A range of different modifiers has been used but the most common is methanol although this is rather toxic for food applications. To alleviate this problem, ethanol has been used as an alternative in a number of applications. The disadvantage of using supercritical carbon dioxide for extraction is that it involves expensive equipment operating at high pressures and puts additional costs onto the analytical procedure. Other gases have been used in supercritical fluid extraction methods including freons and nitrous oxide, which are particularly useful for the extraction of polar compounds. Owing to environmental considerations these are rarely used.

Another new extraction technique is that involving the solvents containing phytosol, which is based on the compound 1,1,1,2-tetrafluoroethane. These solvents are non-flammable, non-toxic, have a neutral pH and are liquid at low temperatures and pressures such as those found in aerosol cans. The processed sample is placed into a heavy extraction vessel with a valve inlet that can take an aerosol can containing phytosol solvent. A measured quantity of solvent is added to the extraction vessel and allowed to mix with the food sample. This process is rather similar to using a supercritical fluid but does not involve the high pressures or temperatures. By releasing the valve on the extraction vessel the phytosol solvent is pushed into a second collection vessel under pressure. When the pressure is released the phytosol solvent evaporates leaving the pesticide residue in the collection vessel. In order to present the pesticide residue to the biosensor the residue must be dissolved in a small amount of a solvent that is compatible with the biological layer of the biosensor. This may entail dissolving the residue in a small amount of solvent such as methanol or ethanol and then making the volume up with an aqueous buffer solution suitable for presenting the sample to the biosensor. This particular extraction procedure is simple, inexpensive, does not require complicated equipment and is easily adapted to interface with portable analytical biosensor modules.

For a rapid, low-cost, portable detection system for pesticide residues in food there has to be the amalgamation of an extraction process and an analytical device based on a biosensor. It is expected that the complete analytical process, from sample introduction to presentation of the result, should take less than 30 minutes with minimal intervention from the operator.

5.7 Future trends

As with all areas of technology, the field of biosensors is moving forward at a terrific pace. There are a range of new technologies being developed at the moment to enhance the performance of rapid detection and measurement of pesticides. We have seen in this chapter how technology ranges from expensive sophisticated instrumentation requiring highly skilled personnel and dedicated laboratory space, to small portable units that can be operated on-site by unskilled personnel. Using one biosensor, information about a single pesticide can be obtained if the biological layer has the specificity to that particular pesticide. By introducing more than one biosensor in the device, multi-analyte detection and measurement are achievable. Using pattern recognition technologies, such as neural networks, the integration of many biosensors will lead to the simultaneous detection of a number of different pesticides. These technologies are being used for both antibody-based biosensors and enzyme-based biosensors.

The logical extension to having multiple biosensors in a device is to incorporate the active surfaces onto a single chip thereby reducing the amount of fluidics in the instrument. The challenge here is to develop isolated transducer elements on the chip on which the different biological layers are immobilised. With new techniques in nanotechnology and microengineered machines (MEM technology) this will soon be possible.

While array technology develops, new transducer technology is also being developed for use with biosensors. Magnetic technology is being developed in competition with optical, electrochemical and piezoelectric transducers. Magnetic biosensors will have the advantage that no chemistry or enzyme reaction is required nor is there any need for optical systems. The magnetic transducer will respond directly to magnetic or paramagnetic material associated with the biosensor surface. This has the advantage of potentially reducing the size and enhancing the portability of the device.

Looking further ahead it is possible to foresee the integration of other technologies such as radio telemetry into biosensors that can be left on-site. For continuous and on-line monitoring of food in the manufacturing process, biosensors could also be incorporated with robotic technology. It can be seen that the development of biosensors has been an important technological advance in monitoring pesticide residues in food. This is a core technology that goes beyond the detection and measurement of pesticide residues but can be employed for the detection and measurement of any other compound where a biological interaction can be integrated into the biosensor.

5.8 Sources of further information and advice

There are many websites giving information and further details of pesticide-related topics. The following websites are a small selection giving infor-

mation regarding the measurement and the impact of pesticides in food and the environment.

http://www.pesticides.gov.uk/
http://www.defra.gov.uk/
http://www.environment-agency.gov.uk/
http://www.hgca.co.uk/
http://www.fsis.usda.gov/
http://www.epa.gov/pesticides
http://www.pesticideinfo.org/

5.9 Further reading

A selection of review articles and scientific papers relating to areas discussed in the text is given below for further information.

5.9.1 Selection of review articles

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ANDREOU V G and CLONIS Y D (2002), A portable fiber-optic pesticide biosensor based on immobilized cholinesterase and sol–gel entrapped bromcresol purple for in-field use, *Biosensors and Bioelectronics*, **17** (1–2), 61–9.

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6

Risk management in the supply chain

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6.1 Introduction

The rising demand for fresh and minimally processed fruit and vegetables benefits from the ongoing trend for globally sourcing supply chains. A summary is given of the recent trends in the increasingly complex development of supply chains, development of globalisation, holistic process optimisation approaches and strategic cooperation. The accelerated progress of information and communication technologies is discussed in this context as a backbone, i.e. for the decoupling of information and product flows throughout the chain, enabling traceability and availability of quality- and process-related information at any step in the supply chain.

The demands of quality and risk management form another section of this chapter. The evolution of good manufacturing practice should form the predominant part of the self-control system of farmers and operators. A summary of risks for fresh and minimally processed fruit and vegetables is given, as well as an introduction to adequate risk analysis, which aims to avoid misinterpretation of hazard analyses.

The critical points in the supply chain of fresh and minimally processed fruit and vegetables are explained in the chapter's main section. A number of relevant pieces of EU legislation have been pronounced recently, concerning good manufacturing practice, additives and pesticides, hazard analysis, traceability and labelling. A compilation of relevant paragraphs is presented in several tables, taking into account special provisions regarding organic production, genetically modified organisms and allergenic substances. The legal requirements are compared to selected checkpoints of

relevant quality programs. This overview aims to support the management of the fruit and vegetable industries in their responsibility for establishing and maintaining a number of documented procedures and records. In addition, potential difficulties for smaller growers and operators are identified. Finally, an outlook on future trends is given, directed towards the development of the supply chain infrastructure as well as the emerging risks at processing and management level, and of risks relating to hurdle technology concepts.

6.2 The supply chain for fresh and minimally processed fruit and vegetables

The term 'supply chain' describes a chain of delivery, supply or company-spanning added value (Busch and Dangelmaier, 2002). An exchange of goods, services, information and/or money takes place throughout the chain. This exchange may be directed towards the consumer (downstream), the supplier (upstream) or bi-directionally. An ideal supply chain ranges from the origin of a product or of its transformed compounds to the consumer or final customer at the 'point of consumption' (Cooper *et al*., 1997; Knight *et al*., 2002). Looking at the recent developments in food supply chains, two individual types may be distinguished as follows:

- non-integrated supply chains (inward-looking, single-enterprise)
- integrated supply chains (outward-focused, dynamic and multi-enterprise extended).

6.2.1 Non-integrated supply chains

Non-integrated supply chains concern the two archetypes:

- where the grower or producer is the salesman as well
- where the produce is directly bought up by intermediaries (i.e. merchants, wholesalers).

Direct selling of fruits and vegetables may be handled via on-farm shops, at open air markets or by contracted street hawkers. This type of producer–consumer relationship is quite common in less structured rural regions, but has become more attractive for periurban growers as well. The latter benefit from the rising demand of the urban population for fresh fruit and vegetables, and commonly sell regional produce such as asparagus, strawberries, cherries, and so on in this way. Further, the increasing share of organic production has become an important driver for this segment. A recent trend is the establishment of farm shops in cities. Such shops are carried and supplied by farmer associations which – in opposition to individual producers – can offer an extended variety of products.

In the second case the products are supplied directly towards an intermediary, i.e. a wholesaler. The producer merely acts as a supplier, without establishing a closer collaboration in the spirit of chain-spanning improvements.

6.2.2 Integrated supply chains

However, most of today's food is supplied by outward-focused, dynamic and multi-enterprise chains. With respect to minimally processed fruit and vegetables, such a chain includes few or all of the following partners:

- the primary producer (i.e. farmer or grower) with its suppliers (seeds, plants, fertilisers, pesticides, packaging, etc.)
- the intermediaries (i.e. marketing organisations, merchants, brokers, wholesalers)
- the processors
- the retailers and caterers
- the consumers.

Additionally, the 'inter-nodal' logistics services (i.e. hauliers, information flows) belong to the chain as well as inspection and customs services, representing government institutions.

In the still ongoing process of further concentration, globally acting suppliers have emerged, using their market power to rule the entire chain. To give an example, the EU top five retailers have a market share of 41% and in Germany the top ten share 83% of the market (Mau, 2003). In particular with respect to the smaller producers, it is of importance that sourcing and procurement initiatives deliver higher margins with the least effort and are recognised as being the fastest way of achieving bottom line results in the chain. A 5% reduction in purchase costs can result in a 50% increase in profit margin in highly integrated supply chains (Ntlhoro, 2003).

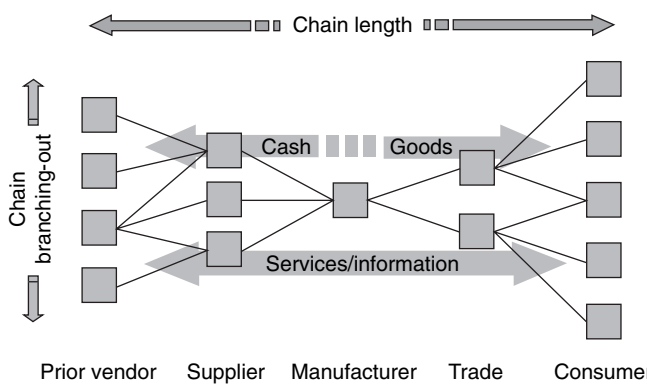

Fig. 6.1 Model of a food supply chain. (*Source*: Horváth, 2004: 7)

Consequently, the globally acting suppliers use their ruling position for stringent pricing negotiations with the producers.

In this process, the members in the chain establish symbiotic cooperation according to one or more of the following schemes (Horváth, 2004, see Fig. 6.1):

- horizontal cooperation, at same level of supply chain (in same sector/branch)
- vertical cooperation, in consecutive steps of the supply chain (between supplier and customer), i.e. licensed/contracted production, franchising, strategic alliances, joint ventures
- diagonal cooperation, merging different sectors/branches at different levels of the supply chain towards a cooperation network (virtual enterprise).

As shown in Fig. 6.2, this process goes in line with an adequate evolution of supply chain management concepts.

The first step aims to establish longer term relationships between suppliers and customers, resulting in reduced costs and improved quality. The second step targets the optimisation of both goods and information flow, with an increasing demand for both internal as well as chain spanning coordination of the operative (strategic) management. Results may be shorter production cycles or a rapid turnover of merchandise. At the third step, strategic key processes are analysed and optimised in a chain-spanning approach. As a result, the borderlines between internal structures are dissolved.

The overall aim of such cooperation is an improved position in the market, with a larger impact on competition as a result of:

- substantial savings in costs by increased productivity and reduced inventory and cycle time (optimisation of quality, price, costs and time)
- increased customer satisfaction and profits for all members

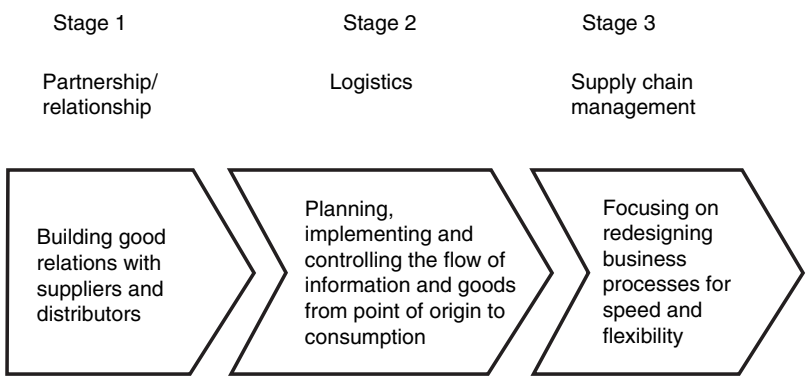

Fig. 6.2 Evolution of supply chain management. (*Source*: Horváth, 2004: 27)

- a holistic approach of added value (economies of speed (timesaving), of scale (moneysaving), of scope (networking effects))
- improved continuity of material, information and cash flows
- creation of transparency and know-how transfer
- reduction of weak information flows
- optimisation of complex chain management.

Eastham *et al*. (2001) describe this evolution in supply chain management as follows: 'Supply chain management seeks to break down the barriers that exist between each of the units in the supply chain in order to achieve higher levels of service and substantial savings in costs. Successful supply chain management coordinates and integrates all of these activities into a seamless process. It embraces the different members in the chain. In addition to the departments within the organisation, these members include suppliers, distributors and transportation carriers, third party logistics companies and information systems providers'.

The involved partners remain legally and economically autonomous in this process, but can:

- focus on their core competences
- increase their flexibility
- tap further savings.

6.2.3 Future trends in supply chain management

Most discussion of future trends is based on the assumption of a stable society with surpluses in supply and manufacturing, being dominated by consumer demand. This may apply to the western hemisphere. This is not the case in the developing world where populations are increasing, potable water is running out and the use of productive land is limited (Lillford and Howker, 2000). However, driven by economic rules, the trend towards outward-focused, dynamic and multi-enterprise supply chains in line with processes of further concentration and of establishing strategic cooperation is going onwards. The following factors may be considered as key drivers (Hahn and Kaufmann, 2002):

- globalisation with enlarged sourcing and sales markets
- increased division of labour at international placements
- growing client and consumer demands with respect to quality, time and price
- accelerated progress in information and communication technologies.

These factors affect both length and branching out of such integrated chains as well as the rapid evolution of an advanced supply chain management with its logistics elements. Key terms such as efficient consumer response (ECR) may represent this evolution. ECR is a grocery industry rationalisation effort to reduce the cost of delivering food products. It is

a reaction to the extreme competition burdened on food producers caused by:

- largely saturated and fragmented food markets
- cyclically fluctuating demand
- unsteady customer and consumer expectations.

ECR is based on a set of basic technologies (i.e. barcodes, standards of electronic data interchange (EDI), logistics standard processes (cross-docking, vendor managed inventory (VMI)) as well as on marketing-driven optimised supply (category management) (Horvàth, 2004; Eggers, 2000). It aims to optimise a holistic process in cooperative distribution systems in line with increased turnover and margins for the members involved by:

- accelerating any material and immaterial flows in the distribution system
- ensuring permanent disposability of the product, to avoid out-of-stock situations
- providing increased satisfaction for clients by supply on demand
- faster adaptation to market changes
- decreasing stocks throughout the chain with reduced stocking costs
- improved efficiency of marketing
- simplified control by balanced/continuous product flow
- greater efficiency of company-spanning production control and capacity planning.

Efficient replenishment is the most remarkable component of ECR. It consists of an automated ordering system using point of sale (POS) data. Efficient replenishment is based on EDI and supported by just-in-time (JIT) logistics. Production and supply are tailored to the actual demand. Another key expression, collaborative planning, forecasting and replenishment (CPFR) means doing this in a collaborative, chain-spanning planning process (Mau, 2003).

Contemporary and future logistics include the supply of the physical objects in line with a growing share of product-related information, where the information flow may go ahead, in line or follow behind the object. This separate handling of object and information in line with hurrying ahead information related to the supplied products is one of the most essential prerequisites of modern supply chain management. Consequently, the accelerated progress of information and communication technologies (IT) is the backbone of this development. Further benefits from using modern information technology are (Horváth, 2004):

- communication efficiency (i.e. immediate communication, short response, immediate error recovery and troubleshooting, no distortion of message, controlled information for day to day business, improved relationships through the chain, etc.)

- knowledge transfer (i.e. easy access to centralised data, allowing development and use of best practices, multi-skilled personnel, etc.)
- quick problem solving (i.e. access to advice, increased productivity, visibility allows proactivity, real time information).

In this context, the development will lead towards even more capable and integrated systems aiming to support an efficient supply chain management.

One approach which stands for this development is 'CyberTrade Xchange' (Siriram, van der Merwe, 2003). Such an electronic operating environment (EOE) serves as a neutral marketplace which supports collaborative commerce. One benefit of such a host platform is that external participants (buying companies) can use e-procurement applications without running their own system. So, there would not be a need to deal with software installation and maintenance. Such approaches will evolve as extended integration between buyers and suppliers. With respect to fresh and minimally-processed fruit and vegetables current efforts such as the Dutch 'The greenery' platform (URL: www.thegreenery.com), or the EUREKA project 'German-Polish Virtual Logistics Broker System' (URL: www.vdp-system.de; www.eureka.be) represent this trend.

6.2.4 Challenges to management in the supply chain

The evolution trends linked with progressing supply chain integration have a deep impact on the management of the primary producers and processors. To deal with these changes, each member of the chain has to manage both its operational processes (material flow, manufacturing practice) and strategic processes (development of new products, choice of suppliers and service providers, location of production, storage and distribution) while taking into account aspects of the entire chain (Corsten and Gössinger, 2001).

The management has to overcome several organisational barriers in order to establish a chain-oriented quality management. At the level of the partners (organisations), individual quality management activities have to be linked to a joint approach. At the production stage, the mentality and culture of the organisation at different levels of production should be taken into account. And finally, there are still different languages, different production methods, quality and information standards as well as differences in executing (EU) legislation between the nations. To deal with these barriers the challenge for food chains is (Schulze Althoff, 2003):

- to follow ISO 9000:2000 *et sqq*. principles
  - process approach
  - continual improvement
  - mutually beneficial supplier relationships

- to implement effective traceability, information standards and IT systems that build on existing IT infrastructure and tracking and tracing identifiers
- to motivate chain links and make them 'get more than give'
- to set up neutral information agents as 'trusted third parties'
- to link the actors across various borders.

However, in most cases consumers are considered to set the starting point for both planning and optimisation throughout the chain (Fig. 6.3). They expect to be supplied with fresh and safe fruit and vegetables:

- with high quality and extended shelf life
- ideally throughout the year
- from an environmentally sound production process
- at a moderate price.

In addition, consumers consider food companies and their management to be responsible for delivering safe food (Ryan, 2003), and have a substantial impact on the development and adoption of new (processing) technologies (Lillford and Howker, 2000).

In reaction to the demand of consumers for fruit and vegetables to be available throughout the year – consequently from different producers, but with constant quality – leading suppliers and retailers have developed numerous quality criteria and quality programmes. These programmes basically have been developed to (SAI, 2004):

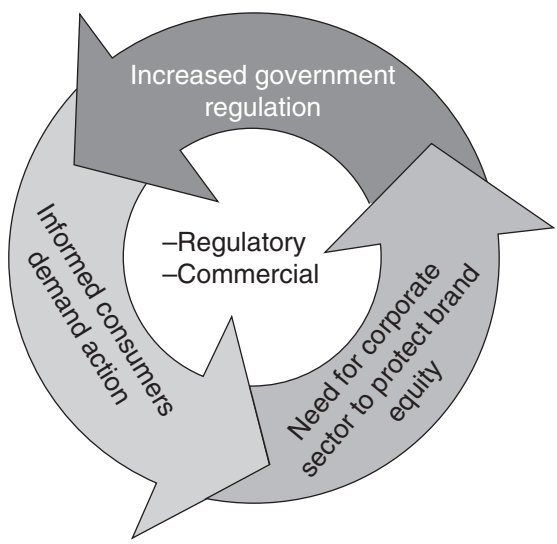

Fig. 6.3 Drivers of market trends. (*Source*: Ryan, 2003)

- provide the base for ensured food safety, by producing high-quality agricultural raw materials and by supporting innovations to improve their quality and safety
- secure adequate food supplies, to meet current and future food demand, by producing high yielding and healthy crops and animals, while increasing efficiency and keeping resource and external input requirements as low as possible
- protect and possibly improve the natural environment and resources, by minimising any adverse effects from agricultural activities on soil, water, air and biodiversity, optimising the use of renewable resources and caring for animal welfare
- support economically viable and responsible farming systems, enabling local communities to protect and improve their livelihood, safeguard their environment and improve their well-being.

To meet these aims, several normative regulations have been developed which usually contain of a set of general regulations and of checkpoints which have to be maintained by the producers if they want to enter the value chain. Prominent initiatives arose from:

- the British Retail Consortium, in particular the 'BRC Global Standard – Food' (BRC, 2003)
- the Euro-Retailer Produce (EUREP) Working Groups, with the support from producer organisations outside the EU (EUREPGAP certification documents, in particular the 'Control Points & Compliance Criteria Fruit and Vegetables' (EUREPGAP, 2004))
- the Global Food Safety Initiative, in particular the GFSI Guidance Document (GFSI, 2003)
- the Bundesverband Deutscher Handelsverbände, in particular the 'International Food Standard' (BDH, 2003)
- the Assured Produce Scheme, in particular the Generic Crop Protocol (APS, 2003)
- the Comité du Commerce des céréales, aliments du bétail, oléagineux, huile d'olive, huiles et graisses et agrofournitures (COCERAL), in particular the European Code of Good Trading Practice (COCERAL, 2003).

In the future, growers and (minimal) processors of fruit and vegetables will be unable to enter the value chain without having an audited and certified self-control system for quality control and quality management (EN ISO, 2004). The establishment of synergistically horizontal associations would be an option for smaller producers and processors who are unable to realise the auditing and certification routines for themselves. Such associations should act in the spirit of supply chain partnerships which jointly define and coordinate all processes relevant to safety and quality.

The most complex processes would be those of chain-spanning information management for individually produced batches and charges. This

information management should supply information for the chain partners such as information:

- on cultivation, processing and quality parameters of each charge
- for tracking and tracing of each charge throughout the chain
- on recalls of products
- prognostic information on expected quality and quantity of production
- on measures related to both quality programmes and management (i.e. benchmarking of suppliers, audits, quality aims)
- on self-control systems including risk assessment
- on achieved continuous improvements.

6.3 Quality and risk management in the supply chain

6.3.1 Risks for fresh and minimally processed fruit and vegetables

In general is has to be assured that no unsafe foods are supplied which either are potentially hazardous for health or are not suitable for consumption or contaminated. In this context, the following types of risk are usually distinguished:

- biological risks: pests such as birds, gnawers, cockroaches or flies and their excrement
- chemical risks: residues and contaminants from biogenic metabolism or from the environment (polychlorinated biphenyls, PCBs; BTX (benzene, tolven and xylene), dioxins) or from animal breeding (organochlorpesticides, organophosphorpesticides, animal pharmacy drugs) or cleansing agents
- physical risks: ionising radiation; foreign bodies such as of glass, metals, plastics or wood
- microbiological risks: foodborne diseases pathogens (i.e. *Salmonella*; *Campylobacter*; enterovirulent and enterohemorrhagic *Escherichia coli* (EHEC); noroviruses and rotaviruses; fungi generating mycotoxins (gen. *Aspergillus*, *Penicillium*, *Fusarium*).

Minimally processed, convenient, ready-to-eat, but ambient-stable foods are the trend in industrialised as well as in developing countries, since these foods have appealing fresh-like characteristics and thus a superior sensory quality (Leistner, 2002). Another advantage is that minimal processing procedures can be applied at various stages of the food processing and distribution chain (processing, storage, packaging). However, such minimally processed products are rarely 'commercially sterile' and can undergo rapid deterioration (Table 6.1). To counteract this, minimal processing is frequently associated with hurdle technology concepts where several systems (additives, packaging systems, refrigeration systems) are used to inhibit spoilage. Through the combination of adequate preservation techniques, a

Table 6.1 Risks occurring in selected fresh and processed fruits and vegetables (*Source*: Kramer *et al*., 2003)

Risks	**Strawberry**	fresh	processed	**Orange**	fresh	processed	**Grape**	fresh	processed	**Potato**	fresh
1. Biological											
Bacteria											
E. *coli* (toxins)		+	+			+		+			+
Listeria monocytogenes		+	+		+			+			+
Clostridia (toxins) e.g. *Clostridium perfringens, Clostridium botulinum*		+	+		+			+			+
Bacillus cereus and other bacilli (+toxins)											+
Shigella spp. (+toxins)											+
Staphylococcus aureus (+toxins)		+	+		+	+		+			
Pseudomonas spp.		+	+		+	+		+			+
Yeasts		+	+		+	+		+			
Fungi (Mycotoxins)		+	+		+	+		+	+		+
Aspergillus (aflatoxin, ochratoxin, nidulin, territreme)								+	+		
Penicillium spp. (patulin, ochratoxin, ragulosin, paxilline, roquefortine)		+	+		+	+		+	+		+
Gliocladium fimbriatum (gliotoxin) and Trichoderma spp. (peptaibols)		+	+		+	+		+			
Fusarium spp. and toxins		+	+		+	+		+	+		+
Wet rot (*Rhizopus nigricans*)		+			+			+			
Leather rot (*Phytophtora cactorum*)		+									
Rots											
Green rot (Penicillium spp.)					+			+			
Blue rot (*P. italicum*)					+						
Grey rot (*Botrytis cinerea*)		+						+			
Mildew (*Plasmopara viticola, Uncinula necator*)								+			
Potato wet rot (Erwinia spp.)											+

Table 6.1 *Continued*

Risks	**Strawberry**	fresh	processed	**Orange**	fresh	processed	**Grape**	fresh	processed	**Potato**	fresh
Bacterial brown rot (*Pseudomonas solanacearum*)											+
Potato crust (*Streptomyces scabies*)											+
Dry rot (Fusarium spp.)					+						+
Viruses/contamination (Noro-, rotaviruses etc.)		+	+		+	+		+	+		+
Parasites:											
Nematodes (spoolworms . . .)		+			+			+			
Helminthes (bandworms)		+			+			+			
2. Chemical											
Pesticides (PCBs etc.)		+	+		+	+		+	+		+
Pharmacological residues (medicals, food additives, antibiotic stimuli, coczidiostatics, hormones)											
Heavy metals:											
Pb		+	+		+	+		+	+		+
Cd		+	+		+	+		+	+		+
Hg		+	+		+	+		+	+		+
Additives (nitrate, nitrite etc.)		+	+		+	+		+	+		+
Allergenes (biogene amines, glutamate . . .)		+	+		+	+		+	+		
3. Physical											
Intrinsic contaminants (bones, kernels, stalks)			+			+			+		
Extrinsic contaminants (glass, metal, insects, animals)		+	+		+	+		+	+		+
Radiation		+	+		+	+		+	+		+

series of preservative factors, called hurdles, may be established that cannot be overcome by microorganisms.

Common hurdles are temperature (heating, cooling), water activity (a_w), pH, redox potential and preservatives (chemical agents, bacteriocins). Novel preservative factors include gas packaging, ultra-high pressure treatment, edible coatings and use of bacteriocins. Such hurdles are synergistic in their operation and use combinations of effects (McKenna, 2002).

Minimal processing of fruit

A hurdle concept that has been proved for fruit consists of mild heat treatment (blanching), slight reduction of a_w and pH, and the moderate addition of preservatives (sorbate and sulphite). The blanching of the fruits (partial decontamination with steam) is important for microbial stability because, even though vegetative microorganisms might survive this mild heat treatment, their number is reduced and thus only fewer and lower hurdles are essential. Such applications result in fresh-like fruits which for several months are microbiologically safe and stable at ambient temperature. The number of surviving bacteria, yeasts and moulds decreases rapidly during ambient storage of the products, probably owing to metabolic exhaustion, since they are not able to multiply in stable hurdle technology fruits. In addition, the added sulphite and sorbate are depleted during storage and this is of benefit for the consumer but at the same time stability against microbial activity is also diminished. Therefore, recontamination of the fruit during storage should be avoided by suitable measures (Leistner, 2002).

Minimal processing of vegetables

Minimally processed vegetables (e.g. raw, sliced vegetables or cooked *sous vide* dishes) are heated only mildly or not at all and must be stored under refrigeration. Additional hurdles such as modified-atmosphere packaging or vacuum packaging, possibly the addition of bacteriocins or bacteriostatic spices, or treatment with ultra-high pressure are often applied. For the raw, sliced vegetables *Listeria monocytogenes* is of major concern, whereas, for the *sous vide* dishes, the concern is non-proteolytic *Clostridium botulinum*. Minimally processed vegetables are more risky with respect to safety because they are not ambient-stable foods and thus have to be stored under strict refrigeration. Pathogenic bacteria survive longer in minimally processed vegetables than in minimally processed fruit because metabolic exhaustion hardly takes place during storage (Leistner, 2002).

When fruit and vegetables are processed and preserved only by relatively mild techniques, a new habitat for microbial growth may have come into focus. It has been found that fresh and minimally processed fruit and vegetables are safe and stable under strictly controlled processing and storage conditions (Leistner, 2002). However, information on important

factors affecting the survival and growth of the relevant microorganisms under mild preservation conditions is required (Gorris and Tauscher, 1999). The investigation of behaviour is of particular importance for the application of new, non-thermal processing techniques such as radiation, high-pressure filtration, additives, electric pulses and oscillating magnetic waves. Such knowledge of the behaviour of the most resistant pathogen during the treatment is required to establish effective minimal processing protocols, including the appropriate kinetics for the full extent of the process operating range (Swartzel, 2002).

6.3.2 Risk analysis and the house of food safety

As shown in the sections above, today's management in the food sector has to deal with a wide range of both strategic and operational management and related risks at both enterprise and supply chain levels. With respect to food quality and safety, risk analysis at both enterprise and supply chain level is one of the major responsibilities of management. Management is committed to developing and implementing a quality management system that includes a framework of risk analysis and to improving continually its effectiveness. To develop a process-based quality management system, documented procedures have to be established for (EN ISO, 2000; EN ISO, 2004):

- the control of documents: approving for adequacy prior to issue; reviewing, updating and reapproving; identification of changes and of the current revision status; ensuring availability at point of use; identification and controlled distribution of documents of external origin; preventing use of obsolete documents
- the control of records: definition of controls needed for the identification, storage, protection, retrieval, retention time and disposition of records
- internal audits: planning of an audit programme regarding audit criteria, scope, frequency and methods; responsibilities and requirements for planning and conducting audits, and for reporting results and maintaining records; verification of follow-up actions taken, and reporting of verification results
- corrective actions within the process of continual improvement: reviewing of non-conformities (including customer complaints); determination and implementation of actions needed; records of results of actions taken; reviewing corrective actions
- preventive actions: determination of potential non-conformities and their causes; determination and implementation of actions needed; records of results of actions taken; reviewing corrective actions.

Demands to undertake such continuous improvements in a chain-spanning approach arise from the White Paper on Food Safety of the EU (CEC, 2000)

and from the General Principles of the Codex Alimentarius Commission of the FAO (FAO/WHO, 2001). In this context the risk assessment is a structured process for determining the risk associated with any type of hazard (biological, chemical or physical). It has as its objective a characterisation of the nature and likelihood of harm resulting from human exposure to agents in food. The characterisation of risk typically contains both qualitative and quantitative information. For the future, risk assessments will help operators to develop HACCP plans on a scientific basis.

Risk assessments also play an important role in international trade. Without a systematic risk assessment, countries may set requirements that are not related to food safety and could create artificial barriers to trade. Recognising the importance of a science-based approach to fair trade, the World Trade Organization requires each country's food safety measures to be based on risk assessment. The Codex Alimentarius Commission, which has already established international food safety standards, is now developing principles for using risk assessment in such standards.

Risk assessment has its roots in concerns about toxic chemicals in food. While these assessments are based on toxicology and carcinogenity studies, their application to microbial pathogens (microbiological risk assessment (MRA)) poses some significant difficulties. Unlike chemical, environmental or toxicological contaminants, bacteria can multiply as conditions change throughout the entire supply chain. The development of predictive models and other tools is therefore required to quantify estimates of risk. Another uncertainty arises from lack of information on the relationship between the quantity of a biological agent and the frequency and magnitude of adverse human health effects. Further, there is limited information on exposure assessment – the accounts of foods consumed by populations and their probable contamination.

Risk analysis is a process consisting of three interconnected components:

- *Risk assessment* is a scientifically based process, consisting of four steps:
  - hazard identification (collection, organisation and evaluation of all information pertaining to a pathogen or a nutrient),
  - hazard characterisation (determining the relationship between a pathogen and any adverse effects),
  - exposure assessment (determining how much of pathogen might be ingested in a serving of food),
  - risk characterisation (evaluating the risk and related information).

  The scientific basis to support risk assessment in an independent and transparent manner may be given by data about foodborne diseases, data about costs of foodborne diseases, by identified new risk factors or by sensitivity analyses.
- *Risk management* is a process, distinct from risk assessment, of weighing policy alternatives in consultation with interested parties, considering risk assessment and other legitimate factors, and, if needed, selecting

appropriate prevention and control options. Strategic alternatives should be considered which are based on the results of the risk assessment. The controllability of derived measures (control measures (including registration and traceability), product standards, measuring strategy) must be taken into account as well as their socioeconomic and environmental impact. The scientific basis of supporting risk management may be given by intervention possibilities, analyses of costs-effectiveness, or by realisation and verification (implementation of the risk management decision, monitoring and review).

- *Risk communication* is an interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions. Ideally, all relevant groups such as risk assessors, risk managers, consumers, feed and food business, the academic community and other interested parties should be involved from the start in both internal (enterprise) and external (chain) interactive information exchange of risk-related issues including the explanation of risk assessment findings and the basis of risk management decisions. Science-based intervention may be based on interviews, or of repeated analysis of the factors mentioned above.

All the three components constitute the framework of risk analysis that is directed towards a continuous improvement of the chain-spanning quality management. It is the responsibility of the management to avoid and to control food safety related risks. At this point, the management has to build its house of food safety with the two complementing components of the self-control system (see Fig. 6.4):

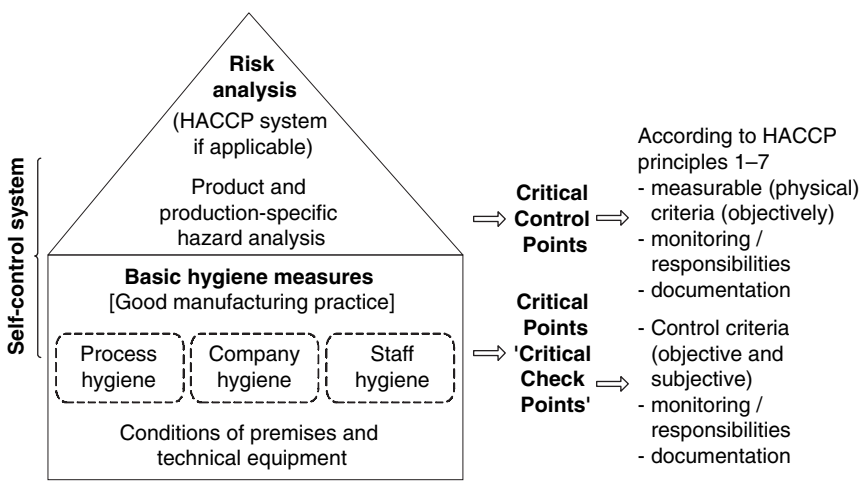

Fig. 6.4 The house of food safety. (Modified from Untermann, 1998)

- basic hygiene measures (process, enterprise and staff hygiene, including duty of care): these measures strongly depend on the qualification and motivation of the entire staff and management. They interdepend on the entire production environment provided by the conditions of technical equipment and of other premises (i.e. choice of suppliers, training). The measures are usually described in product-specific guidelines of good manufacturing practice (APS, 2003; EUREPGAP, 2004; FDA/USDA, 1998). These basic measures should be designed to assure safe products on their own.
- Hazard Analysis and Critical Control Point (HACCP) system: this system includes both product and production-specific preventive measures based on a specific hazard analysis (FAO/WHO, 2001; Untermann, 1998). Such a system should only be implemented as far as necessary and when applicable (CEC, 2004).

6.3.3 Misinterpretation of risk-related issues

Although the HACCP system was developed in the USA around 1960, it became increasingly known throughout the world after 1985, in the course of public discussion on food contamination and intoxication. For HACCP systems, seven principles have been developed which are structured in three elements (Untermann, 1998):

- hazard identification and risk assessment: principle 1 (conduct hazard analysis)
- measures to control the identified hazards: principles 2 (determine Critical Control Points), 3 (critical limits), 4 (monitoring) and 5 (corrective action)
- verification and documentation of the system: principles 6 and 7.

Although the application of a HACCP system is described in detail in Chapter 7 of this book it should be pointed out that in practice the HACCP system and its related terms such as 'Critical Control Point (CCP)' sometimes are subject to divergent interpretation in a misleading way. In those cases, a purely formal compliance with HACCP principles is sought, and measures and critical limits are defined which are not in agreement with the original Codex Alimentarius approach. For example, the implementation of basic process hygiene measures sometimes may be declared as a 'HACCP concept' although a specific hazard analysis is not carried out. Such misinterpretation will cause a large number of 'costly check points' (10 or even more in practice) instead of CCPs, leading to a decline in the effectiveness of food safety concepts and in this way to a watering down of the HACCP approach (Untermann, 1998). In case of doubt, the Codex Alimentarius (FAO/WHO, 2001) should be preferred for further consultation instead of derived directives or guidelines.

A CCP should be identified according to the Codex Alimentarius principles 1 to 7, as a step in the production or processing process where it is possible and essential (despite the implemented basic hygiene measures) to bring a hazard under control, i.e. to eliminate it or to reduce it to an acceptable level. Examples may be the refrigeration of fresh fruit and vegetables during storage with a defined and controlled temperature and time regime to reduce the occurrence of pathogens, or the high-pressure treatment at a certain pressure within a predefined time interval. In consequence, CCPs can be identified in the post-harvest supply chain, for processing, storage and supply. It is not common so far to set CCPs in primary production although there is a trend to apply HACCP principles (APS, 2003; EC, 2004a). For the position in the chain occupied by primary production, a number of produce-related 'Critical *Check*points' have been identified and described in the above-named quality programmes (i.e. EUREPGAP, 2004; BRC, 2003). Such programmes aim to qualify the basic hygiene management of the individual growers because the retailers and wholesalers who establish such programmes intend to market produce of equal quality grown under equal production standards in a globally sourcing supply chain. As a consequence, a number of CCPs are defined as 'KO' criteria what means that a grower or operator will not gain approval if it does not meet any of such criteria. To give an example, the 'KO' criteria in the BDH (2003) are:

- identification of a handleable number of CCPs, being relevant to the process concerned
- establishment of a system to monitor control of the CCPs. Each CCP shall be controlled, records shall be kept for a defined period
- the company's director shall ensure that all employees are aware of their responsibilities and mechanisms are in place to monitor the effectiveness of their operation
- evidence shall be available in the organisation of the ability to trace any product back to the raw material supplier and to track it to the supplied customer, including ascertaining the date of supply
- corrective actions shall be undertaken in a timely manner to prevent further occurrence of non-conformity.

6.4 Critical points in the supply chain

6.4.1 General principles and requirements of European legislation

The production of fresh and minimally processed fruit and vegetables is extensively regulated by recent European legislation. With the Regulation EC 178/2002 (EC, 2002), the EU has laid down general principles and requirements of food law which apply to all stages of production, processing and distribution. One general objective is the high level of protection of human health, taking into account the protection of plant health and of

the environment. To assure this, all measures taken shall be based on the five principles of:

- risk analysis
- precaution
- protection of consumers' interests
- public consultation, and
- public information.

Unsafe food that is injurious to health, or unfit for human consumption shall not be placed on the market. In this context, the probable immediate, short-term or even long-term effects of food on the health of consuming persons, and on subsequent generations shall be taken into account, as well as specific adverse health effects such as allergies (CEC, 2004a).

Responsibilities

The responsibilities of every food operator are defined in the Articles 17 and 19 of Regulation EC 178/2002. Within the entire businesses under control, the operator has to ensure that foods satisfy the requirements of any relevant food law which are relevant to its activities and shall verify that all requirements are met. If the operator considers or has reason to believe that food is not in compliance with safety requirements, the operator shall immediately initiate procedures to withdraw the food from the market and shall inform the competent authorities thereof. If food may already have reached the consumer, the operator shall inform the consumers of the reason for the withdrawal, and if necessary, recall products already supplied to the consumers when other measures are not sufficient. Further, operators shall collaborate with the competent authorities on action taken to avoid or reduce risks. If unsafe food is part of a batch of the same class, it shall be presumed that all food in that batch is also unsafe, unless there is no evidence of unsafeness resulting from a detailed assessment (Article 14).

Traceability and labelling

Another issue of EU Regulation 178/2002 concerns traceability. According to Article 18, traceability shall be established at all stages of production, processing and distribution. The food business operators shall be able to identify any person from whom they have been supplied with a food, or substance to be incorporated into a food, and in addition to identify the other businesses to which their products have been supplied. Systems and procedures shall be in place which allow for this information to be made available to competent authorities on demand. All requirements of the Regulation EC 178/2002 shall be effective by January 2005.

Special requirements relate to traceability of genetically modified organisms (GMO) and to food that may cause allergies. With Regulation EC 1830/2003 the labelling of any food consisting of or containing GMOs is

required unless the technically unavoidable proportion of GMO does not exceed 0.5–0.9% (EC, 2003b).

With respect to allergenic substances, the basic regulation to be noticed is Directive 2000/13/EC on labelling, presentation and advertising of foodstuffs (EC, 2000). It details which ingredients shall be designated. The respective annexes have been updated by Directive 2003/89/EC (EC, 2003c) with regard to substances which are recognised to cause hypersensitivity such as soybeans, milk products (lactic acid), nuts, celery, mustard, sesame seeds or sulphur dioxide and sulphites.

Consequently, the use of potentially allergenic substances as additives or processing aids for minimal processing should be avoided if they remain present in the finished product, even if in altered form. Anyway, if substances listed in the annexes of the amended Directive 2000/13/EC are used for minimal processing the labelling requirements should be observed. A summary of significant EU legislation concerning traceability and labelling, and how these issues are considered in quality programmes such as the 'BRC Global Standard – Food' is given in Table 6.2.

6.4.2 European regulation EC 852/2004 on food hygiene

The basic requirements of food hygiene are established by the EU Regulation EC 852/2004 on the hygiene of foodstuffs (EC, 2004a) which covers all stages of production, processing and distribution. The regulation mainly contains of a set of obligations for food business operators (Articles 3 to 6) and of guides to good practice (Articles 7 to 9, with Annexes). According to the Regulation, operators shall ensure that all production stages under their control satisfy the relevant hygiene requirements. As appropriate, they shall adopt specific hygiene measures such as:

- compliance with microbiological criteria
- temperature control requirements
- maintenance of the cold chain
- sampling and analysis.

Further, operators which carry out any stage after primary production shall put in place permanent procedures based on the seven HACCP principles as contained in the Codex Alimentarius. While establishing HACCP-based procedures should not initially apply to primary production so far, the feasibility of such an extension will be one element of the next reviews by the EU. In consequence, operators at the level of primary production are already encouraged to apply such HACCP principles as far as possible (considerations 10–12, 14–15). A recommended guide for conducting hazard analyses in the primary production is given with the 'Generic Crop Protocol' of Assured Produce Ltd. (APS, 2003) in line with the DG SANCO working document (CEC, 2004b). An overview on the specific provisions to HACCP and related checkpoints in established quality programmes is given in Table 6.3.

Table 6.2 Specific provisions of European legislation for traceability and labelling, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s))

	1	2	3	4
Regulation EC 178/2002 (EC, 2002)				
Article 18 – Traceability				
1. Traceability of food, feed, food-producing animals, and any other substance intended to be, or expected to be, incorporated into a food or feed established at all stages of production, processing and distribution.	+	+	+	+
2. Business operators able to identify any person from whom they have been supplied with a food . . . , or any substance intended to be, or expected to be, incorporated into a food . . . operators have in place systems and procedures . . . [to make available this information] to competent authorities . . .	+	+	+	+
3. Business operators have in place systems and procedures to identify the other businesses to which their products have been supplied. This information is available to competent authorities on demand.	+	+	+	+
4. Food which is placed on the market or is likely to be placed on the market in the Community adequately labelled . . . to facilitate traceability, through relevant documentation or information . . .	+	+	+	+
Regulation EC 852/2004 (EC, 2004a)				
Annex I Part B Par. 2 Guides to good hygiene practice should include appropriate information on hazards . . . and actions to control hazards, including relevant measures . . . Examples . . . may include:				
(c) the correct and appropriate use of plant protection products and biocides and their traceability			+	
Regulation EC 1830/2003 (EC, 2003b)				
Article 4 – Traceability and labelling requirements for products consisting of or containing GMOs				
1. At the first stage of the placing on the market . . . operators shall ensure that the following information is transmitted in writing to the operator receiving the product: (a) that it contains or consists of GMOs; (b) the unique identifier(s) assigned to those GMOs . . .				
2. At all subsequent stages . . . operators ensure that information received in accordance with Par. 1 is transmitted in writing to the operators receiving the products.		+	+	
7. Paragraphs 1 to 6 shall not apply to traces of GMOs . . . in a proportion no higher than the thresholds established in accordance with Article 21(2) or (3) [0.9%] of Directive 2001/18/EC . . .				

Table 6.2 *Continued*

	1	2	3	4
8. Paragraphs 1 to 6 shall not apply to traces of GMOs in products intended for direct use as food, feed or for processing in a proportion no higher than the thresholds established for those GMOs in accordance with Articles 12 [0.9%], 24 [0.9%] or 47 [0.5%] of Regulation (EC) No 1829/2003 . . .				
Article 5 – Traceability requirements for products for food and feed produced from GMOs				
1. . . . operators ensure that following information is transmitted in writing to the operator receiving the product: (a) an indication of each of the food ingredients which is produced from GMOs; (c) in case . . . [that] . . . no list of ingredients exists, an indication that product is produced from GMOs.				
2. . . . operators have in place systems and standardised procedures to allow the holding of the information specified in paragraph 1 and the identification, for a period of five years from each transaction . . .		+	+	
4. Paragraphs 1, 2 and 3 shall not apply to traces of GMOs in products . . . in a proportion no higher than the thresholds established . . . with Articles 12 [0.9%], 24 [0.9%] or 47 [0.5%] of Regulation (EC) No 1829/2003, provided that these traces of GMOs are adventitious or technically unavoidable				
Directive 2000/13/EC (EC, 2000)				
Article 3				
1. . . . indication of the following particulars alone shall be compulsory on the labelling of [processed] foodstuffs: (1) name under which the product is sold; (2) list of ingredients; (3) quantity of certain ingredients . . .; (4) in case of prepackaged foodstuffs, the net quantity; (5) date of minimum durability or, [for] . . . highly perishable [food], the 'use by' date; (6) any special storage conditions or conditions of use; (7) name or business name and address of the manufacturer or packager . . . (8) particulars of the place of origin or provenance . . .; (9) instructions for use . . .				
Article 5				
3. The name under which the product is sold shall include or be accompanied by particulars as to the physical condition of the foodstuff or the specific treatment which it has undergone (e.g. powdered, freeze-dried, deep-frozen, concentrated, smoked) in all cases where omission of such information could create confusion in the mind of the purchaser. Any foodstuff which has been treated with ionising radiation must bear . . . the following indications: 'irradiated' or 'treated with ionising radiation'.				
Article 6				
2.(a) Ingredients need not be listed in the case of fresh fruit and vegetables, including potatoes, which have not been peeled, cut or similarly treated				

Table 6.2 *Continued*

1	2	3	4

Regulation EC 907/2004 (EC, 2004b)
Article 1
Stickers individually affixed on product shall be such as, when removed, neither to leave visible traces of glue, nor to lead to skin defects.

Selected checkpoints of relevant quality programmes

- Product traceable back to and trackable from the farm where it has been grown
- Traceability system regularly tested to ensure traceability from raw material source to finished product
- Identification through a code marking on container and product, to identify the source of any out-sourced product, ingredient or service
- Record of purchaser and delivery destination for all product supplied
- Effective documented product recall procedure in place to ensure that all potential risks to the quality, safety and legality are controlled. Procedure shall be regularly reviewed, and if necessary, revised to ensure currency
- Evidence of annual residue testing . . . traceable to the farm
- Compliance of GMO's planting with all applicable legislation in the country of production
- Documentation of any planting, use or production from genetic modification
- Suppliers' declaration on use of GMO's and/or presence of allergenes available
- Identification of products containing GMO's assured at any time
- Adequate labelling for the identification of products containing GMO's and/or allergenes
- A system in place to ensure the identification of most common allergens in food and food additives

Further relevant EU regulation:

Council Directive 89/396/EEC of 14 June 1989 on indications or marks identifying the lot to which a foodstuff belongs, Official Journal L 186, 30 June 1989, 21–22 (EC, 1989)

Commission Regulation (EC) 907/2004 of 29 April 2004 amending the marketing standards applicable for fresh fruit and vegetables with regards to presentation and labelling, Official Journal L 163, 30 April 2004, 50–55 (EC, 2004c)

GMO:

Commission Regulation (EC) 1829/2003 of 22 September 2003 on genetically modified food and feed, Official Journal L 268, 18 December 2003, 1–23 (EC, 2003a)

Allergens:

Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs, Official Journal of the European Union L 308, 25 November 2003, 15–18 (EC, 1989)

Table 6.3 Specific provisions of EU Regulation 852/2004 to HACCP, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s))

	1	2	3	4
HACCP				
Article 1 – Scope				
1(d) general implementation of procedures based on the HACCP principles, together with the application of good hygiene practice, should reinforce food business operators' responsibility;				
1(e) guides to good practice are a valuable instrument to aid food business operators at all levels of the food chain with compliance with food hygiene rules and with the application of the HACCP principles;				
Article 5 – Hazard analysis and critical control points				
1. Food business operators shall put in place, implement and maintain a permanent procedure or procedures based on the HACCP principles.	+	+		+
2. The HACCP principles referred to in paragraph 1 consist of the following:				
(a) identifying any hazards that must be prevented, eliminated or reduced to acceptable levels;				
(b) identifying the critical control points at the step or steps at which control is essential to prevent or eliminate a hazard or to reduce it to acceptable levels;				
(c) establishing critical limits at critical control points which separate acceptability from unacceptability for the prevention, elimination or reduction of identified hazards;				
(d) establishing and implementing effective monitoring procedures at critical control points;				
(e) establishing corrective actions when monitoring indicates that a critical control point is not under control;	+	+		+
(f) establishing procedures, which shall be carried out regularly, to verify that the measures outlined in subparagraphs (a) to (e) are working effectively;				
(g) establishing documents and records commensurate with the nature and size of the food business to demonstrate the effective application of the measures outlined in subparagraphs (a) to (f). When any modification is made in the product, process, or any step, food business operators shall review the procedure and make the necessary changes to it.				
3. Paragraph 1 shall apply only to food business operators carrying out any stage of production, processing and distribution of food after primary production and those associated operations listed in Annex I.				
4. Food business operators shall:				
(a) provide the competent authority with evidence of their compliance with paragraph 1 in the manner that the competent authority requires, taking account of the nature and size of the food business;				

Table 6.3 *Continued*

	1	2	3	4
(b) ensure that any documents describing the procedures developed in accordance with this Article are up-to-date at all times;	+	+		+
(c) retain any other documents and records for an appropriate period.				

Selected checkpoints of relevant quality programmes

- The basis of the company's food safety control system shall be a HACCP plan which shall be systematic, comprehensive, thorough, fully implemented and maintained and shall be based on the Codex Alimentarius HACCP principles. 1. Assemble HACCP team; 2. Describe product; 3. Identify intended product use; 4. Construct flow diagram; 5. On-site confirmation of flow diagram; 6. List all potential hazards associated with each step, conduct a hazard analysis, and consider any measures to control identified hazards. (Principle 1); 7. Determine Critical Control Points (Principle 2); 8. Establish critical limits for each CCP (Principle 3). Criteria often used include measurements of temperature, time, moisture level, pH, A_w, or available chlorine; 9. Establish a monitoring system for each CCP (Principle 4); 10. Establish corrective actions (Principle 5); 11. Establish verification procedures (Principle 6); 12. Establish documentation and record keeping (Principle 7)
- Identification of a handleable number of relevant CCPs, implementation of a system for the control of CCPs, and of record-keeping
- The HACCP system shall be specific to the application, practical to implement and effective in controlling the associated hazards of the operation. This will include all existing and new products and the HACCP system shall be regularly and appropriately reviewed
- HACCP System shall be developed, reviewed and managed by a multidisciplinary team. The HACCP team leader or nominated team representative shall be able to demonstrate competence in understanding HACCP principles and their application. Key personnel identified as HACCP team members shall have adequate training and experience

Regulation EC 852/2004 shall apply by 1 January 2006. With it, a framework is set for the development of both EU and national guides to good practice for hygiene and for the application of HACCP principles. Such national and Community guides should refer to the relevant codes of practice of the Codex Alimentarius, i.e. FAO/WHO (2001) as well as to detailed recommendations in the two Annexes of the Regulation 852/2004.

Annex I applies to primary production and its associated operations of transport, storage and handling of primary products at the place of production, provided that this handling does not substantially alter their nature. Further, it applies to transport operations to deliver primary products from the place of production to an establishment. The Annex I consists of two parts:

- part A, with provisions for general hygiene, and for record-keeping
- part B, with recommendations for guides to good hygiene practice. Such guides should include appropriate information on hazards that may arise in primary production and associated operations, and actions to control hazards.

The requirements of Annex I and their relation to relevant checkpoints are summarised in the Tables 6.4 and 6.5.

Table 6.4 Provisions and recommendations of relevant EU Regulations 2092/91, 850/2004 and 852/2004 on hygiene in primary production, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – CORECAL; + – equivalent/similar checkpoint(s); (+) – notice recommended)

		1	2	3	4
EC 852/2004 Hygiene provisions					
2.	To protect primary products against contamination as far as possible, having regard to any processing that primary products will subsequently undergo	(+)	+	+	+
3.	To comply with appropriate Community and national legislative provisions relating to the control of hazards in primary production and associated operations, including:	(+)	+	+	+
	(a) measures to control contamination arising from the air, soil, water, feed, fertilisers, veterinary medicinal products, plant protection products and biocides and the storage, handling and disposal of waste	(+)	+	+	+
	(b) measures relating to plant health that have implications for human health, including programmes for the monitoring and control of zoonoses and zoonotic agents	(+)	+	+	+
5.	To take adequate measures, as appropriate:	(+)	+	+	+
	(a) To keep clean and, where necessary after cleaning, to disinfect, in an appropriate manner, facilities, equipment, containers, crates, vehicles and vessels	(+)	+	+	+
	(b) To ensure hygienic production, transport and storage conditions for, and the cleanliness of, plant products	(+)	+	+	
	(c) To use potable water, or clean water, whenever necessary to prevent contamination	(+)	+	+	
	(d) To ensure that staff handling foodstuffs are in good health and undergo training on health risks	(+)	+	+	+
	(e) as far as possible to prevent animals and pests from causing contamination	(+)		+	+
	(f) to store and handle wastes and hazardous substances so as to prevent contamination	(+)	+	+	+
	(g) to take account of the results of any relevant analyses carried out on samples taken from plants or other samples that have importance to human health			+	+
	(h) to use plant protection products and biocides correctly, as required by the relevant legislation			+	
6.	To take appropriate remedial action when informed of problems identified during official controls			+	

Table 6.4 *Continued*

	1	2	3	4
EEC 2092/91 Article 6 – Rules of organic production (EC, 1991)				
1. The organic production method implies that . . .				
(a) at least the requirements of Annex I and, where appropriate, the detailed rules relating thereto, must be satisfied;				
(b) only products composed of substances listed in Annexes I and II may be used as plant-protection products, detergents, fertilisers, or soil conditioners . . .				
2. By way of derogation from paragraph 1 (b), seeds treated with products not included in Annex II and authorised . . . may be used in so far as users . . . were unable to obtain on the market non-treated seed of an appropriate variety of the species in question.				
EC 852/2004 Guides to good hygiene practice should include				
2. Appropriate information on hazards that may arise in primary production and associated operations and actions to control hazards, including relevant measures set out in Community and national legislation or national and Community programmes. Examples of such hazards and measures may include:	(+)	+		+
(a) the control of contamination such as mycotoxins, heavy metals and radioactive material	(+)	+		+
(b) the use of water, organic waste and fertilisers	(+)		+	
(c) the correct and appropriate use of plant protection products and biocides and their traceability	(+)		+	
(g) protective measures to prevent contagious diseases transmissible to humans through food, and any obligation to notify the competent authority	(+)	+	+	+
(h) procedures, practices and methods to ensure that food is produced, handled, packed, stored and transported under appropriate hygienic conditions, including effective cleaning and pest-control measures relating to record-keeping	(+)	+	+	
(j) measures relating to record-keeping	(+)	+	+	+
EC 850/2004 (EC, 2004d) **on persistent organic pollutants [and on prohibited plant protection products]**	+	+	+	
Article 3 – Control of production, placing on the market and use				
1. The production, placing on the market and use of substances listed in Annex I, whether on their own, in preparations or as constituents of articles, shall be prohibited.				
Article 6 – Release reduction, minimisation and elimination				
1. Within two years . . . Member States shall draw up and maintain release inventories for the substances listed in Annex III into air, water and land . . .				
Article 7 – Waste management				
1. Producers and holders of waste shall undertake all reasonable efforts to avoid, where feasible, contamination of this waste with substances listed in Annex IV.				

Table 6.4 *Continued*

	1	2	3	4
Annex I – List of substances subject to prohibitions	+	+	+	
Aldrin, Chlordane, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex, Toxaphene, Polychlorinated				
Biphenyls (PCB), DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane), Chlordecone, Hexabromobiphenyl, HCH, including lindane				
[completion list of prohibited substances from **79/117/EEC** (EC, 1979)]				
A. Mercury compounds: 1. Mercuric oxide; 2. Mercurous chloride (Calomel); 3. Other inorganic mercury compounds; 5. Alkoxyalkyl and aryl mercury compounds				
B. Persistent organo-chlorine compounds: 9. Camphechlor				
C. Other compounds: 1. Ethylene oxide; 2. Nitrofen; 3. 1,2-Dibromoethane; 4. 1,2-Dichloroethane; 5. Dinoseb, its acetate and salts; 6. Binapacryl; 7. Captafol; 8. Dicofol; 9. (a) Maleic hydrazide and its salts . . .; (b) Choline, potassium and sodium salts . . . 10. Quintozene				
Annex III – List of substances subject to release reduction provisions				
Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDF), Hexachlorobenzene (HCB), Polychlorinated biphenyls (PCB), Polycyclic aromatic hydrocarbons (PAHs)				
Annex IV – List of substances subject to waste management provisions set out in Article 7				
Substances of Annex I, and Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDF)				

Selected checkpoints of relevant quality programmes

- Documented, up to date hygiene risk assessment and analysis for harvest and pre-farm gate transport processes, documented hygiene procedure implemented
- Evidence of annual residue testing, or participation in a third party crop protection product residue monitoring system, traceable to the farm
- Detailed records of the pest control inspections, recommendations and necessary action undertaken
- Hygiene cleaning and maintenance procedures for farm vehicles used for transport, and a cleaning schedule to prevent harvested produce contamination in place, with records on taken actions
- No use of untreated sewage water for irrigation/fertigation. Treated sewage water quality complies with the WHO published Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture 1989
- Records for monitoring re-circulated washing water for filtering, pH, concentration and exposure levels of disinfectants
- Records (attendance certificates) for training/instructions to all workers operating dangerous or complex equipment, and of the person responsible for fertilising
- Records of use of official collection and disposal systems
- Documented annual list of used and approved crop protection products updated by any changes in crop protection product legislation, product inventory updated at least every 3 months and readily available

Table 6.4 *Continued*

- Records of all crop protection product applications including the crop name and variety, application location, date, product trade name and active ingredient(s), identifying the operator applying, justification for application, technical authorisation for application, applied product quantity, used application machinery, and the preharvest interval
- Documented record of use of only officially registered or permitted post harvest biocides, waxes and crop protection products. Reference to the EUREPGAP guideline Annex 2 and FAO International Code of Conduct on the Distribution and Use of Pesticides. Evidence that no substances have been used . . . that are banned in the EU . . .
- Documented application records for post-harvest biocides, waxes and crop protection products include produce identity, location of application, application dates, type of treatment, applied product trade name, applied product quantity, the operator's name, and the justification for application
- Risk assessment for organic fertiliser before application
- Site-referenced records of any fertiliser application by date, quantity, nutrients, application method, operator etc.
- Documented records that handling facilities and equipment are cleaned and maintained to prevent contamination according to a cleaning schedule
- On-farm storage areas must be cleaned, and temperature and humidity control maintained and documented
- Evidence that cleaning agents, lubricants etc. that may come into contact with produce are authorised for use in the food industry, and that dose rates are followed correctly
- Documented action procedure in the event of a maximum residue level (MRL) being exceeded, including remedial steps and actions, communication to customers, product tracking exercise, etc.
- Maintained record of all programmed internal audits and associated corrective actions. All corrective action verified to ensure satisfactory completion, and accurately documented, assigning responsibility and accountability

Further relevant legislation and guides:

Council Directive 79/117/EEC of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances, Official Journal of the European Union L 033, 8 Feb 1979, 36–40, and its referring amendments (EC, 1979)

International Code of Conduct on the Distribution and Use of Pesticides (revised version), Rome, Food and Agriculture Organization of the United Nations (FAO, 2002)

Table 6.5 Provisions of EU Regulation 852/2004 Annex I Part A for record-keeping in primary production, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – CORECAL; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4
Record-keeping				
7. To keep and retain records relating to measures put in place to control hazards in an appropriate manner and for an appropriate period, commensurate with the nature and size of the food business. To make relevant information contained in records available to the competent authority and receiving food business operators on request	(+)	(+)	+	(+)
9. To keep and retain records on:	(+)	(+)	+	(+)
(a) any use of plant protection products and biocides				
(b) any occurrence of pests or diseases that may affect the safety of products of plant origin	(+)	(+)	+	(+)
(c) the results of any relevant analyses carried out on samples taken from plants or other samples that have importance to human health	(+)	(+)	+	(+)
10. Record-keeping may be assisted by veterinarians, agronomists, or farm technicians				

Selected checkpoints of relevant quality programmes

- Requested records accessible for inspection and kept for a minimum of two years
- Internal self-inspection documented and recorded
- Documented complaints procedure including a record of actions taken
- Record of seed treatments
- Records of crop protection product treatments on in-house nursery propagation
- Documentation of any planting, use or production from genetic modification
- Documented site management recording system for each field, orchard or greenhouse
- Site-referenced records of any fertiliser or crop protection product application
- Documented records of use of official collection and disposal systems
- Records (attendance certificates) for training or instructions
- Documented records for monitoring re-circulated washing water
- Documented application records for post-harvest biocides, waxes and crop protection products

Annex II of EC 852/2004 applies to all subsequent stages of production, processing and distribution of food. It is structured into 12 chapters of which 11 are relevant to fresh and minimally processed fruit and vegetables. The following requirements of the Annex II are of relevance towards fresh and minimally processed fruits and vegetables. They are summarised in the corresponding Tables 6.6 to 6.16:

- Chapter I: general requirements for food premises (see Table 6.6)
- Chapter II: specific requirements for rooms where foodstuffs are prepared, treated or processed (see Table 6.7)
- Chapter IV: requirements for transport (see Table 6.8)
- Chapter V: requirements for equipment (see Table 6.9)
- Chapter VI: requirements for the handling of food waste, non-edible by-products and other refuse (see Table 6.10)
- Chapter VII: requirements for water supply (see Table 6.11)
- Chapter VIII: requirements for personal hygiene (see Table 6.12)
- Chapter IX: provisions applicable to foodstuffs (see Table 6.13)
- Chapter X: requirements for wrapping and packaging of foodstuffs (see Table 6.14)
- Chapter XI: requirements for heat treatment (see Table 6.15)
- Chapter XII: requirements for training (see Table 6.16)

Chapters V to XII apply to all stages of production, processing and distribution of food.

First of all, Tables 6.6. to 6.16 contain the main requirements of EU Regulation 852/2004 on hygiene. In addition, applicable requirements of other relevant EU legislation are listed, to give an up-to-date and comprehensive overview of current legislation. Further, this overview is compared to the demands of the relevant quality programmes as listed at the end of Section 6.2.4. In the lower parts of Tables 6.6 to 6.16, a survey of essential checkpoints of several quality programmes is given that targets the management responsibilities for documented procedures and record-keeping. With this structure, the tables may provide a basis for internal self-evaluation of food business operators.

6.4.3 Critical points in the production of fresh and minimally processed fruit and vegetables

Discussion of critical points relies on the remarks which have been given in Sections 6.3.2 and 6.3.3 of this chapter. With this understanding, 'critical points' are equivalent to terms such as 'major/minor must control points' and 'critical failure points' and 'foundation/higher level criteria' which are checkpoints of the certification procedures of individual quality programs (BRC, 2003; EUREPGAP, 2004; APS, 2003; BDH, 2003; GFSI, 2003).

In Tables 6.6 to 6.16, a selection of such checkpoints regarding the different fields of production and processing are listed in comparison to the

Table 6.6 General requirements of EU Regulation 852/2004 Annex II Chapter I for food premises, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

		1	2	3	4
General requirements for food premises					
1.	Premises are kept clean and maintained in good repair and condition	+	+	(+)	+
2.	Layout, design, construction, siting and size of food premises are to:				
	(a) permit adequate maintenance, cleaning or disinfection, and provide adequate working space to allow for hygienic performance	+		(+)	+
	(b) protect against the accumulation of dirt, contact with toxic materials, the shedding of particles into food and the formation of condensation or undesirable mould on surfaces	+		(+)	+
	(c) permit good food hygiene practices, including protection against contamination and, in particular, pest control	+		(+)	+
	(d) where necessary, provide suitable temperature-controlled handling and storage conditions of sufficient capacity, allowing temperatures to be monitored and, where necessary, to record them				+
3.	Adequate number of flush lavatories available and connected to an effective drainage system. Lavatories are not to open directly into rooms in which food is handled	+	+	(+)	+
4.	Adequate number of washbasins available, suitably located and designated. Washbasins for cleaning hands provided with hot and cold running water, materials for cleaning hands and for hygienic drying. Where necessary, the facilities for washing food separated from hand-washing facility	+	+	(+)	+
5.	Suitable and sufficient natural or mechanical ventilation. No mechanical airflow from a contaminated area to a clean area. Filters and other parts accessible for cleaning or replacement	+	+		+
6.	Sanitary conveniences with adequate natural or mechanical ventilation			(+)	
7.	Food premises with adequate natural and/or artificial lighting	+	+		+
8.	Adequate drainage facilities, designed and constructed to avoid the risk of contamination. Fully or partially open drainage channels designed such that no waste flows from a contaminated area towards or into a clean area				+
9.	Adequate changing facilities for personnel	+	+	(+)	+
10.	Cleaning agents and disinfectants not stored in areas where food is handled	+	+	(+)	+

Table 6.6 *Continued*

Selected checkpoints of relevant quality programmes

- Process flow from intake to despatch arranged to prevent product contamination
- Premises allow sufficient working space and storage to enable all operations to be carried out properly under safe hygienic conditions
- Regular inspection and treatment of premises to deter and eradicate infestation
- Detailed records of the pest control inspections, recommendations and necessary action undertaken shall be kept
- Cleaning and housekeeping in accordance with documented procedures
- In circumstances where temperature and/or time control is critical to product safety, quality attribute or legality (e.g. thermal processing, freezing or chilling), temperature and/or time recording equipment, linked to a suitable failure alert system, shall be used to monitor at an appropriate frequency the process status
- Personnel shall enter a high risk operation via a specially designated changing facility, and shall follow appropriately specified procedures for donning visually distinctive clean overalls, headwear and footwear
- Access to clean toilets and hand washing facilities in the vicinity, toilets shall not open directly into production, packing or storage areas
- The effectiveness of the cleaning and sanitation procedures shall be verified
- Adequate ventilation in product storage and processing environments, to prevent condensation
- Lights should be protected, preferably glass should be absent. All bulbs and lights, including those on electric fly killer units, where they constitute a risk to product, shall be protected by shatterproof plastic diffusers or sleeve covers. For high-temperature lights, where plastic covers are not viable, a fine mesh metal screen shall be fitted. Where full protection cannot be provided, the glass management system shall take this into account
- Adequate covered drainage should be in place, which flows away from high risk areas
- Where appropriate, changing facilities shall be sited to allow personnel (whether staff, visitor or contractor) direct access, without recourse to any external area, to the production, packing or storage area
- Entry to high-risk production areas should be via a specifically designated changing facility and follow specified procedures
- Documentary evidence that cleaning agents, lubricants etc. that may come into contact with produce are authorised for use in the food industry, and that dose rates are followed correctly

Table 6.7 Specific requirements of EU Regulation 852/2004 Annex II Chapter II for rooms where foodstuffs are prepared, treated or processed, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s))

	1	2	3	4
Specific requirements in rooms where foodstuffs are prepared, treated or processed				
1. Adequate design and layout to permit good food hygiene practices, including protection against contamination between and during operations. In particular:				
(a) Floor surfaces maintained in a sound condition and easy to clean and to disinfect where necessary. Use of impervious, non-absorbent, washable and non-toxic materials unless evidence that other materials used are appropriate. Adequate surface drainage where appropriate.	+	+		+
(b) Wall surfaces maintained in a sound condition and easy to clean and to disinfect where necessary. Use of impervious, non-absorbent, washable and non-toxic materials, smooth surface up to a height appropriate for the operations unless evidence that other materials used are appropriate.	+	+		+
(c) Ceilings, interior roof surface, or overhead fixtures constructed and finished to prevent accumulation of dirt and to reduce condensation, growth of undesirable mould and shedding of particles.	+	+		+
(d) Windows and other openings constructed to prevent the accumulation of dirt. Fitted with insect-proof screens which can be easily removed for cleaning where necessary. Where open windows would result in contamination, windows are to remain closed and fixed during production.	+	+		+
(e) Doors easy to clean and to disinfect where necerssary. Use of smooth and non-absorbent surfaces unless evidence that other materials used are appropriate.	+	+		+
(f) Surfaces including equipment surfaces in areas where foods are handled and in particular those in contact with food maintained in a sound condition and easy to clean and to disinfect where necessary. Use of smooth, washable corrosion-resistant and non-toxic materials, unless evidence that that other materials used are appropriate.				
2. Adequate facilities for cleaning, disinfecting and storage of working utensils and equipment where necessary. Facilities constructed of corrosion-resistant materials, easy to clean and having adequate supply of hot and cold water.	+	+	+	+
3. Adequate provision for washing food. Every sink or other facility with adequate supply of hot and/or cold potable water consistent with the requirements of Chapter VII and kept clean and disinfected where necessary.	+	+	+	+

Table 6.7 *Continued*

Selected checkpoints of relevant quality programmes (also to be noted for reconstruction and extension!)

- Walls, floors and ceilings should have easy access and be easy to clean and impervious
- Walls, floors and ceilings should be easy to clean and impervious
- Wall/floor junctions and corners should be coved to facilitate cleaning
- Floors shall have adequate falls to cope with the flow of any water or effluent towards suitable drainage
- Where windows are designed to be opened for ventilation purposes, they shall be adequately screened to prevent the ingress of pests
- Where external doors to raw material handling, processing, packing and storage areas are kept open, suitable precautions shall be taken to prevent pest ingress. Doors, in these areas, shall be close fitting or adequately proofed
- External doors linked to production areas need to be close fitting and adequately proofed
- Facilities for tray and utensil washing and general purpose cleaning adequately segregated from production activities, where appropriate
- Equipment shall be positioned so as to give easy access under, inside and around it for cleaning, maintenance or servicing
- Washing water potable or declared suitable by competent authorities
- Documented records for monitoring re-circulated washing water for filtering, pH, concentration and exposure levels of disinfectants

Table 6.8 Specific requirements of EU Regulation 852/2004 Annex II Chapter IV on transport, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; 5 – COCERAL; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4	5
Transport					
1. Conveyances or containers for transporting foodstuffs kept clean and maintained in good repair and condition, designed and constructed to permit adequate cleaning or disinfection where necessary	+	+	+	+	+
2. No use of receptacles in vehicles or containers for transporting anything other than foodstuffs where this may result in contamination					
3. Effective separation of products where conveyances or containers are used for transporting anything in addition to, or for transporting different foodstuffs at the same time					
5. Effective cleaning between loads where conveyances or containers are used for transporting anything other than foodstuffs or for transporting different foodstuffs					+
6. Foodstuffs in conveyances or containers placed and protected to minimise the risk of contamination	+	+			+
7. Conveyances or containers being capable of maintaining foodstuffs at appropriate temperatures and allow those temperatures to be monitored where necessary	+	+	+	+	+

Table 6.8 *Continued*

Selected checkpoints of relevant quality programmes

- All vehicles used for the transportation of raw materials (including packaging), intermediate/semi processed (primary) product and finished (primary) product suitable for the purpose, maintained in good repair and clean
- Documented hygiene cleaning and maintaintainance procedures for farm vehicles used for transport, and a cleaning schedule to prevent harvested produce contamination is in place
- Documented, up-to-date hygiene risk assessment and analysis for harvest and pre-farm gate transport process
- Appropriate treatment, decontamination and/or cleaning procedures must be documented in case goods listed in Annex 2 (COCERAL, 2003) have been amongst the previous three loads
- Procedures shall, where appropriate, be in place in the case of vehicle breakdown. These procedures shall ensure product safety, legality and quality
- Where contract refrigerated transport is used, documented procedures shall be in place to ensure product temperature requirements are met
- Appropriate control and registration procedures at the point of entry
- Instructions for storage, labelling and delivery implemented

Table 6.9 Specific requirements of EU Regulation 852/2004 Annex II Chapter V for equipment, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

		1	2	3	4
Equipment requirements					
1.	All articles, fittings and equipment with which food comes into contact are to:				
	(a) be effectively cleaned and disinfected where necessary, at a frequency sufficient to avoid any risk of contamination	+	+	+	+
	(b) be so constructed, and of such materials and kept in good order, repair and condition to minimise any risk of contamination	+	+		+
	(c) be so constructed, and of such materials and kept in good order, repair and condition to enable them to be kept clean and, where necessary, disinfected, with the exception of non-returnable containers and packaging	(+)	+		+
	(d) installed in a manner to allow adequate cleaning of the equipment and the surrounding area	+	+		+
2.	Equipment fitted with any appropriate control device to guarantee fulfilment of this Regulation's objectives where necessary				
3.	Use of chemical additives for preventing corrosion of equipment and containers in accordance with good practice	(+)	+		+

Selected checkpoints of relevant quality programmes

- Hygiene procedures for (harvesting) containers, tools and equipment implemented
- Equipment designed for the purpose intended and easly to be cleaned
- Equipment positioned so as to give access under, inside and around it for ease of cleaning and servicing

Table 6.10 Specific requirements of EU Regulation 852/2004 Annex II Chapter VI for food waste, non-edible by-products and other refuse, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s))

	1	2	3	4
Food waste, non-edible by-products and other refuse				
1. Removal from rooms where food is present as quickly as possible, to avoid their accumulation	+	+		+
2. Deposition in closable containers, unless evidence that other types of containers or evacuation systems used are appropriate. Containers are to be of appropriate construction, kept in sound condition, be easy to clean and, where necessary, to disinfect	+	+		+
3. Adequate provision is to be made for the storage and disposal. Refuse stores designed and managed in a way to be kept clean and, where necessary, free of animals and pests	+	+		+
4. All waste eliminated in a hygienic and environmentally friendly way in accordance with applicable Community legislation, not constituting a direct or indirect source of contamination	+	+		+

Selected checkpoints of relevant quality programmes

- Systems shall be in place to minimise the accumulation of waste in production areas, and shall prevent the use of unfit materials
- Waste containers for internal and external purposes should be clearly identified and cleaned regularly
- External waste containers should be covered and removed at appropriate frequencies
- Restricted access of domestic animals to facilities

Further relevant EU legislation:

Regulation (EC) No 850/2004 of the European Parliament and of the Council of 29 April 2004 on persistent organic pollutants and amending Directive 79/117/EEC, Official Journal of the European Union L 158, 30 Apr 2004, 7-49 (EC, 2004c)

Refer to Table 6.4

relevant EU legislation. These listings represent particular tasks of management in relation to documentary issues, record-keeping, establishing procedures and controlling processes. The comparison may be of particular benefit for smaller growers and operators because they may have to overcome difficulties which relate to their structure (Mueller *et al*., 2003). Table 6.17 gives examples of specific difficulties with continuous improvements which may be found in practice.

Such difficulties may be found at all stages of the supply chain. A suitable activity to deal with them may be an exchange of experience with adequate operators in the neighbourhood. A joint visit to the processing

Table 6.11 Specific requirements of relevant EU Regulation 852/2004 and EU Directive 98/83 for water supply, their relation to quality programmes, and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4
Water supply: EC 852/2004				
1. (a) Adequate supply of potable water, which is used whenever necessary to ensure that foodstuffs are not contaminated	+	+	(+)	+
2. Where non-potable water is used, it is to circulate in a separate duly identified system. Non-potable water is not to connect with, or allow reflux into, potable water systems				
3. Recycled water used in processing or as an ingredient must be of the same standard as potable water, unless evidence that water quality cannot affect the wholesomeness of the foodstuff in its finished form			(+)	+
4. Ice which comes into contact with food or which may contaminate food is to be made from potable water	+	+		
5. Steam used directly in contact with food must not contain any substance that presents a hazard to health or is likely to contaminate the food	+	+		
6. Where heat treatment is applied to foodstuffs in hermetically sealed containers it is to be ensured that water used to cool the containers after heat treatment is not a source of contamination for the foodstuff				
98/83/EC (EC, 1998)				
Article 5 – Quality standards				
1. Member States shall set values applicable to water intended for human consumption for the parameters set out in Annex I.				
2. The values set in accordance with paragraph 1 shall not be less stringent than those set out in Annex I . . .				
3. A Member State shall set values for additional parameters not included in Annex I where the protection of human health within its national territory or part of it so requires . . .				
Article 6 – Point of compliance				
1. The parametric values set in accordance with Article 5 shall be complied with: (d) in the case of water used in a food-production undertaking, at the point where the water is used in the undertaking.	+	+	+	+
Selected checkpoints of relevant quality programmes				
• Potable water should be used and where appropriate checked for contaminants at an appropriate frequency				
• Washing water potable or declared suitable by competent authorities				
• Documented records for monitoring re-circulated washing water for filtering, pH, concentration and exposure levels of disinfectants				
• No use of untreated sewage water for irrigation/fertigation. Treated sewage water quality complies with the WHO published Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture 1989				
• Uncontrolled sewage water flow into irrigation facilities and other water basins should be prohibited				
• Quality of ice, when used in processing, should be managed to prevent cross-contamination				
• The quality of water, steam or ice that comes in contact with food, shall be regularly monitored and shall present no risk to product safety				

Table 6.12 Specific requirements of EU Regulation 852/2004 Annex II Chapter VIII for personal hygiene, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4
Personal hygiene				
1. Every person working in a food-handling area is to maintain a high degree of personal cleanliness and is to wear suitable, clean and, where necessary, protective clothing	+	+	(+)	+
2. No person suffering from, or being a carrier of a disease likely to be transmitted through food or afflicted, for example, with infected wounds, skin infections, sores or diarrhoea is to be permitted to handle food or enter any food-handling area in any capacity if there is any likelihood of direct or indirect contamination. Any person so affected and employed in a food business and who is likely to come into contact with food is to report immediately the illness or symptoms, and if possible their causes, to the food business operator	+	+	(+)	+
Selected checkpoints of relevant quality programmes				
• The company's personal hygiene standards shall be documented and adopted by all personnel, including visitors of the factory. These standards shall be formulated with due regard to risk of product contamination				
• The effectiveness of hygiene procedures with regard to hands shall be checked periodically				
• The company shall ensure that medical screening procedures are in place for all employees, who will be working in areas where product safety could be compromised				
• The company shall have a procedure for the notification by employees, including temporary employees, of any relevant infectious disease or conditions with which they may be suffering, or have been in contact				
• Equipment of workers (incl. subcontractors) with suitable protective clothing in accordance with label instructions, with procedures in place to clean protective clothing after use				
• Recommendations or procedures for the use of protective clothing and equipment in place and used by all workers handling or applying crop protection products				

facilities may result in creative impulses and encourage the establishment of horizontal/vertical partnerships.

An essential step towards a chain-spanning approach would be the establishment of a dialogue on hygiene and safety issues with the preceding and subsequent partners in the chain. It has been found that handing over produce in the supply chain may be subject to substantial complaints concerning safety and quality (Mueller *et al*., 2003). Detailed agreement on supply and delivery specifications, including agreement on both safety and quality-related terms would be of benefit to all partners in the chain.

Table 6.13 Specific provisions of EU Regulation 852/2004 Annex II Chapter IX for foodstuffs, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4
Provisions applicable to foodstuffs				
1. No acceptance of raw materials if they are known to be, or might reasonably be expected to be, contaminated with parasites, pathogenic microorganisms or toxic, decomposed or foreign substances to such an extent that, even after the hygienic application of normal sorting or preparatory or processing procedures, the final product would be unfit for human consumption	+	+		
2. Raw materials and all ingredients stored in appropriate conditions designed to prevent harmful deterioration and protect them from contamination	+	+	(+)	+
3. To protect food against any contamination likely to render the food unfit for human consumption, or injurious to health or contaminated in such a way that it would be unreasonable to expect it to be consumed	+	+		
4. Adequate procedures in place to control pests, and to prevent domestic animals from having access to places where food is prepared, handled or stored	+	+		
5. Raw materials, ingredients, intermediate products and finished products likely to support the reproduction of pathogenic micro-organisms or the formation of toxins are not to be kept at temperatures that might result in a risk to health. The cold chain is not to be interrupted. However, limited periods outside temperature control are permitted, to accommodate the practicalities of handling during preparation, transport, storage, display and service of food, provided that it does not result in a risk to health. Food businesses manufacturing, handling and wrapping processed foodstuffs are to have suitable rooms, large enough for the separate storage of raw materials from processed material and sufficient separate refrigerated storage	+	+		+
6. Where foodstuffs are held at chilled temperatures they are to be cooled as quickly as possible following the heat-processing stage, or final preparation stage, to an appropriate temperature				
7. Thawing of foodstuffs in such a way as to minimise the risk of growth of pathogenic microorganisms or the formation of toxins in foods. During thawing, foods are to be subjected to temperatures that would not result in a risk to health. Where run-off liquid from the thawing process may present a risk to health it is to be adequately drained. Following thawing, food is to be handled in such a manner as to minimise the risk of growth of pathogenic microorganisms or the formation of toxins				
8. Hazardous or inedible substances adequately labelled and stored in separate and secure containers	+	+		+

Table 6.13 *Continued*

Selected checkpoints of relevant quality programmes
• Clear procedures for the control of non-conforming material, including rejection, acceptance by concession, or regarding for an alternative use, shall be in place and understood by all authorised personnel
• Documented supplier approval procedure in place based upon risk assessment
• Establish and implement corrective action and reporting procedures, in the event of the monitoring and testing procedure identifying any failure of the metal or foreign body detector. These will include the isolation, quarantining and re-inspection of all food produced since the last acceptance test of the metal or other foreign body detector
• Storage areas must be cleaned, and temperature and humidity control maintained and documented
• Detailed records of the pest control inspections, recommendations and necessary action undertaken shall be kept
• Inspections, recommendations and corrective action of pest control documented
• In circumstances where temperature and/or time control is critical to product safety, quality attribute or legality, temperature and/or time recording equipment, linked to a suitable failure alert system, shall be used to monitor at an appropriate frequency the process status
• Procedures shall be in place to record actions taken when the prescribed measuring and monitoring devices are found not to be operating within specified limits
• Where materials require special handling procedures (e.g. allergens), these shall be in place to ensure that product safety, legality and quality are maintained
• Corrective actions shall be undertaken in a timely manner to prevent further occurrence of non-conformity, and shall be accurately documented and revised, assigning responsibility and accountability
• Raw materials, work in progress, packaging and finished goods should be adequately labelled to allow effective stock rotation based on first in first out principle

The above aspects apply to the minimal processing of fruits and vegetables as well. It could be illustrated by Section 6.3.1 that minimally processed fruit and vegetables are safe and stable under strictly controlled processing and storage conditions. As minimal processing techniques commonly go in line with the application of an adequate hurdle concept throughout various stages of the food chain, compliance with required process parameters, their control and monitoring are of particular importance in keeping the product safe.

As a consequence it would be essential to identify and to monitor CCPs for the application of minimal processing techniques and hurdle concepts at the corresponding stages in the chain, to eliminate safely the occurrence of microbial risks. Such monitoring should include the kinetics and

Table 6.14 Specific provisions of EU Regulation 852/2004 Annex II Chapter X for wrapping and packaging of foodstuffs, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4
Wrapping and packaging of foodstuffs				
1. Material for wrapping and packaging are not to be a source of contamination	+	+	(+)	+
2. Wrapping materials stored in such a manner that they are not exposed to a risk of contamination	+	+	(+)	+
3. Wrapping and packaging operations carried out so as to avoid contamination of the products. Where appropriate and in particular in the case of cans and glass jars, integrity of the container's construction and its cleanliness is to be assured	+	+	(+)	+
4. Wrapping and packaging material re-used for foodstuffs is to be easy to clean and, where necessary, to disinfect				
Selected checkpoints of relevant quality programmes				
• Where packaging materials pose a product safety risk special handling procedures shall be in place to prevent product contamination or spoilage. Records shall be maintained of failures and corrective actions taken				
• Procedures shall be in place to prevent contamination and cross-contamination of raw materials, packaging and finished product				
• Packaging should be removed from outer packaging outside production areas to eliminate risks of contamination				
• Packaging . . . should be adequately labelled to allow effective stock rotation based on first in first out principle				
• Packaging . . . shall be stored so as to minimise the risk of infestation. Where stored product pests are considered a risk, appropriate measures shall be included in the control programme				
• Procedures shall be in place to confirm that product packaging conforms to specification, shall comply with relevant food safety legislation and suitability for use				
• Any part used packaging materials shall be effectively protected before being returned to storage				

treatment parameters which are required to eliminate the relevant micro-organisms. At the present state of the art, the implementation of minimal processing and hurdle technologies for fruit and vegetables may result in a higher number of CCPs than for 'conventional' processing techniques.

6.5 Future trends

Minimally processed, convenient, ready-to-eat but ambient-stable foods are the trend in industrialised as well as in developing countries. Consumers

Table 6.15 Specific provisions of EU Regulation 852/2004 Annex II Chapter XI for heat treatment, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s); (+) – notice recommended)

	1	2	3	4
Heat treatment				
Requirements apply only to food placed on the market in hermetically sealed containers:				
1. Any heat treatment process used is:				
(a) to raise every party of the product treated to a given temperature for a given period of time				
(b) to prevent the product from becoming contaminated during the process				
2. To ensure that the process employed achieves the desired objectives, food business operators are to check regularly the main relevant parameters (particularly temperature, pressure, sealing and microbiology), including by the use of automatic devices	+	+		
3. The process used should conform to an internationally recognised standard (for example, pasteurisation, ultra high temperature or sterilisation)				+
Selected checkpoints of relevant quality programmes				
• In circumstances where temperature and/or time control is critical to product safety, quality attribute or legality (e.g. thermal processing, freezing or chilling), continuous real time temperature recording equipment, linked to an automatic alarm system, shall be used to monitor the process status at an appropriate frequency				
• A full description of the product should be drawn up, including relevant safety information such as composition, physical/chemical structure (including A_w, pH, etc.), microcidal/static treatments (heat treatment, freezing, brining, smoking, etc.), packaging, durability and storage conditions and method of distribution				

prefer minimally processed foods, since these foods have appealing fresh-like characteristics and thus a superior sensory quality (Leistner, 2002). A recent development is minimally processed and bagged/wrapped fruit and vegetables, such as fruit cut fresh at the supermarket and peeled and washed baby carrots. The rising demand for such foods and for home meal replacements or carry-out food, are all certain to continue increasing sales of fresh and minimally processed fruit and vegetables, largely because the newer trend provides greater profit margins for retailers (Mintel, 2003). This trend is likely to continue because of the increasing availability and variety of fresh fruit and vegetables all year round, based on the establishment of globally sourcing supply chains. On this basis, retailers have greater

Table 6.16 Specific provisions of EU Regulation 852/2004 Annex II Chapter XII for training, their relation to quality programmes and selected checkpoints (1 – BRC; 2 – IFS; 3 – EUREPGAP; 4 – GFSI; + – equivalent/similar checkpoint(s))

	1	2	3	4
Training				
Food business operators are to ensure:				
1. that food handlers are supervised and instructed and/or trained in food hygiene matters commensurate with their work activity	+	+	+	+
2. that those responsible for the development and maintenance of the HACCP procedure or for the operation of relevant guides have received adequate training in the application of the HACCP principles	+	+		+
3. compliance with any requirements of national law concerning training programmes for persons working in certain food sectors	+	+	+	+
Selected checkpoints of relevant quality programmes				
• Adequate training for the required skills should be established, verification of training and review of training needs should be in place				
• Records (attendance certificates) for training or instructions to all workers operating dangerous or complex equipment				
• When appropriate, training for the administering and use of pesticides, herbicides and fungicides should be in place				
• Documentary evidence of training and competence of the person responsible for fertilising				
• Assistance for implementation of IPM systems through documented training or qualified advice				
• Keys and access to crop protection product store are limited to workers with formal training				
• A training matrix showing all personnel and job roles should be in place. The document should specify the level of training for all personnel and their competence to carry out specific tasks				
• The Company shall have documented training procedures and full training records				
• Key personnel identified as HACCP team members shall have adequate training and experience				

incentives to promote new creations of greater-margin fresh or minimally processed products.

On the other hand it has been illustrated that 'commercially sterile' products can barely be produced by applying just one of the state-of-the-art minimal processing techniques. As such foods would undergo rapid deterioration, minimal processing is frequently associated with hurdle concepts where additional techniques are applied at several stages in the production chain. As a consequence it would be necessary to identify and to monitor a greater number of CCPs to keep hazards under control. This will lead to increasing requirements of the self-control systems of growers and opera-

Table 6.17 Potential difficulties of smaller growers and operators regarding the evolution of their self-control systems

Aspect	Potential difficulties
HACCP	
HACCP study	No systematic approach, irreproducible
Probability of risks	Formulated too abstract; risk or severity not calculated
Corrective action	Incomplete procedures
Traceability	Problems with bulk goods such as spices; lack of records; first-in-first-out (FIFO) principle neglected
Management	
Responsibility of senior management	Delegation to lower hierarchy; lack of management review
Management system	Lacking evidence of improvements because no key figures are recorded (i.e. productivity/performance, deviations from product formulation, complaints)
Investigation of customer satisfaction	Not only in relation to price, product quality and delivery reliability but also innovative ability, flexibility, capacity reserves, acceptance of audits
Human resources	Inadequate number of staff for all tasks; executive producer overburdened; no appointee for quality management
Production/processing	
Control	No care about tasks; duties neglected due to lack of time
Malfunction of production line	No procedures for handling of produce
Raw material sampling	No procedure and/or infeasible (no space)
Glass breakage	No procedures; lacking awareness (eyeglasses, control computer monitors, raw material bottles)
Product release	No examination routines; checking parameters not appropriate; no agreement on product supply and delivery specifications
Access regulation for contracted craftsmen or waste disposal services	Usually well regulated, own staff concerned about hygiene, but no instructions given to contracted persons

tors in general, and in particular of risk analysis efforts, including product and production specific hazard analyses. Further, the application of HACCP principles will be expanded to farm level. Specific hygiene requirements for primary production will be developed within the next few years. It should be expected that requirements set for relevant quality programmes will constitute the standard for hygiene guides applicable to all growers and operators in the future (CEC, 2004a and b; EN ISO, 2004; EC, 2004a).

Smaller growers and operators may have difficulties in dealing with the related challenges and costs of the evolution of their quality control and

management. To maintain access to the value chain, the establishment of further grower and operator associations is likely in the frame of horizontal and vertical supply chain integration and mutually beneficial supplier relationships.

Further impact on the development of minimal processing may arise from recent legislation regarding the labelling of treatments (i.e. irradiation) and additives, including used processing aids (i.e. acids, lactobacteria). The growing public concern about additives, processing aids and other substances with allergenic effects has made them subject to labelling rules, providing appropriate information for consumers suffering from food allergies, in particular about substances recognised as causing hypersensitivity. These trends are in line with continued rapid development of complex information management structures throughout the supply chain. All process and quality-related information for each product batch and charge will be accessible at any place in the chain.

Finally, the design and application of hurdle technology concepts is expected to be a challenging field for further development, in particular to reduce the number of processing steps without compromising safety. To assure this, appropriate risk evaluation methods for the new techniques will be developed as well.

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7

Good agricultural practice and HACCP in fruit and vegetable cultivation

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7.1 Introduction

A principal and entirely reasonable expectation of consumers is that the food they purchase will be of the required quality and safe to eat. Responsibility for ensuring that food complies with quality and safety requirements rests with those who produce and process food products for the food marketplace and with those who sell products to consumers. All food businesses bear a moral obligation to provide consumers with products which meet quality and safety requirements. Coordinate legal responsibilities are usually enshrined in food laws aimed at protecting consumers from foodborne harm. Though food businesses may intend to produce food products that are of the required quality and safe to eat, thereby complying with legislation, regulatory emphasis is increasingly being placed on the ability of such businesses also to be able to demonstrate continuously their competence in managing the production of safe food. Food businesses must, therefore, be able to show the possession and operation of food safety management systems capable of ensuring that consumers are protected at all times. For growers of fruit and vegetables much of the control exercised over quality and some issues of food safety (amongst other things) is best achieved through the application of good agricultural practice, or GAP, which provides a systematic approach to planning and managing most aspects of crop production. For food safety management specifically, the method most widely accepted and considered as the best way to protect consumers from foodborne harm is provided by the hazard analysis critical control point, or HACCP, system.

This chapter concerns food safety management and expressly the application of the HACCP systems approach to food safety management in relation to the production of fruit and vegetables. The key objectives of the chapter are (1) to outline the concept of GAP and establish its role as a prerequisite for the development and operation of HACCP systems, and (2) to describe the process by which HACCP systems may be developed for managing food safety in the production of fruit and vegetables. It is not the intention here to discuss issues of food safety management specific to particular types of fruit or vegetables. The intention is to convey an understanding of GAP and HACCP sufficient to enable the reader to see how they are applied in the production of safe fruit and vegetables. The safety of such products is of course paramount. They may constitute raw materials for further processing, for example in the production of minimally processed bagged salads and manufactured and formulated foods, or they may be released directly into the food marketplace for immediate consumption and the domestic preparation of meals. At no time during production, harvesting, post-harvest handling and processing can fruit and vegetables be allowed to represent a source of hazard for consumers and any hazards that may arise must be controlled effectively. This chapter will explain how this can be achieved.

7.2 Perspectives on food quality and safety

Although the focus of this chapter is on food safety, it is also a chapter about food quality. Logically, food safety is a subset of food quality. Though a food product may not be of the right quality, it may still be safe to eat; however, a food product that is not safe to eat will automatically fail to be of the right quality. Consequently, when issues of food quality and safety are considered, food safety must be paramount, as the failure to control food safety inevitably means the failure to control quality.

Numerous definitions of the term 'quality' have been devised. One of the simplest and most memorable was given by Crosby (1984) who said that quality 'has to be defined as conformance to requirements'. This is echoed by the International Organisation for Standardization (ISO) which states that quality is 'The degree to which a set of inherent characteristics fulfils requirements' (ISO 2000a). In the production of food products, including fruit and vegetables, quality must be defined in terms which enable those who produce the products and those who buy them to agree that quality has, or has not, been achieved. Thus, parameters must be set which encompass the quality requirements – including food safety requirements – to which a product is expected to conform and, in this respect, production systems must be designed, implemented, operated and maintained appropriately to ensure that quality parameters are consistently met. It must be remembered that if quality cannot be measured it cannot be controlled. This

means that the parameters used both to describe the quality (and safety) of products and to enable their production must be defined in measurable terms. It is common practice to define quality parameters in documented product specifications which describe products, and often aspects of production, in quantitative terms. Product specifications enable products to be assessed for their conformance to, and ability to fulfil, requirements. Specifications are a statement of requirements that the producer must comply with if the customer is to be satisfied. Frequently they form the basis of contracts between customers and their suppliers.

Issues of food safety are an important part of product specifications. They are usually embraced by the inclusion of food safety parameters to which products are required to conform. A description of the food safety systems, for example HACCP systems, used to manage food safety during production may also be included. Although food safety has been recognised as a subset of quality, it is conventional and good practice in food production to manage it separately from non-safety aspects of quality. The justification for this will be stated later. For now, it is important to recognise that product specifications may present this distinction and that some elements of specifications may relate to food safety parameters and food safety management, while others will be concerned with issues of quality separate from food safety.

Although food businesses bear a moral obligation to provide safe food, and food safety requirements are usually encompassed by product specifications agreed between suppliers and purchasers, for the benefit of consumers, many countries establish legal requirements for food businesses to provide safe food. Issues of food safety in the production and processing of food for public consumption are governed by the publication of food law and the enforcement of penalties for breaking the law. In the United Kingdom (UK), for example, the Food Safety Act 1990 is the principal piece of food legislation. As an enabling act it also facilitates the publication of regulations that permit food safety authorities to provide specific forms of consumer protection, for example general food hygiene or temperature control regulations, speedily and without the usual complications of the legislative processes.

The Food Safety Act itself contains key clauses which express the nature of offences and defences. In essence, Section 8 of the Act makes it an offence to make for release to the marketplace, or to release to the marketplace, or to advertise for sale or sell food which is not safe to eat. Section 14 of the Act states that 'Any person who sells to the purchaser's prejudice any food which is not of the nature, substance or quality demanded by the purchaser shall be guilty of an offence'. Atwood (2000) says that this section has proven its flexibility and has been used more than others in past and current food laws. He recognises the term 'nature' to refer to the type, variety or species of a food: thus, savin sold as saffron has been in breach of the law. The term 'substance' can refer to the composition of the food

and, hence, adulterants and improper ingredients, but it may also be taken to refer to contaminants which make food unsafe (though this is also addressed by Section 8). The term 'quality' encompasses issues of compositional quality, or the failure to meet compositional standards, and also issues of decomposition and failure to conform to shelf-life expectations. Significantly, Section 21 of the Act introduced what has become known as the 'due diligence defence', but which is a proposition containing two concepts, not one. Section 21 states that in the event of prosecution under the Act 'it shall be a defence for the person charged to prove that he took all reasonable precautions and exercised all due diligence to avoid the commission of the offence by himself or by a person under his control'. The term 'reasonable precautions' refers to the design and implementation of a system of food safety control adequate to ensure the production of safe food. The term 'due diligence' means operating and maintaining the system effectively. It is generally recognised that the design, implementation, maintenance and effective operation of HACCP systems ought to meet the requirements of the 'due diligence' defence. In the UK, food businesses that are competently and knowledgeably managed, routinely operate HACCP systems for the protection of consumers, and the businesses themselves. It is perhaps worrying that not all those who manage food businesses, particularly small food businesses, have the knowledge or competence to implement and maintain effective HACCP systems. Consequently, from time to time, food poisoning incidents occur which could have been prevented.

The requirement for food businesses in the UK to identify the hazards associated with food processing and handling and to recognise and control points critical to food safety is stated in the Food Safety (General Food Hygiene) Regulations 1995, which exist under the Food Safety Act 1990. In effect, the General Food Hygiene Regulations require all food businesses to implement the first five of the '7 Principles of HACCP'. These regulations derive their authority from the European Union (EU) Directive 93/43 'on the hygiene of foodstuffs'. At the time of writing there is no requirement for food businesses in the UK to implement all seven principles of HACCP. The exception is certain kinds of meat businesses which are required to implement all 7 Principles, following a serious food poisoning outbreak concerning *Escherichia coli* O157:H7 (see Section 7.6). Apart from requirements established in law, another factor influencing the adoption of HACCP is the globalisation of the food supply system and the part being played by the major supermarket businesses. In many ways the supermarkets may be a greater influence than legislation in the way HACCP is now used at all levels of the food supply system, including those occupied by farmers and growers. Throughout history, as the food supply system developed at local, regional and international levels, the system itself was defined mainly as an entity that was supply driven. Consumers were offered the agricultural food products that farmers and growers could produce and

which food processors – domestic and commercial – could convert into foodstuffs, at whatever quality could be achieved.

Near the end of the 20th century significant changes began to occur in the nature and structure of the food supply system, changes of a kind that are now erasing the model that has been in operation for generations. The concept of a food supply system structured according to consumers' demands is now frequently proposed, with the supermarkets – as the self-appointed representatives of consumers in matters of food – playing a central role in moulding the concept. The supermarkets, as perhaps the most powerful entities in the food chain, now routinely dictate the standards to be met by farmers, growers and food processors, using a justification embodied in the oft stated expression that in what they do 'supermarkets only act in response to consumers' demands'. This provides the constant reaffirmation that the food supply system is structured and organised solely for the good of consumers and, therefore, for the common good of society. Yet, surely such a position is open to challenge? Reason suggests that the word 'demand' is used as economists use it; meaning that when a product sells at the required level then the demand for it is confirmed. In other words, the consumers' demand for a product is proven by consumers being willing to buy it.

The idea that consumers demand products which supermarkets then source and stock would seem to be flawed, especially when the conduct of supermarkets is analysed in detail. For instance, it is the common experience of consumers to find that products they have bought for years are no longer stocked because, in relative terms, the ability of the products to generate the required margin has fallen below that which may be obtained from other products occupying the same shelf space. Evidence of this may have been witnessed in the UK in early 2004 when Coca-Cola's product Dasani™ – a water made by reverse osmosis processing tap water – was launched. At the time of the launch many popular brands of mineral water disappeared from supermarket shelves as the bottles of processed tap water took valuable space. It is doubtful that consumers demanded a reverse osmosis tap water in place of well-established mineral waters and that, in response to the demand, the supermarkets and Coca-Cola strived to meet consumer requirements. Indeed, one might wonder if many consumers even know what reverse osmosis processed tap water is. The appeal to reason suggests that when Dasani™ was launched the bottled mineral waters normally bought by consumers were substituted by the tap water-based product, because, potentially, it offered greater levels of profitability for both the supermarkets and the manufacturer. The success of Dasani™ would then clearly be dependent upon the consumers' willingness to buy a tap water-based product they had not demanded and had not even heard of until its launch, instead of the bottled mineral waters they normally bought.

Observations such as this, as well as observations of other forms of supermarket conduct, challenge the notion that today consumers define the

nature of the food supply system. Rather it would seem that the supermarkets decide which food products will gain access to the food marketplace, and from where, and by gaining such power they now seem able to determine the nature and dynamics of the world's food supply system, as well as, to a great extent, the food cultures of the countries in which they operate. The supermarkets have become focal points of authority and control in the food supply system, and in being able to decide which food products shall be made available to consumers they have gained significant levels of domination over farmers and growers, as well as food processors and manufacturers. They stand between those who produce food and those who consume it and in this there are advantages and disadvantages for both groups. Importantly, the supermarkets have become one of the most influential agents in the setting of standards for quality and food safety applied throughout the food supply system.

In taking command of the food supply system many supermarkets have sought to incorporate their suppliers into vertically integrated supply chains, structured and managed appropriately to exercise control over both the quality of products passing up through the chains and the prices of products when they are finally presented to consumers. The nature of competition between supermarkets is manifested in many ways, but the two most contested components of their offering to consumers are quality and price. In this there is a certain irony. During the time that supermarkets have gained dominance over the food supply system many aspects of the quality of food products have increased, although this may not be due entirely to the influence of supermarkets. Developments in food science and technology, and in specialisations such as food hygiene and packaging, may inevitably have improved food quality, with or without the influence of supermarkets. We can be sure though that the almost continuous 'price wars' between supermarkets risk driving down certain aspects of food quality. For instance, to enable a lower selling price, the 'quality of design' of many products must be reduced, often through the manipulation of composition and ingredients, thereby ensuring some, albeit often small, increase in profit margin. Quality and price are the principal battlefields of the world's major supermarket chains and associated with and forming part of quality is the matter of food safety. Although supermarkets may place constant pressure on their suppliers to achieve low production costs, thereby risking compromises on aspects of quality not associated with food safety, there can be no compromises in food safety itself.

As the supermarkets have established global food supply chains, they have also sought to specify standards for the production, processing, packaging, storage and distribution of foodstuffs destined for supermarket sale. Many supermarket businesses consequently require farmers and growers to operate according to nationally and internationally accepted standards for farm assurance, while food processors and manufacturers are required to operate in compliance with international quality system standards such

as ISO 9001:2000 (Quality management systems – Requirements), or, commonly in the UK, the British Retail Consortium's (BRC) Technical Standard for Companies Supplying Retailer Branded Food Products. Indeed, some supermarkets, such as Tesco in the UK, have established their own farm assurance scheme and standards. Although HACCP may not form an explicit part of some farm assurance schemes, increasingly its use as the means of food safety management at farm level is being expressed as a requirement by supermarkets. This is particularly so with respect to the production of fruit and vegetables which may receive only minimal processing before sale to consumers. The growers of fruit and vegetables may independently recognise the benefits to be gained from the implementation of HACCP within their businesses, but such a requirement may inevitably be imposed on them by supermarkets, or, indeed, by any of the major food processors with whom they and the supermarkets do business.

7.3 Food safety and the grower

Like the farmer who produces animals destined for food processing, the grower of fruit and vegetables forms an integral part of the food supply system. Today the activities of farming and growing can no longer be thought of as separate from the food industry, as they once were by those who, colloquially speaking, could not see beyond the farm gate. Food quality and safety as perceived by the consumer depend in many ways upon what happens in farming and growing. As the primary point of food production, the farm is often thought of as the first step in the food supply chain. From there the products of agriculture pass into primary processing and food manufacture of varying degrees of complexity, to be sold to consumers in the retail food marketplace, or through food service operations. It is often presumed, therefore, that food safety starts on the farm, but this is wrong. The UK's bovine spongiform encephalopathy (BSE) crisis in the 1990s brought the realisation that issues of food safety can arise before the farm. The BSE crisis, the cattle disease itself and probably the human equivalent, variant Creutzfeld Jacobs Disease (vCJD), appear to have been the effects of causes that occurred before agriculture. BSE seems to have been a consequence of practices undertaken by the animal feed industry involving the use of cattle and sheep remains in the compounding of feedstuffs for cattle, practices that seem to have denied questions of both a biological and moral nature concerning the wisdom of using materials of animal origin to feed herbivores. BSE seems to have been a consequence of an activity undertaken for commercial benefit by an agricultural inputs industry, but which seems to have been hazardous to both humans and cattle. Importantly, it has served to raise awareness that issues of food safety can arise at the level of the agricultural inputs industry and not just in farming or food processing.

From the perspective of growers of fruit and vegetables many agricultural inputs require careful thought about their use, and careful management and control of their use, because they are potentially hazardous when applied purposely to agriculturally produced food products. A case in point is found in the use of pesticides. The term 'pesticides' is generally taken to encompass herbicides, insecticides and fungicides. Pesticides are toxic compounds used in the growing of fruits and vegetables (as well as cereals and flowers) to reduce or eliminate target organisms, thereby increasing crop yield and quality. Simplistically, they may function on the surface of a plant or its fruiting body, or systemically throughout the plant structure. Many pesticides have been shown to be carcinogenic, mutagenic and teratogenic. At times, public health authorities express concern about the ability of some pesticides to act as endocrine disruptors, causing, for instance, the impairment of brain and other bodily functions, disruption in the development of the brain and reproductive system, weakening of the immune system and the development of behaviour disorders. Also of concern is the degree to which pesticides are absorbed by the body and deposited in fatty tissues and the liver, as was demonstrated by the organochlorine compound, DDT, amongst others. Apart from concerns about the toxicity of some active agents in pesticides and their effects on human health, there may also be reason to be concerned about other compounds present in pesticides for technical purposes. Some pesticides contain polyacrylamide to aid the adhesion of the active agents to plant leaves. Polyacrylamide is converted to acrylamide at high temperatures such as those used in food processing and cooking. Recently the presence of acrylamide in fried and baked foods has caused concern (FSA, 2003) because of its carcinogenic potential.

Warnings about the dangers of pesticides for human health and biodiversity have been well reported over the years. In the 1960s Carson (1964) was writing of the harm that pesticides can bring. Pesticide residues in foodstuffs are a potential source of harm for consumers if they are present beyond certain accepted levels, usually described as the maximum residues levels (MRLs). The World Health Organization estimates the occurrence of some 25 million cases of pesticide poisoning globally each year, with some 20 000 deaths, but mainly in developing countries (Anon, 2002). Illness and deaths from pesticides are usually the result of mistakes, poor education in pesticide handling and use, environmental contamination, and illegal use or illegal levels of residues in foods. Harm to consumers as a result of the legal and correct use of pesticides is highly unlikely and, according to Grierson (1997), there have been no such cases in the United States of America (USA). The risk exists, however, that pesticide residues may be present in fruit and vegetables, or materials derived from fruit and vegetables, when they are ultimately consumed. It is imperative, therefore, that fruit and vegetable growers take appropriate precautions to prevent the harms to consumers that may be associated with pesticides.

When used at the proper rates and with observation of the required pre-harvest intervals, pesticides ought to represent only a small risk, if at all, to consumers. Concerns are expressed about the so-called 'cocktail effect' and the notion that different pesticide residues in foodstuffs may act synergistically to cause disease when individually the pesticide residues might not. In most countries the types of pesticides that may be used and the conditions under which they can be used are prescribed by law. In the UK the Control of Pesticides Regulations 1986 and the associated Plant Protection Products Regulations 1995 govern the sale, supply, storage, advertisement and use of pesticides. Although the control of pesticide use in developed countries may be generally effective, questions might be asked about their use in developing countries, especially in the context of a global food supply system. Increasingly western supermarkets source fresh produce from developing countries because of the financial benefits to be gained from trading with countries with low labour and production costs and, in some instances, with either poorly developed regulatory frameworks or the inability to police regulations effectively. Concerns must inevitably be raised about the possibility of fruit and vegetables grown in some Third World countries being potentially unsafe to eat as the result of flawed regulation and control relative to that practised in developed countries. Globalisation and the movement of food throughout the world makes pesticide control a critical issue for all consumers, not just from the perspective of food safety, but also for moral reasons in respect of the health and safety of field and pack-house workers exposed to pesticides in the production of cheap produce for western supermarket chains.

The avoidance of harm to consumers from pesticides applied to crops represents one element of the requirements for food safety management for growers of fruit and vegetables. Another concerns the exercise of appropriate controls in the use of pesticides in relation to crop storage and handling facilities. Pesticides used for the fumigation of stores, or the control and elimination of pests in the vicinity of stores and crop handling facilities, must be managed suitably to ensure the prevention of hazards to consumers. Other potential sources of hazard for consumers may exist indirectly in relation to the production of fruit and vegetables. For example, fuel oils may, at times, contaminate products and chemical compounds such as dioxins derived from, for example the burning of paper sacks next to growing crops, may potentially increase the risk of foodborne harm to consumers. Apart from chemical hazards, physical hazards, for example glass and metal, also need to be considered, although they are, perhaps, more easily identified and controlled. They may arise from various field operations and in the storage and processing of fruits and vegetables.

Attention must also be given to biological hazards and significantly microbiological hazards. Disease caused by pesticides may take years or even decades to become apparent. Even then, to say that a specific pesticide has caused a disease depends on the ability to establish a link between

the two, which may not be easy. In contrast, bacterial food poisoning usually presents itself very quickly and is more readily identifiable because of the characteristic symptoms associated with the different bacterial pathogens and the possibility of identifying causative organisms quickly using laboratory techniques. The sources of microbial food poisoning in fruit and vegetable production are mainly animal manure and irrigation water. For instance, in the use of manures, the potential exists for crops to be contaminated by *E. coli* O157:H7 and other pathogenic bacterial species, such as *Campylobacter* spp., *Salmonella* spp. and *Shigella* spp., whereas similar risks exist in the use of irrigation water which has been contaminated with faeces of either human or animal origin. The examination of information sources concerning outbreaks of food poisoning, for example the US Food and Drug Administration's *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook* (see Section 7.8 for details), will reveal that, for example, bacterial food poisoning outbreaks have occurred from *Campylobacter jejuni* associated with lettuce, *E. coli* O157:H7 associated with mixed greens, brussels sprouts, melons and apple juice, *Listeria monocytogenes* associated with cabbage, *Shigella* associated with onions and lettuce and *Salmonella* associated with a variety of crop products including almonds, brussels sprouts, melon and tomatoes.

Various sources and kinds of foodborne hazard can be problematic for the production of fruit and vegetables, and all must be controlled such that they represent no harm to consumers. Growers must be competent in the management of their operations and the products they produce, and they must establish strategies and methods for the control of hazards. In this respect, GAP provides the strategies and methods required for the management of sources of hazards, while HACCP provides the means by which hazards can be identified and controlled.

7.4 Good agricultural practice

In many ways GAP is the agricultural industry's equivalent of good manufacturing practice (GMP) used by the food manufacturing industry. The Institute of Food Science and Technology (IFST, 1998) says that GMP consists of 'effective manufacturing operations' and 'effective food control'. GMP is concerned with establishing a systematic approach to the control of both food production operations and food products such that customer requirements stated in the form of product specifications are consistently met. GMP correlates with the development and use of quality assurance systems and incorporated quality control procedures. Its aim is to ensure that customer requirements are clearly defined and understood in the form of product specifications, and that the procedures and operational methods required to meet customer requirements are combined in the form of a

quality plan implemented through the organisation for quality management and quality assurance.

Quality management has been defined as the 'Coordinated activities [used] to direct and control an organisation with regard to quality' (ISO, 2000a). Quality management is the systematic control of quality planning and operational activities required to meet the objectives of the business. It encompasses quality assurance (QA) which is defined as that 'part of quality management focused on providing confidence that quality requirements will be fulfilled' (ISO, 2000a) and which exists to ensure customer requirements are met. Quality management and quality assurance are strategic activities, whereas quality control (QC), which is 'part of quality management focussed on fulfilling quality requirements' (ISO, 2000a), may be thought of as a tactical activity. QC is involved in, for example, inspections and measurements undertaken to ensure processes are operating correctly and products meet specifications. GAP is thus the application of quality management and quality assurance at farm level and it resembles GMP in that it can be seen to provide effective production operations and effective product control.

Since the 1980s we have seen significant developments in the field of GAP, although other terminology may be used. Developments in farm assurance are effectively developments in GAP, as are developments in integrated farm management (another term for farm assurance) which incorporates integrated crop management (ICM) and integrated pest management (IPM). Various forces have driven the development of farm assurance in the UK and amongst them perhaps the BSE crisis and the supermarkets have been the most significant. As has already been suggested, the BSE crisis brought with it the realisation that issues of food safety span the whole of the food supply chain and that what happens at the beginning of the chain can have dramatic and disastrous effects at the end. This emphasised the need for the use of quality and food safety management techniques at farm level, which is a perspective that some of the major supermarket chains had already been developing. Various supermarket chains have been instrumental in establishing farm assurance schemes and standards which have given them control over their supply chains, right down to farm level and the level of the agricultural input industries in the case of, for instance, animal feed suppliers. A significant benefit for supermarkets in requiring suppliers to work to farm assurance requirements concerns the concept of 'due diligence' as conveyed by the Food Safety Act 1990. It ensures that in respect of food safety they can say they took 'reasonable precautions' and 'acted diligently' in the provision of food to consumers and, thus, stand a reduced chance of inheriting food safety problems and the associated accountability. This is not an unreasonable objective when operating in a complex, global food supply system.

With the development of farm assurance in the UK we have seen the proliferation of schemes applying to a wide range of agricultural produce. We have also seen many inspection and assessment organisations established, whose function is to audit agricultural enterprises to determine compliance with schemes. A new industry has been spawned. Varying degrees of complexity are associated with different farm assurance schemes and, at times, certainly in the UK, confusion exists about the status of schemes, with some recognised nationally and internationally, and some not. A common source of criticism concerning farm assurance is that not all schemes appear to be equally rigorous, with variability in the way standards encompassed by different schemes need to be adhered to and in the way compliance with schemes is inspected. Questions are inevitably raised about the common sense of establishing a single, internationally accepted standard for the modelling of farm assurance schemes. It is, perhaps, surprising that such a standard has not yet been devised for the benefit of consistency in the interpretation of QA principles at farm level, and for reasons of efficiency. In this respect there would seem to be significant scope for the rationalisation of farm assurance schemes and associated standards. This said, although their application may be different, the various farm assurance schemes and integrated farm management (IFM) standards all possess certain common elements and are effectively based on the same principles of GAP. The principal objective of GAP is the management of agricultural resources to fulfil the human needs of agriculture while, at the same time, (1) protecting the environment and preventing pollution, (2) maintaining and enhancing the quality of the soil such that food production remains sustainable, (3) maximising the welfare of animals in food production systems, (4) maintaining and enhancing the amenity value of the land and landscape, and (5) promoting conditions that restore and enhance biodiversity. Fundamental to the evolving belief system that drives developments in GAP is the notion of agricultural sustainability. Embedded in this are objectives concerning the reduction of off-farm inputs and the reduction of the negative externalities of agricultural food production. Here we find resonance with the belief system that drives organic agriculture and, indeed, elements of the organic philosophy of agricultural food production and environmentalism seem to be finding their way into approaches to GAP.

The concept of externalities is important to GAP. Mautner (1997) defines 'externality' as 'A consequence considered irrelevant in deliberation or evaluation; especially, a cost or benefit not included in the accounts. Things that have value but no price – e.g. environmental beauty – are, from the standpoint of accountancy, externalities'. Pretty (2002) says that externalities are the side effects of economic activity and are external to markets, so their costs are not included in the prices paid by producers and consumers. As intensive agriculture has developed, so too have negative externalities which are not accounted for in the balance sheets of farming or the agricultural inputs industries, or reflected in the price of food in the super-

markets. Such externalities range from the loss of plant and animal species to the pollution of watercourses and the atmosphere. Many of the externalities of intensive agriculture are paid for by society as a whole through, for instance, local and national taxes which fund remedial activities, for example purifying drinking water. Some externalities cannot readily be valued in financial terms and are borne by the animals we farm and by nature as a whole. For example, how can the suffering of animals in intensive production systems or the loss of wild habitats and species be assigned a monetary value? It is not easy, but work is now being done to calculate the external costs of agricultural food production and Pretty *et al*. (2000) estimate the external costs of UK agriculture in 1996 to have been £2343 million. Of this £120 million is the cost of removing pesticides from drinking water and £71 million the cost of removing nitrates, phosphates and soil. The use of GAP offers farmers and growers a systematic approach to the management of quality and safety at farm level and the opportunity to work towards the reduction of negative externalities of agricultural food production. Significantly, it provides a foundation for the development of sustainable methods of food production. But, what is the scope of GAP and how is it structured?

7.4.1 The structure and scope of GAP

Many organisations have developed approaches to GAP, including the LEAF (Linking Environment and Farming) organisation which proposes IFM incorporating ICM and IPM (LEAF, 2000). EUREPGAP (see Section 7.8 for information) has proposed a range of GMP protocols designed for different kinds of agricultural enterprises. The *Protocol for fresh fruit and vegetables* (EUREPGAP, 2001) provides a framework for the application of GAP in the production of, for example, fruit, vegetables, potatoes, salads, etc. It describes the elements of best practice that ought to be employed in the growing and harvesting of fresh produce. Essentially, the protocol gives a reasoned and intelligent structure and scope for a specific area of crop production. Because of the subject matter of this chapter, it has been used to inform this section. The elements of GAP applied to fruit and vegetable production ought to include:

1 **Traceability**: using methods of identification and traceability to allow products to be traceable from the point of consumption to the point of production on the farm; using methods of identification and traceability to enable relevant inputs to crop production to be traced to suppliers.
2 **Record keeping**: maintaining and retaining records concerning crop production and associated activities.
3 **Varieties and rootstocks**: choosing varieties and rootstocks; ensuring seed quality; selecting according to pest and disease resistance or

tolerance; using appropriate seed treatments and dressings; managing nursery stock; complying with legal requirements concerning genetically modified organisms.

4 **Site history and management**: monitoring and recording site history, the rotations of crops and the use of off-farm inputs as well as on-farm resources such as organic manures.

5 **Soil and substrate management**: mapping soil types; using appropriate methods of cultivation; avoiding soil erosion; justifying and controlling soil fumigation.

6 **Fertilizer usage**: using fertilizers according to nutrient requirements; using fertilizers on the basis of competence and knowledge; keeping records of applications; timing use and frequency of applications; planning the maintenance and calibration of application equipment; controlling the storage of fertilizers; controlling the use of organic manures.

7 **Irrigation**: using irrigation when appropriate; using efficient methods and machinery for irrigation; using irrigation water of appropriate quality; sourcing irrigation water from environmentally sound supplies.

8 **Crop protection**: selecting methods of crop protection appropriate to pests and diseases; applying IPM to avoid chemical use; when chemicals must be used, selecting and applying them appropriately and legally; taking adequate advice on chemical use; keeping records of applications; training staff in safe chemical use and providing proper instructions; providing protective clothing and equipment; observing pre-harvest intervals; maintaining and calibrating sprayers; ensuring appropriate and controlled disposal of surplus chemicals; using analyses of spray chemicals appropriately and maintaining traceability; controlling storage of pesticides and disposal of empty containers; controlling disposal of obsolete pesticides.

9 **Harvesting**: establishing procedures and staff practices to prevent the microbial, chemical or physical contamination of produce during harvesting; establishing procedures and staff practices to prevent the microbial, chemical or physical contamination of produce during packing; controlling pests and preventing contamination by pests; controlling and cleaning re-usable crates.

10 **Post-harvest treatments**: using only appropriate, legally approved post-harvest chemicals; using potable water for washing operations.

11 **Waste and pollution management, recycling and re-use**: establishing plans and procedures for controlling waste and pollutants; identifying waste and pollutants; disposing of wastes in approved, legal ways; planning for, and operating systems for recycling and the re-use of materials whenever possible.

12 **Worker health, safety and welfare**: assessing health and safety risks to staff; training staff in safe working practices and first aid procedures; providing first aid facilities and equipment; providing health checks for

staff handling pesticides; maintaining hygiene standards in all areas of the business and in all operations; training staff in hygienic practices; ensuring adequate staff welfare, including observing employment regulations, maintaining appropriate and adequate conditions of employment, e.g., staff facilities, working hours, etc.; establishing policies for employment; providing adequate housing, etc.

13 **Environmental issues**: assessing the impact of agricultural practices on the environment; establishing a policy and conservation management plan for protecting wildlife and enhancing biodiversity on the farm.

14 **Complaints**: establishing a policy and procedure for receiving and handling complaints; keeping records of complaints, their management and resolution.

15 **Internal audit**: establishing procedures for scheduling, managing, conducting and recording internal audits and for implementing and verifying corrective action.

Of the various activities undertaken through the implementation of GAP some are critical, some are important and some are recommended. Judgement and experience are needed to categorise activities correctly, though the organisations that publish GAP (farm assurance/IFM) standards usually indicate the categories. EUREPGAP recognises the distinction between activities and categorises them as 'major musts', 'minor musts' and 'recommendations'.

Although HACCP is not an integral part of GAP, it operates in conjunction with GAP and in the case of agricultural enterprises, GAP provides the foundation for HACCP. The value of GAP to HACCP is found significantly in the way it ensures that critical and prerequisite requirements are established such that HACCP systems can then be implemented and operated effectively and, importantly, without complication and unnecessary costs.

7.5 The hazard analysis critical control point system

The hazard analysis critical control point (HACCP) system was originally developed for the US space programme as a means of making safe food for astronauts. It is now recommended as the best way to make safe food products, by the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) of the United Nations (UN) as well as the governments of many nations and many food industry representative bodies. The HACCP system is a food safety management system, not a quality system. HACCP is compatible with documented quality management systems, such as those complying with the requirements of the International Standard ISO 9001:2000 (ISO, 2000b). Indeed, any food businesses that develops its quality systems against ISO 9001 would be expected to use HACCP systems

integrated into the overall quality management system designed to ensure compliance with, and the satisfaction of customer requirements. The international standard, ISO 15161, Guidelines on the application of ISO 9001:2000 for the food and drink industry, explains the interrelationship between HACCP and quality systems developed in compliance with ISO 9001:2000.

Although the concept of HACCP can be translated for the management of quality factors not associated with food safety, HACCP itself should be used only for food safety management. HACCP systems should not be corrupted for the management of both food safety and non-food safety aspects of quality. The liberal reinterpretation of HACCP as a method of managing food safety as well as the broader issues of quality assurance can lead to complication and confusion, to the detriment of the interests of both customers and consumers. Although food safety is logically an element of food quality, clarity of purpose must be maintained when it comes to keeping consumers safe from foodborne harm.

7.5.1 Categorising hazards

HACCP concerns foodborne hazards and the purpose of HACCP is to either prevent or eliminate hazards, or to reduce them to acceptable levels. In the context of food, a hazard is defined as 'A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect' (CCFH, 1997). Biological hazards include:

- foodborne disease causing bacteria, e.g. *Vibrio cholerae*, *Mycobacterium tuberculosis*, and viruses
- infective food poisoning bacteria, e.g. *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*
- intoxicating food poisoning bacteria, e.g. *Staphylococcus aureus*, *Clostridium botulinum*
- toxigenic fungi, e.g. *Aspergillus flavus*, *Aspergillus clavatus*, *Fusarium spp.*
- food poisoning viruses, e.g. Norwalk virus, hepatitis A
- protozoan parasites, e.g. *Cryptosporidium parvum*, *Toxoplasma gondii*
- poisonous plants and plant materials, e.g. deadly nightshade berries
- poisonous fungi, e.g. *Amanita phalloides*, *Amanita virosa*, *Amanita mascaria*
- allergenic materials, e.g. nuts and wheat gluten.

Microbial pathogens are the main cause for concern when considering biological hazards in food, as most food poisoning is caused by pathogenic bacteria. Some bacterial pathogens are not serious in terms of their effects on consumers, often causing only short-term inconvenience with symptoms such as vomiting and diarrhoea. Others are very serious and may cause immediate and acute effects, or long-lasting illness or death. *E. coli* O157:H7

and some other pathogenic *E. colis* are linked to renal failure and death, most often in children and other sensitive consumers. *Campylobacter* spp. are linked to Guillain-Barré syndrome (paralysis in adults and children) and *Listeria monocytogenes* can cause meningitis and abortion. These organisms may be found in organic manures and the environment in which fruit and vegetables are grown. The website of the Food and Drug Administration in the USA gives a comprehensive account of microbial food poisoning organisms, as well as other sources of foodborne harms, see Section 7.8 for details.

Chemical hazards have already been discussed a little. Depending on their nature they may apparently cause no significant harm, they may cause short-term illness from which a full recovery is usual, or they may cause long-term illness and even death. The health effects of some chemical contaminants of foodstuffs are well documented; however, the effects, or freedom from effects, of some others, particularly pesticides, may be a matter of conjecture rather than proven science. Consequently, when pesticides are used records should be kept of their use according to the manufacturers' recommendations and legal requirements. This allows traceability to the manufacturers and assists in the transference of accountability, in the event that litigation arises concerning adverse health effects or environmental damage linked to the use of pesticides. The proper use of GAP and HACCP in relation to the growing of fruit and vegetables will necessitate the keeping of such records. Many kinds of chemical hazards may be associated with agricultural food production, including:

- naturally occurring environmental contaminants, e.g. heavy metals
- industrial contaminants, e.g. dioxins, PCBs
- pesticide (insecticide, herbicide and fungicide) residues in fruit, vegetables and cereals, etc.
- nitrates (mainly leafy crops) and other fertilizer residues
- pesticides and nitrates in drinking water
- residues of veterinary medicines and zootechnical substances in animal products, e.g. meat, milk, eggs.
- contaminants arising from the handling, storage and processing of foodstuffs, e.g. grain treatment compounds, machine lubricants, cleaning agents, rodenticides and other pest control poisons
- contaminants arising from food packaging, e.g. plasticisers and other packaging material additives, adhesives, inks, metals leached from cans.

Clearly, not all listed above will be associated with the production of fruit and vegetables.

Physical hazards can be problematic to the agricultural production of food and may cause difficulties in the post-harvest activities of fruit and vegetable production. Consequently, they may be of concern to further processors and food manufacturers who use fruit and vegetables as raw materials, as well as ultimately to consumers. Physical hazards include:

- slicing hazards – sharp glass fragments, sharp plastic fragments, wood splinters, sharp metal filings and swarf
- dental hazards – glass particles, pieces of wood, pieces of hard plastic, stones, metal fragments and parts, e.g. nuts, washers
- choking hazards – wood, stones, metal fragments, string, nuts, e.g. peanuts.

7.5.2 Applying the HACCP concept

The HACCP concept is based on the development of HACCP systems using the '7 Principles of HACCP', which are:

Principle 1: Conduct a hazard analysis.
Principle 2: Determine the critical control points (CCPs).
Principle 3: Establish critical limit(s).
Principle 4: Establish a system to monitor control of the CCP(s).
Principle 5: Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
Principle 6: Establish procedures for verification to confirm the HACCP system is working effectively.
Principle 7: Establish documentation concerning all procedures and records appropriate to these principles and their application.

The HACCP concept is employed in a series of logical steps. A HACCP study is carried out to yield a HACCP plan which is implemented, operated and maintained as a HACCP system. A HACCP study involves the collection of information about the food product under consideration and the way it is produced, and the evaluation of the information to establish the HACCP plan. A HACCP plan is defined as 'A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain *under* consideration' (CCFH, 1997). A HACCP system is defined as 'A system which identifies, evaluates and controls hazards which are significant for food safety' (CCFH, 1997). HACCP systems are conventionally developed in relation to a specific food product and its associated production process. Some authorities recommend the use of generic HACCP systems, i.e. the styling of a food safety management system based on HACCP methodology for a given type of food product, such that the generic system can be used to ensure food safety in any circumstance where the product is made. Caution should be taken with generic HACCP systems. Although two different production processes making the same product may apparently present the same hazards, which may then apparently be controlled in the same ways, the possibility exists that intrinsic features of one or the other process may give rise to unique forms of hazards. The standard methods of control advocated by the generic approach may then be inadequate.

Generic HACCP systems have the potential to miss important and unusual things and should be dealt with guardedly, if at all.

7.5.3 Developing and implementing HACCP systems

The development and implementation of HACCP systems starts with the HACCP study which is based on the 7 Principles of HACCP, but elaborated as a twelve stage process, although variation in the number of stages is possible.

Stage 1: Assemble the HACCP team (and define the scope and terms of reference of the study)

HACCP studies are usually carried out by HACCP teams which bring a mix of expertise and experience to the task. In a food factory the team may consist of a production manager familiar with the production process, an engineer who can supply knowledge of the processing equipment and a technical manager with expertise in the product itself and foodborne hazards. In the context of fruit and vegetable growing it may be that a team cannot easily be constructed, though in post-harvest processing and packhouse operations there may be a sufficient number of people with appropriate knowledge and expertise to form a team. The development of HACCP teams and associated judgements will depend on local circumstances and, in some cases, it may be appropriate to draw in external expertise, for example consultants or university academics, to assist in the HACCP study.

The terms of reference of the HACCP study must be agreed before the study commences. This means deciding which product and associated production process is to be the subject of the study and which kinds of hazard are to be considered. It is usual to carry out product-based studies which focus on the hazards most likely to be associated with the product and the way it is produced. In some instances, however, large or complex processes may be used to make a number of very similar products and then it may be appropriate to carry out a process-based study. In the production of fruit and vegetables it is likely that the HACCP study will be product based, although considerable focus will be given to both the pre-harvest production and post-harvest processing activities. With regard to the types of hazard to be encompassed by the study it may be that all three classes are considered, that is, microbial, chemical and physical. It may be convenient though to consider only one class at a time, depending on the experience of those carrying out the study. Also, it may be strategic to focus only on the hazards considered to be of critical importance, for example chemical hazards in the form of pesticides.

The scope of the study is defined to set the limits of the HACCP system. In this, two perspectives are important. First, consideration should be given

to the extent of control a business may be able to exercise over the resulting HACCP system. A grower cannot conceivably control the agricultural inputs businesses that supply materials such as seed, fertilizer or pesticides. Therefore, the scope of the HACCP study cannot encompass the inputs businesses. What they provide must be controlled, for example, by the agreement of product specifications and recognition of the accountability of the inputs businesses to provide products that represent no harm to consumers when used correctly. Effectively, the scope of the grower's HACCP system will begin at the inputs end of the farming enterprise and end at the outputs end, covering all that can reasonably be controlled by the grower. Secondly, the scope may be moderated by the size of the production system under consideration. A large and complex production system may represent too great a structure to consider in one go and the HACCP study may, therefore, be broken down into a series of small studies which are ultimately joined together to form the overall HACCP system.

Stage 2: Describe the product

In food manufacture it is usual to gather information that allows an accurate description of the product to be formed. Thus, the composition of the product, its ingredients, the way it is packaged and the way it is preserved must all be taken into account. This information is needed for the identification of:

- ingredients that might give rise to hazards, e.g. high risk food materials
- intrinsic food safety factors, e.g. pH, salt-in-moisture content, water activity (a_w), etc.
- safety factors arising from the method of processing and/or packaging, e.g. the use of vacuum packaging or MAP (modified atmosphere packaging)
- the identification of ingredients that might harm sensitive consumers, e.g. peanuts affecting consumers with nut allergy or wheat gluten affecting coeliacs.

In the context of fruit and vegetables, consideration must be given to both intrinsic and extrinsic features of the product which might represent a source of harm for consumers. The presence of seed-stones in some dried fruit may be considered an intrinsic hazard because of the potential to cause choking. In this respect, at times the product itself may be considered a hazard. For instance, nuts are a choking hazard for young children (see Stage 3). The possibility of stones or pieces of wood being entrained in sun dried fruit may be a form of extrinsic hazard that needs recognising and controlling. When pesticides and other chemicals, for example sprout suppressants used on potatoes, are used on fruit and vegetables and are likely to be present as residues in the final products, the chemicals become part of the product and may be likened to the ingredients in formulated foods. When we eat foods the chemicals from which they are comprised have the

potential to find their way into every cell of our bodies. In the case of nutrients this is what we hope will happen. The potential exists, however, for the chemical residues present on fruit and vegetables also to find their way into the cells of consumers' bodies, perhaps with unknown and unpredictable consequences which may be detrimental to health and longevity. Such factors must be taken into account in the development of food safety management systems. At this stage of the HACCP study the 'product description' of a fruit or vegetable may be quite simple and require no more than the identification of the product itself. Alternatively, it may require a more detailed form of information gathering, taking into account intrinsic features of the product that might be considered hazardous (when evaluated at Stage 6, the hazards analysis stage) or extrinsic factors, such as the application of pesticides or other chemicals, which become part of the product and which may have the potential to harm consumers.

Stage 3: Identify the intended use of the product

The intended use of the product must be identified to ascertain whether any hazards might be introduced or develop as a result of the way the product is handled, stored, prepared or used by the consumer, or by organisations making the product available to consumers, for example food service businesses. Additionally, the potential for the product to represent a hazard for sensitive consumer groups must also be assessed. For example, when it has been identified that young children may gain access to nuts, an appropriate warning may be given on packaging to draw attention to the possible choking hazard. With some food products links between the intended use and possible hazards resulting from use can be relatively easily established. For instance, in the case of products such as raw chicken, which may be contaminated by *Salmonella enteritidis* PT4 or *Campylobacter* spp., an immediate concern is raised by the possibility of consumers cross-contaminating the chicken they have cooked and made safe, with pathogenic bacteria derived from the raw chicken and contaminating work surfaces and utensils. Appropriate warnings may be given on product packaging advising consumers to cook chicken properly and to avoid post-cooking contamination. In the case of fruit and vegetables such links may seem harder to envisage; however, this does not mean they do not exist. For example, might pesticide residues on the skins of peaches or nectarines harm consumers if they are not washed before being eaten whole? Is it possible that the skins of potatoes intended for peeling could contain pesticide residues that might harm consumers if the potatoes are baked and the skins are eaten? Is there the possibility of a fruit or vegetable being stored badly and giving rise to mould growth and the development of mycotoxins, for example patulin resulting from the growth of *Aspergillus clavatus*, sometimes associated with apples and apple juice?

Apart from considering the possibility of hazards being associated with, or arising from the way the product is used, thought should be given to the

intended market for the product. Although some may not consider this to be directly an issue of food safety, to certain consumer groups it is of great importance. For example, although products containing pesticide residues within MRLs may be considered by most authorities to be safe, to some consumers any product containing any amount of pesticide may be considered potentially hazardous. Thus, products intended for the organic food marketplace must be free of pesticides (and most other applied chemical compounds). Such factors need to be thought about and managed appropriately. At this stage of the HACCP study a process should be undertaken to collect information of various forms, to be assessed at the later hazard analysis stage, in relation to the nature of hazards and their effects on consumers, amongst other things.

Stage 4: Construct a flow diagram

Stages 2 and 3 of the HACCP study provide information used to identify the hazards that might be involved directly with the product. This stage of the study concerns the provision of information that will be used to identify hazards that might arise from the production process. A flow diagram of the production process should be prepared detailing all of the inputs to, and outputs from the process, as well as production conditions and parameters (a generalised flow diagram is shown in Fig. 7.1). In fruit and vegetable production inputs include seed, seed treatment agents, irrigation water, manure, fertilizers, pesticides, etc. They also include, for example, water used in post-harvest operations such as hydrocooling to remove field heat, or to remove soil and other contaminants, such as separating vegetables from stones by floatation methods. Of course, the product is the principal output from the process, but other outputs include product rejected by grading owing to damage or deterioration, waste botanical materials, soil, stones and wood separated from products, etc. Many have the potential to give rise to, or constitute hazards if they are not handled appropriately and become involved with the product. The production process itself will consist of a variety of activities and operations such as seed treatment and propagation, root stock propagation, field or site preparation, fertilizer applications, planting, growing, irrigation, pesticide applications, harvesting, post-harvest handling and post-harvest pre-treatments, for example cleaning, trimming, washing in chlorinated water, as well as storage and transport, and so on, which must be assessed at Stage 6 for their potential to be the source of hazards. The nature of the flow diagram may be influenced by the scope and terms of reference of the HACCP study. In a study which has been confined to a particular class of hazard, or even source of hazard, for example pesticides, the flow diagram may be constrained such that it is concerned only with fruit and vegetable production operations in relation to the specific class or source of hazard. Flow diagrams should be logically and systematically structured and contain sufficient information in enough

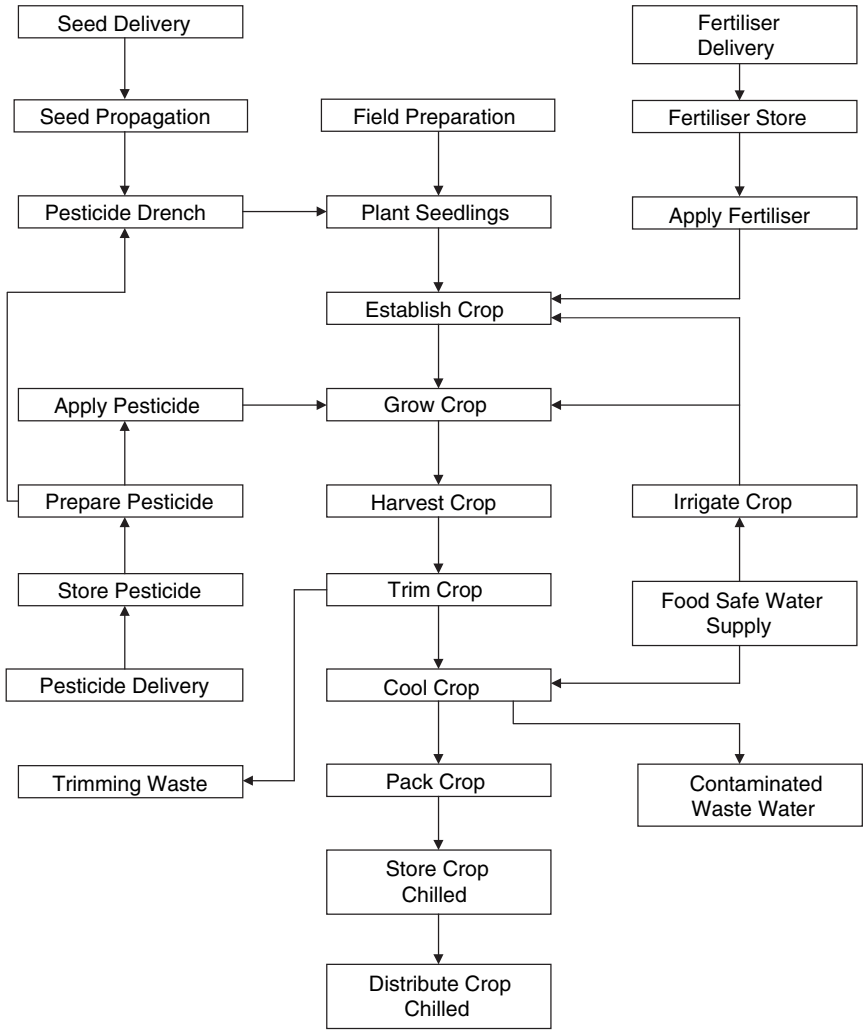

Fig. 7.1 Generalised flow diagram for field crop production. (Note: a working flow diagram would present greater detail, giving process parameters etc.).

detail to allow the evaluation of hazards without constant reference to additional information.

Stage 5: Confirm the flow diagram

The flow diagram will be developed using information about the production process and operations carried out as part of the process. In fruit and vegetable production many sources of information may be used, including

information about seeds, root-stocks, and so on; information about seed pre-treatments; pesticide data sheets; government information and regulations about pesticides; fertilizer data sheets; procedures and agricultural practices concerning field preparation, seed drilling or planting, fertilizer application, irrigation, harvesting, post-harvest handling and storage and so on; as well as post-harvest cleaning, grading, processing, and so on. Whatever the theoretical flow diagram shows, the completed diagram must be confirmed as an accurate representation of the production process. It should therefore be compared with what actually happens. Given the nature of fruit and vegetable production it may not be practical to observe a complete cycle involving, for instance, growing, harvesting and post-harvest handling or processing. Consequently, the whole process may be broken into a series of HACCP studies creating HACCP systems with limited scope, which are ultimately 'stitched' together as a complete HACCP system with a scope covering the whole production process. Until a complete production cycle has been experienced it may be necessary to refer to procedures, records and experience to establish the accuracy and veracity of the flow diagram.

Stage 6: Hazard analysis

At this stage the hazards associated with the product examined by the study, those associated with its intended use and those intrinsic to, or arising from, its production process are now identified and analysed. Identification means putting a name to every hazard that might arise as a result of factors concerning the product itself – what it is and the way it is used – or those concerning the way the product is made. Analysis means understanding the nature of each hazard identified, assessing the risks associated with them in the context of the product and the way it is used, and identifying the most appropriate method of control, or the preventive measures, for each hazard. The hazards identified should be analysed in turn and in this respect it is important to identify each fully. For instance, the term 'pathogenic bacterium' says nothing about a microbial hazard that enables the precise identification of control measures, or an assessment of the consequences it may present for affected consumers. Naming a bacterial pathogen allows specific control measures to be chosen and the consequences to be determined precisely. Risk assessment should properly be based on an assessment of the occurrence of a given hazard and the severity of effects for consumers. Thus, a hazard that has only a small chance of occurring but could be extremely harmful to consumers, for example causing permanent injury or death, would score more highly on a risk index than would a hazard that might occur more frequently, but which caused only minor harm, for example food poisoning symptoms lasting 24 hours. Hazards scoring highly on a risk index demand a great degree of attention and confidence in control, but this does not mean that those that score less should not be adequately controlled. The aim of HACCP is, of course, to make safe food, that

is, food that causes consumers no harm, irrespective of the kind of hazards involved.

Risk analysis is not easy and risk can be difficult to quantify, but it is a necessary part of HACCP and has the benefit of allowing hazards that prove to be of negligible risk to be dispensed with. There is no point in spending time and effort controlling hazards that are unlikely to arise. When the nature of each hazard has been evaluated and adequately understood, control methods can be determined either to prevent or eliminate each hazard identified, or to reduce each to acceptable levels. In this respect judgement and reference to good science will be necessary. For example, *E. coli* O157:H7 has a very low infective dose level, so it must be prevented or eliminated. Reduction to an acceptable level is not an option. On the other hand, when eliminating stones from dried fruit by visual inspection and sorting, it may not be possible to see the smallest of stones, but very small stones may be considered to fall below the hazard threshold. The hazard has then been reduced to an acceptable level. Risk assessment in the production of fruit and vegetables will concern various hazards, such as pesticides, other chemicals used in conjunction with crops, as well as microbial and physical contamination. So, in relation to pesticide use, it may be important to recognise as part of the risk assessment when and how frequently pesticides are used and the nature of consequences for consumers (a) if they are used correctly according to recommended and legal requirements and (b) if they are inadvertently used at levels exceeding recommended and legal requirements.

Stage 7: Identify critical control points (CCPs)

At this stage of the HACCP study the critical control points or CCPs must be identified. CCPs are 'A step [in a process] at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level' (CCFH, 1997). The control or preventive measures are applied at CCPs and, effectively, they represent the last line of defence in ensuring product safety. Each hazard identified in Stage 6 must be assessed in relation to each process step identified in the flow diagram, to decide at which step the appropriate control or preventive measures should be applied. The steps where the measures are applied constitute CCPs. Experience and judgement are important to the identification of CCPs; however, use of the CCP decision tree (Fig. 7.2) is also beneficial, especially when experience is limited.

In fruit and vegetable production CCPs are likely to concern, for example, activities involving checking the concentration and application rates of pesticides or removing physical contaminants from produce. When applying hazard control measures at CCPs it is important not to incorporate into the HACCP plan activities that should be dealt with by GAP. For instance, it may be thought that the activity of calibrating sprayers constitutes a CCP. Similarly, it may be thought that the activity of checking that

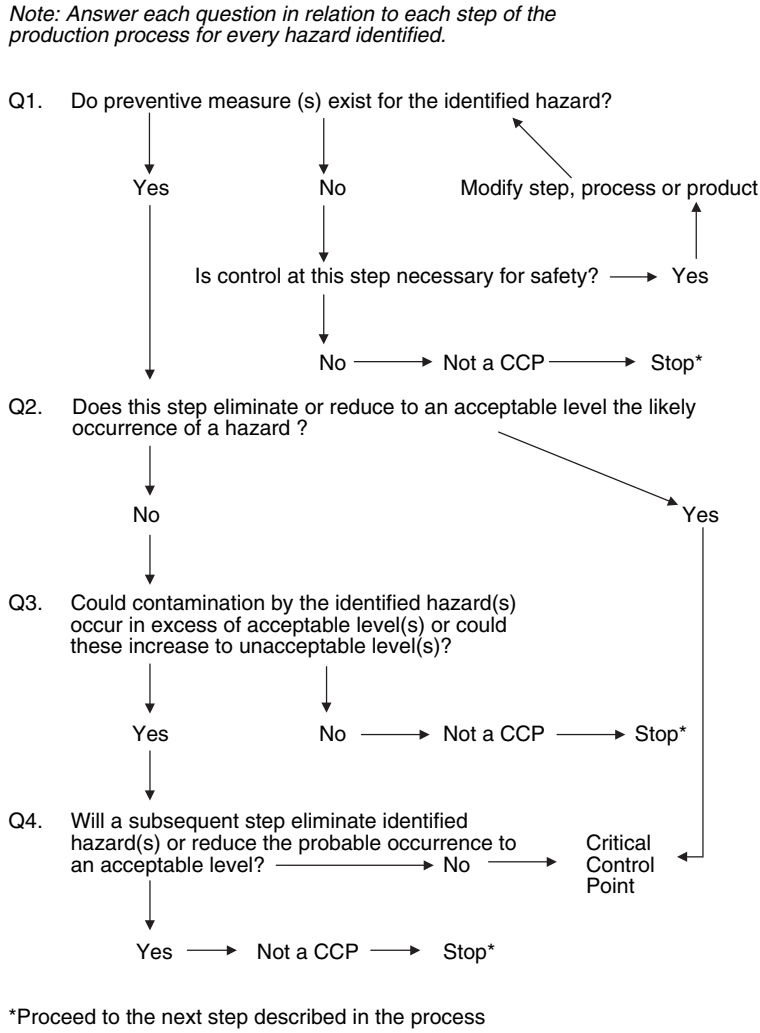

Fig. 7.2 The CCP decision tree. (Adapted from FLAIR, undated. *HACCP User Guide.* Concerted Action No. 7. Food Linked Agro Industrial Research, 191, Rue de Vaugirard – 75015, Paris).

irrigation water is suitable for use and not faecally contaminated is also a CCP. These activities are better dealt with under GAP. It is good agricultural practice to spray crops using properly maintained and calibrated equipment, just as it is good manufacturing practice to pasteurise milk with a well-maintained and calibrated milk pasteuriser and not badly maintained and uncalibrated plant. Indeed, there would be no point in trying to pasteurise milk with the latter. Similarly, the source and quality of irrigation

water should be known and its freedom from contamination should be established as a standard practice, before it is used. This is a matter for GAP, not HACCP. Many HACCP systems are overcomplicated by the incorporation of CCPs which are really GAPs or GMPs and which, with proper and competent evaluation, would be separated from inclusion within HACCP. It should always be remembered that every CCP identified entails the application of routine control and monitoring procedures, as well as corrective action procedures. CCPs therefore generate management costs and should, wisely, be limited to those that are exactly what is required to achieve food safety.

Stage 8: Set critical limits

A critical limit is 'A criterion which separates acceptability from unacceptability' (CCFH, 1997). Critical limits establish parameters for the operation of control or preventive measures at CCPs. They may concern quantitative values such as time, temperature, pH, a_w, concentration, application rates, and so on, or qualitative values, for example observations of occurrences proven to indicate food safety. In fruit and vegetable production many sources of information may be used to establish critical limits. For example, in the use of pesticides, values for concentration and application rates may be set as quantitative critical limits, as may intervals between application and harvesting. Legally defined MRLs may also serve as critical limits. In some instances critical limits may be set subjectively. For instance, in establishing a critical limit for the physical contamination of fruit by wood and stones, it may be that the only practical limit is the absence of contamination as established by visual inspection. Critical limits must be set in relation to specific hazards and provided they are adhered to the product can be regarded as safe. Records of control activities at CCPs and adherence to critical limits should be kept for HACCP system verification and maintenance, and to provide evidence of due diligence and the production of safe food.

Stage 9: Establish monitoring systems

The purpose of monitoring is (1) to confirm that the controls exercised at CCPs remain effective and (2) to detect when control has been lost. Monitoring commonly involves a planned sequence of measurements or observations used to show the HACCP system is operating effectively and that safe food is being produced. Monitoring methods should be kept as simple as possible and may be quantitative, for example taking a temperature measurement, or qualitative, for example making a visual observation. They are often linked to the nature of the control or preventive measures applied at CCPs. In fruit and vegetable production, monitoring activities may be carried out to confirm, for instance, that the correct pesticides were used and applied at the correct concentration or rate, and that pre-harvest intervals were correct. The measurement of pesticides residues in crops could be used as a

monitoring method to confirm that the control methods of correct application and pre-harvest interval were used. Such measurements are not normally carried out by growers. They are more commonly undertaken as part of government crop surveillance programmes designed to check that MRLs are not exceeded. The value of such programmes to the issue of real-time control at farm level is limited. Monitoring methods that give a rapid understanding of the status of CCPs are to be desired and, in any case, they must be such that the status is known within acceptable timescales.

When monitoring shows that a CCP has gone out of control, appropriate action must be taken quickly to restore control and deal with implicated product (see Step 10). If the timescale between the point at which control is lost and the point of its discovery is too great, too much potentially non-conforming product may be produced to be handled practically and/or the costs involved may be prohibitive. Such a perspective often dictates that rather than using monitoring just to indicate whether or not a CCP is under control, it is used to show when a CCP is going out of control, thereby allowing preventive action to be taken to maintain control, rather than corrective action to restore it. The HACCP plan should identify how each CCP is to be monitored, the frequency of monitoring and who is responsible for ensuring that monitoring is carried out. Records of monitoring activities should be maintained for HACCP system operation, verification and maintenance, as well as for due diligence purposes.

Stage 10: Establish corrective action procedures

When monitoring shows that control has been lost at a CCP, corrective action must be taken immediately, (a) to restore control to the CCP and (b) to identify, segregate and establish by suitable methods whether or not implicated product is safe. Unsafe product must be dealt with by approved methods such that it represents no potential hazard to consumers. In principle, when a HACCP system is operated properly there ought to be no need to take corrective action, because (in an ideal world) CCPs would not be allowed to go out of control. The loss of control is an eventuality that must be planned for and, consequently, corrective action procedures must be written for each CCP. Planned and documented corrective action ensures that staff know immediately what should be done when control is lost, thereby avoiding the problems associated with debate and indecision at the time of an emergency. The staff responsible for taking corrective action and controlling implicated product should be identified in the HACCP plan, as should the staff responsible for verifying that corrective action has been successful. Records should be kept of corrective action taken, and of the management and disposition of implicated product.

Stage 11: Validate the HACCP plan and implement and verify the HACCP system

A completed HACCP plan is implemented as a HACCP system, but prior to implementation the plan must be validated. Following implementation the

operational HACCP system is then verified to confirm its suitability and effectiveness. Validation involves answering the question: Will the system work when we put it into practice? (ILSI 1999). Validation is defined (CCFH 1997) as 'Obtaining evidence that the HACCP plan is [likely to be] effective'. In practice validation is an assessment of the completeness of the plan – checking that the elements of the plan are complete – and that the decisions and assumptions made during the study are sound, that scientific and technical information used to form the plan are correct and that the plan itself is adequate to create a workable and effective food safety management system. A series of validation activities have been recommended (ILSI 1999) to enable the collection of objective evidence that confirms the adequacy of the plan within the context of the 7 Principles of HACCP:

Principle 1 (Hazard analysis): Confirm (a) that the skills of those undertaking the HACCP study were adequate for the task, (b) that the flow diagram was suitable for the purposes of the study and (c) that all significant hazards and appropriate preventive measures have been identified.

Principle 2 (Identify CCPs): Confirm (a) that CCPs have been identified for the application of preventive measures for each significant hazard and (b) that the CCPs are at the right stages of the process.

Principle 3 (Critical limits): Confirm (a) that critical limits are established for each hazard and (b) that the limits are appropriate to the preventive measures applied at the relevant CCPs.

Principle 4 (Monitoring): Confirm (a) that monitoring methods and systems can demonstrate the effectiveness of preventive measures, and (b) that procedures exist for the calibration of monitoring methods and systems, as appropriate.

Principle 5 (Corrective action): Confirm (a) that corrective action procedures exist for each preventive measure and CCP, (b) that procedures exist to prevent non-conforming product reaching customers and (c) that responsibility for taking and verifying corrective action is identified, as well as that for approving the disposition of non-conforming product.

Principle 6 (Verification): Confirm that procedures and a plan for the verification of the HACCP system have been established.

Principle 7 (Documentation): Confirm that documentation describing the entire HACCP system and records required to support the system have been established.

The objective of validation is to confirm that the HACCP plan will yield a HACCP system capable of producing safe food and protecting consumers. Standard auditing techniques, as used for internal quality audit, can be used for validating the HACCP plan.

The process of verification is carried out on an operational HACCP system. The purpose of verification is to answer the question: Are we doing what we planned to do? (ILSI 1999). Verification is defined (CCFH 1997) as 'The application of methods, procedures, tests and other evaluations, in

addition to monitoring, to determine compliance with the HACCP plan'. It provides confirmation that what the HACCP plan says will be done, has been done, and that the HACCP system has been implemented according to the requirements of the plan. The process of verification should be carried out according to defined procedures and using appropriate methods for checking, for instance, the suitability and effectiveness of controls at CCPs and monitoring methods. Verification will also utilise standard audit techniques. The objectives of verification should be to confirm:

- that hazard analysis and risk assessment were carried out properly and that adequate control or preventive measures have been identified (also a check on validation);
- that CCPs have been identified properly and correctly, and that the critical limits have been set properly (also a check on validation);
- that the control of hazards at CCPs is effective and records of control are kept;
- that monitoring methods are effective and monitoring methods are kept;
- that corrective action procedures work properly and customers/consumers are prevented from receiving non-conforming product, and that records of corrective action are kept;
- that the verification procedures are carried out properly;
- that documentation covering the entire HACCP system has been established and that records supporting the system are completed and retained for appropriate periods of time.

In growing fruit and vegetables the length of production cycles may make it necessary to spread verification activities over the year in order to complete a full verification cycle. Alternatively, if the full production system is covered by a sequence of HACCP systems with limited scope, in recognition of the difficulties of dealing with a production cycle of a year or more, then verification will be arranged to match the scope of each HACCP system. The activity of verification will drive modifications and improvements to the HACCP plan and HACCP system. In crop production it is important to take into account and analyse fully any deviations from production plans caused by, for example, the weather, pest infestations or crop disease and to establish their effects on the results of verification and their implications for changes that are thought to be necessary to the HACCP plan.

Stage 12: Establish and maintain documentation

A variety of documents will be collected and produced during the HACCP study. They constitute the HACCP system documentation. In the production and post-harvest processing of fruit and vegetables, documents such as seed specifications, pesticide and fertilizer data sheets, procedures for pesticide preparation and application, product specifications, raw materials

processing specifications, storage instructions, and so on, all form part of the HACCP system documentation. The process flow diagram will be of significance and the HACCP control chart (Table 7.1) will be central to the HACCP plan and system. This defines the operation and control of the HACCP system. Other important documents will be the CCP control and monitoring procedures, corrective action procedures, verification procedures and, of course, procedures for controlling HACCP system documentation. Records must also be kept as part of the system documentation, including CCP control and monitoring records, corrective action records, system validation and verification records and records relating to changes to the HACCP plan and system.

7.5.4 Implementing, maintaining and improving HACCP systems

Once the HACCP plan is complete it must be implemented and operated before it can be verified. Then it must be run as the HACCP system and it must be maintained. It is important to realise that the plan and system are not static, unchangeable entities. Fruit and vegetable production systems change. Production methods change, as do, for example, inputs to production systems, such as pesticides. New hazards are also recognised from time to time. The HACCP plan and system must be capable of responding to changes. Modifications to both must be made when implications for food safety and the protection of consumers arise. Mortimore and Wallace (2001) define an eight step approach to implementation. Adaptation of this approach (taken from Early, 2002, 2004) to emphasise the implementation of preventive measures, or confirmation of their adequacy if they already exist, leads to a ten step process, as follows:

1. **Determine the approach to implementation** – the HACCP system may be implemented as a complete system or broken down into more manageable units.
2. **Agree the activities to be undertaken and the timetable** – this requires the identification of implementation activities, those responsible for them and a timetable for completion. Project management techniques, e.g. Gantt charts, can be useful.
3. **Confirm the existence of adequate control or preventive measures, or implement measures, as necessary** – control or preventive measures may exist as part of an operational process or may have been identified in the HACCP study and require implementation. Either way they must be confirmed to exist.
4. **Conduct training in the operation of control or preventive measures or confirm adequate operations exist** – preventive measures must be shown to be effective. Staff training may be needed for the operation of new preventive measures.
5. **Set up CCP monitoring methods** – methods for monitoring the control of CCPs must be established.

Table 7.1 Example HACCP control chart for field crop production

Process step	Step no.	CCP no.	Hazard	Control measure	Critical limit(s)	Monitoring** Procedure	Frequency	Corrective action** Procedure
Fertilizer delivery	1							
Fertilizer in store	2							
Seed delivery	3	1	Unacceptable pesticide residues in seed	Seed meets specification	As stated in specification	Check certificate of analysis on delivery	Each delivery	Reject delivery Review supplier
Seed propagation	4							
Pesticide drench	5	2	Microbial pathogens in drench water, e.g. *E.coli* O157, *Salmonella*	Use potable water	Absence of named pathogens	Confirm water quality with supplier	Annually	Agree measures for improvement with supplier
Field preparation	6	3	Contamination with pathogens from un-rotted manure	Check history of manure use on site	No un-rotted manure used in past two years	Confirm site history	Prior to site preparation	Use site only if free from manure deposits or choose another site
Fertilizer application (P & K) during field preparation	7							
Planting of seedlings	8							
Irrigation during crop establishment and growth	9	4	Microbial pathogens in water, e.g. *E.coli* O157, *Salmonella*	Use clean water	Absence of named pathogens	Confirm water quality with supplier	Prior to use	Agree measures for improvement with supplier or use another source
Fertilizer (N) application during growth	10							

Pesticide preparation	11	5	Pesticide(s) prepared at excessive concentration giving unacceptable residue levels	Use calibrated equipment for preparation	As defined by manufacturer for named pesticide(s)	Observe preparation procedures complied with & and check usage of pesticides against stocks	Periodically when pesticide(s) used	Calibrate equipment, revise procedures, or test crop for excess residue, according to nature of failure
		6	Microbial pathogens in water	Use clean water	Absence of pathogens	Confirm water quality with supplier	Prior to use	Agree measures for improvement with supplier or use another source
Pesticide application	12	7	Pesticide(s) applied at excessive concentration giving unacceptable residue levels	Apply with appropriate and calibrated equipment. Use trained staff for job	As defined by manufacturer for named pesticide(s) and/or legally defined limits	Check pesticide usage and observe application procedure	Periodically when pesticide(s) used	Calibrate equipment, revise procedures, or test crop for excess residue, according to nature of failure
Pesticide post-application period	13	8	Pesticide(s) remain in crop at unacceptable residue levels	Observe pre-harvest interval. Do not harvest until interval elapses	Pre-harvest interval as advised by manufacturer for named pesticide(s)	Record dates of pesticide applications and observe interval to harvest	Each crop	Test crop for excess residue and reject if levels exceed requirements
Harvesting (glass control)	14	9	Contamination with glass from machinery	Glass policy – only use glass when needed & care taken when glass involved	No glass contamination of product	Check all glass for damage	Daily	Segregate and check implicated product before approving for use
Harvesting (wood control)	15	10	Contamination with glass from packaging	Care taken when wood is involved	No wood contamination of product	Check packaging materials for damage	Daily	Segregate and check implicated product before approving for use
Harvesting (staff) control	16	11	Contamination with microbial pathogens from staff	Good personal hygiene practiced by staff	Staff adhering to personal hygiene policy	Observation and supervision of staff	Continuous	Appropriate management of staff breaking the rules

Table 7.1 *Continued*

Process step	Step no.	CCP no.	Hazard	Control measure	Critical limit(s)	Monitoring** Procedure	Frequency	Corrective action** Procedure
Storage	17	12	Growth of microbial pathogens on produce	Select temperature & humidity suitable to prevent growth	Adequate temperature & humidity to maintain product quality, but unsuitable for microbial growth	Check storage temperature and humidity	Daily	Segregate and check implicated product before approving for use. Rectify temperature and humidity
Transport	18	13	Contamination with microbial pathogens from transport vehicles	Use only approved vehicles and hauliers. Check vehicles before use	Vehicles clean, hygienic and fit for use	Check records of vehicle inspection	Daily	Agree measures for improvement with haulier or use another approved haulier
		14	Growth of microbial pathogens	Check temperature & humidity suitable to prevent growth prior to despatch	Temperature/ humidity suitable to maintain product quality, but unsuitable for microbial growth	Check records of vehicle temperature and humidity assessment	Daily	Review control procedures. Agree measures for improvement with haulier or use another approved haulier

Note: This table is not as detailed as is possible – it is for illustrative purposes only.
** The responsibility for monitoring and corrective action would normally be given.

6. **Conduct training in CCP monitoring** – staff must be adequately trained and competent in CCP monitoring activities.
7. **Complete 'once-only' activities** – the activities needed to finish the HACCP system must be completed, e.g. procedure writing, creating records, establishing document and record control systems, process engineering and modification, and staff training.
8. **Confirm the monitoring systems are in place** – confirmation must be made that monitoring systems are in place and operating adequately according to effective procedures used by trained staff.
9. **Confirm implementation is complete and operate the HACCP system** – when confirmation is made that implementation activities have been completed the HACCP system can become fully operational.
10. **Audit to confirm adequate implementation** – correct implementation of the HACCP system should be confirmed by audit using standard QA auditing techniques. The system will need to be run for an agreed time to generate records that allow the state of implementation and operational effectiveness to be confirmed. One (or more) complete production cycle may be needed to have complete confidence in the system.

Apart from encompassing necessary changes, the maintenance and improvement of HACCP plans and systems requires the annual review of both the plan and the operational system. Confirmation must be made that the plan and system still meet all food safety requirements and that in operation as a HACCP system, the HACCP plan is being faithfully adhered to. For this reason audits of the system documentation and the operational system should be carried out on a scheduled basis, with corrective action taken to rectify non-compliances.

7.6 HACCP and GAP development

As a result of its international recognition and, importantly, its common sense approach to food safety management and practical benefit, the use of HACCP at all levels of the food supply chain is increasingly being encouraged by official, technical and commercial organisations. Indeed, it is considered that HACCP will become the benchmark for food safety management, as advocated by Codex Alimentarius, and that the adoption of Codex standards by WTO (World Trade Organization) member countries will exempt them from justifying their sanitary measures under the WTO's Sanitary and Phytosanitary (SPS) Agreement (Mayes, 2001). There may be advantages in this for WTO member countries, but the global acceptance of HACCP raises questions about the achievement of standard, globally accepted interpretations and methods of implementation. The problem of achieving a standard interpretation and application of the 7 Principles of

HACCP through the development, implementation and maintenance of HACCP plans and systems has been recognised by the International Organization for Standardisation. At the time of writing the ISO's technical committee, ISO TC 34 – the technical committee for Food Products – is working on the development of an international standard, ISO 22000, on Food Safety Management – Requirements for organisations throughout the food chain. The standard is expected to comply with the 7 Principles of HACCP as established by Codex Alimentarius and to provide a means by which voluntary international standards for food safety management may be harmonised. It should also provide the means by which fully documented and auditable food safety management systems can be designed, developed, implemented and maintained. Use of the standard is intended to be voluntary, but the food safety systems developed in compliance with it may be validated through internal audit and, essentially, self-certification, or they may be extrinsically audited and certificated through third-party processes. It is intended that ISO 22000 will be aligned with both ISO 9001:2000 and ISO 14001:1996 (Environmental Management Systems – Specification for guidance and use), thereby enabling the integration of quality, food safety management and environmental management systems within food production businesses. It is also expected of ISO 22000 that it will provide a standardised means of communicating HACCP requirements throughout the world. The date for the publication of the new food safety management standard was set for mid-2004, but it is likely that it may now be seen in 2005. Alongside the development of this standard, TC 34 is also developing a standard for food product traceability, ISO 22519, Traceability Systems in the Food Chain – General principles for design and development.

Although a voluntary international standard for food safety management may be published and generally accepted, some food businesses still need particular encouragement to use HACCP. At the time of writing all food businesses within the EU are required to implement the first five of the 7 Principles of HACCP, as explained earlier. In the UK some kinds of meat businesses are required to implement all seven principles, following recommendations made in the 'Pennington Report' (Stationery Office, 1997), which resulted from an investigation into an outbreak of *E. coli* O157:H7 poisoning associated with cooked meat products in 1996/97. On the 29 April 2004 new EU food hygiene legislation (Regulation (EC) No. 852/2004) was adopted. This consolidated and simplified preceding EU food hygiene legislation. The new legislation will apply from 1 January 2006 and, significantly, it will require all food businesses in the EU to implement all 7 Principles of HACCP. Importantly, the legislation states that the use of HACCP in primary production (farming and growing) is not yet a generally practical proposition. Thus, the use of HACCP at farm level will not yet be a legal requirement, although its use can be sensible and practical in the case of certain enterprises, e.g., those concerned with the growing of fruit and vegetables. Even though EU law will soon require HACCP to be

applied completely by food businesses, problems are likely to be encountered as a result of inconsistent methods of staff training in HACCP system development and maintenance, and in the way the use of HACCP is 'policed' by enforcement authorities, particularly in different EU countries. Perhaps alongside the development of an international standard for the development and maintenance of food safety management systems, there is need for standards covering (a) the training of HACCP practitioners, and (b) the inspection and auditing of HACCP systems by enforcement officers.

Whilst emphasis may be placed at an international level on the development of standards for food safety management and food traceability systems, it may become increasing important that an international standard for GAP be developed. If the complexity and, often, confusion that surrounds the subject of quality assurance applied at farm level is to be reduced and, ideally, eliminated, perhaps the only route to this objective is by the production of an international standard for GAP. A standard that embodies the elements of quality assurance and good management practice in the operation of animal and crop production businesses, interlinking with different standards, or subordinate standards, for different types of agricultural produce, would seem to offer a more coherent and consistent approach to farm assurance than does the current plethora of standards operated by many different bodies with many different interests. Whether such a development will occur remains to be seen.

7.7 Conclusion

This chapter has examined the use of GAP and HACCP with reference to fruit and vegetable production. As a broad management tool capable of encompassing quality, hygiene and elements of food safety, as well as other dimensions of the business of growing fruit and vegetables, GAP has much to offer and, perhaps, provides broader business benefits than does HACCP. HACCP is strictly concerned with food safety management and, in this respect, it should only be used where there is an identified need to manage food safety. By function, HACCP requires the identification of the hazards associated with products and processes, which have the potential to harm consumers. If hazards are thought to exist use HACCP to identify and control them to safeguard consumers. If hazards cannot be identified, then the growers of fruit and vegetables may find GAP sufficient to meet their needs.

7.8 Sources of information (worldwide) and training (in the UK)

CAMPDEN AND CHORLEYWOOD FOOD RESEARCH ASSOCIATION (CCFRA), Chipping Campden, Gloucestershire, GL55 6LD, United Kingdom. http://www.campden.co.uk (Information and training).

EUREPGAP c/o FoodPlus GmbH, Spichernstrasse 55 D-50672 Koln, Germany. www.eurep.org.

EUROPEAN COMMISSION FOOD SAFETY website. Food Safety: From the Farm to the Fork. http://www.europa.eu.int/comm/food/index_en.html.

the FOOD BUSINESS INITIATIVE, Harper Adams University College, Newport, Shropshire, TF10 8NB, United Kingdom. http://www.foodbusinessinitiative.co.uk (Information and training on HACCP, ICM, AMTRA & BASIS).

ILSI (International Life Sciences Institute) USA. One Thomas Circle, 9th Floor, Washington DC, 20005, USA. http://www.ilsi.org/

ILSI (International Life Sciences Institute) Europe. Avenue E. Mounier 83, Box 6, B-1200 Brussels, Belgium. http://europe.ilsi.org/

UK PESTICIDE SAFETY DIRECTORATE. PSD, Mallard House, Kings Pool, York, YO1 7PX, United Kingdom. http://www.pesticides.gov.uk/

US FOOD & DRUG ADMINISTRATION, 5600 Fishers Lane, Rockville, MD 20857-0001, USA. http://www.fda.gov/default.htm. See also: the *USFDA Foodborne Pathogenic Microorganisms and Natural Toxins Handbook* (Bad Bug Book) at, http://vm.cfsan.fda.gov/~mow/intro.html

US GOVERNMENT CENTERS FOR DISEASE CONTROL. Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA., 30333, USA http://www.cdc.gov/

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8

Implementing on-farm food safety programs in fruit and vegetable cultivation

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8.1 Introduction

The phone rings at a farmer's house. A buyer at the other end says that health officials have linked an outbreak of foodborne illness to a specific commodity – the farmer's commodity – and are requesting that the producer provide documentation supporting the safety of the product. In the meantime, a public statement will be issued and sales will most likely decline; further, borders for exports could be closed.

Canadian fresh fruit and vegetable producers have only been implicated as the source of two outbreaks of foodborne illness since 1981 (Sewell and Farber, 2001; Strauss *et al*., 2002). However, this lack of identifiable outbreaks in the past provides little in the way of protection against future associations, given better surveillance and increasing consumption of fresh fruits and vegetables.

There have been over 400 known produce-related outbreaks in North America since 1990, resulting in over 21 000 illnesses (CSPI, 2004). There have been thousands of outbreaks without identified causes. The level of public and buyer understanding has risen steadily since the mid-1990s. The adoption of an on-farm food safety (OFFS) program can help producers reduce food safety risks and retain, even expand, market share, strengthening relationships with customers and consumers through proactively addressing risks and creating trust.

Nevertheless, any on-farm food safety program has limitations, including: appropriate paperwork and documentation; dissemination of evidence-based information to producers; the role of third-party audits versus producer-driven verification; and, the source and allocation of resources to pay for such programs. Further, while the hazard analysis and critical control point (HACCP), or HACCP-based approach to controlling food safety risks has merit, attempts to turn producers into HACCP experts are often misguided.

HACCP is based on the control of critical points in food production: that control must be verifiable and must have been proved to be verifiable in research studies. Because there is still little known about the mechanisms of how fresh produce becomes contaminated on-farm, HACCP purists argue that it is almost impossible to define true critical control points in fresh fruit and vegetable production, and the terminology is often misused. The US Food and Drug Administration (FDA), the United Fresh Fruit and Vegetable Association and the International Fresh-cut Produce Association suggest that because critical control points are, at this point, unachievable, a true HACCP system is too rigid for the farm. A HACCP-based program that incorporates the principles of carrying out a risk assessment and establishing points of control where good agricultural practices are applied can be effective in reducing risks on the farm. Regardless of the acronym, any program must be practical, credible and cost-effective. Guidelines must be accompanied by aggressive implementation, documentation, verification, incentives and, most importantly, support. Individual producers do not need to become food safety experts, but they do need, and usually want, to do the right thing.

Effective on-farm food safety programs have a mechanism to effectively and efficiently document risk-reduction practices. The documentation provides a quick reference to specific practices for interested buyers or for regulators in the case of an outbreak. The documentation medium does not matter, whether it is a checklist that is posted on the wall, a computer spreadsheet or a notebook, as long as it is accessible, complete and current. Verification provides a producer with a record of how well an on-farm food safety program is being implemented, can reveal potential areas of concern and, over time, can provide data that demonstrate continuous improvement in terms of risk reduction. It is not enough to provide a set of guidelines from a government agency or producer association and expect growers to comply with standards. Industry organizations and their producer members must be provided with on-going, evidence-based information, a dialogue of support that can promote the adoption of new practices. Effective on-farm food safety programs require a variety of components that alone are meaningless, but together, provide evidence demonstrating proactive producer-led, risk reduction. In short, on-farm food safety programs should be clearly

designed so producers can: say what they do; do what they say; and verify that it works.

8.2 Systems controlling foodborne illnesses

Outbreaks of foodborne illness, traced to a variety of different foods, can be found worldwide. It has been estimated that there are 76 million incidents of foodborne illnesses in the USA each year (representing approximately one-in-four citizens (Mead *et al*., 1999)); Australian authorities have also validated this estimate (one-in-four citizens, (OzFoodNet Working Group, 2002)).

There has been a continued rise in reported outbreaks of foodborne illness associated with the consumption of fresh fruits and vegetables. Bacteria, viruses and parasites on fruits and vegetables have been linked with illness. In Canada, 18 outbreaks were documented from 1981 to 2000, with approximately 2000 people affected and 18 deaths. Alfalfa sprouts, cantaloupe, lettuce, raspberries and parsley are included amongst the implicated vehicles. The very nature of produce that makes it healthy – fresh and consumed raw – is what makes fresh produce a high-risk food for microbial contamination. Without the microbiological kill step provided by cooking, produce is vulnerable to contamination from the farm-to-fork.

Pathogens can contaminate at any point along the food chain, at the farm, packing shed, processing plant, transportation vehicle, retail store or food service operation, and the home. By understanding where potential problems exist, it is possible to develop strategies to reduce risks of contamination (Tauxe *et al*., 1997). Raw produce can become contaminated with pathogenic and non-pathogenic microorganisms at a number of different stages, by several means, from production through to consumption. Laboratory studies have found that fresh produce can support the growth of organisms such as *Salmonella*, *Shigella* and *Escherichia coli* O157 : H7. Consequently, methods of growing, handling, processing, packaging and distribution of fresh produce have received increased attention in terms of identifying and minimizing microbiological hazards. The produce industry has now focused on developing and implementing programs aimed at reducing foodborne disease and illness. Complete HACCP systems can never be implemented in fresh produce operations, as there is no definite kill step, such as pasteurization. Instead, these HACCP-based systems help to identify and reduce the potential for microbial contamination along the entire production and distribution process. A successful program helps to avoid recall campaigns, adverse publicity, loss of sales and serves to enhance public health. There is value in applying the steps of HACCP to fruit and vegetable production, using available scientific information as part of the framework, to reduce the risk of foodborne pathogens.

8.2.1 HACCP-based programs

HACCP is a system of food safety control based on a systematic approach to the identification and assessment of hazards associated with food operations and the definition of means for their control. This approach focuses on prevention and control and is advocated for every stage in the food chain, from primary producers through to the final consumer (California Strawberry Commission, 1998; International Fresh-cut Produce Association and Western Growers Association, 1997; United Fresh Fruit & Vegetable Association, 1997).

HACCP has gained recognition throughout the developed world as the best food safety assurance system currently available. It has been recommended by the US National Academy of Sciences and the World Health Organization's Codex Alimentarius Committee, as well as the US Food and Drug Administration (FDA) as an effective and workable approach to food safety control, which can be incorporated into a total quality management program (US Department of Agriculture Food Safety and Inspection Service, 1989). Besides its preventive nature, the HACCP system exercises control over the manufacturing process at critical stages which are known as critical control points (CCPs), detecting or correcting defects which might have an impact on the safety and wholesomeness of the product before its packaging and distribution (Food Safety Enhancement Program, 1993). Until the introduction of HACCP, end-product testing was used as a means of assessing food safety, whereby a percentage of samples were taken for microbiological, chemical or physical testing to determine if the product met with the customer's acceptance criteria. However, a number of limitations to this approach have been recognized, usually summarized by the maxim, 'You cannot test your way to a safe food supply'. Testing has a role in verification of HACCP plans or in establishing critical limits for CCPs, but is limited by sampling plans that are based on the probability of a fault being identified from a representative number of samples being tested. The HACCP approach to food safety moves away from testing of the final product, and instead emphasizes raw material and process control, providing a structured and systematic approach to the control of identified hazards.

The application of the HACCP system consists of a logical sequence of twelve steps encompassing seven basic principles, which can be implemented in any food industry. Recently, HACCP-based programs have been extended to the on-farm environment as a way to reduce risks associated with commodities before they enter the processing environment. However, there is still little known about the mechanisms whereby produce becomes contaminated, so HACCP purists argue that it is almost impossible to define true critical control points in fresh fruit and vegetable production. The FDA, the United Fresh Fruit and Vegetable Association and the International Fresh-cut Produce Association suggest that because critical control

points are unachievable, a true HACCP system is too rigid for on the farm. A HACCP-based program that incorporates the principles of carrying out a risk assesssment and establishing points of control where good agricultural practices (GAPs) are applied has been shown to work in reducing risks on the farm (Powell *et al*., 2002; Luedtke *et al*., 2003).

Some have suggested that actions controlled by human behavior – such as handwashing, or the application of agricultural chemicals – be considered as CCPs. Others, however, have noted the difficulty in monitoring human behavior versus monitoring pasteurization temperatures or other mechanically monitored activities. Nevertheless, reliance on well-developed and consistently performed standard operating procedures (SOPs) and GAPs can simplify the HACCP-based plan.

The FDA states that growers, packers and shippers are urged to take a proactive role in minimizing food safety hazards potentially associated with fresh produce (US Food and Drug Administration, 1998). Being aware of, and addressing, common risk factors can result in a more effective, cohesive response to emerging concerns about microbial hazards and fresh produce. Furthermore, producer associations should encourage the adoption of safe practices by their partners along the farm-to-table food chain.

Developing an on-farm food safety program for a specific commodity by relying on generic formulations may not be effective for multitude of horticultural commodities. Basing programs on generic horticultural GAPs can work, but the implementation of programs by individual producers must be flexible and adaptable to various types of farms.

Recent public interest in microbial food safety and dietary concerns indicates that food safety risk management systems must be both scientifically credible and publicly accountable. On-farm risk management systems such as food safety programs are becoming the cost of doing business and can enhance public trust if the industry can verify what they say they are doing. To this end, open and transparent communication of the potential risks encountered on the farm, how they are addressed and producer compliance is essential. Buyers and government drive the need for food safety standards. These two groups, and sub-groups within each, possess different needs that can lead to various sets of standards for the same product. Industry-led, on-farm food safety programs can provide the infrastructure to create a dialogue with buyer and government groups regarding action taken to ensure a commodity sector is producing safe food, with results in hand to demonstrate compliance.

The recent North American BSE situation has provided an extreme example of how a food safety issue can have an impact on an industry quickly and extensively through trade restrictions; implementing an on-farm food safety program to reduce potential for food safety risks provides mechanisms to minimize impact when a food safety issue arises.

Nevertheless, one of the primary incentives for on-farm food safety programs is to maintain market share and strengthen relationships with

customers and consumers by enhancing trust by a proactive program. The voluntary approach taken through Environmental Farm Plans in the Canadian province of Ontario is a good model for on-farm food safety compliance: liability concerns ensure that producers participate.

8.3 Existing guidelines and OFFS programs for fresh fruit and vegetables

There are a variety of generic and specific guidelines for safe fresh fruit and vegetable production in North America (for a summary of all on-farm food safety programs see Appendix 1). These programs are generally based on HACCP and many are also based on the US FDA's *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables* published in 1998.

Basing programs on HACCP principles provides scientific credibility for the guidelines only. Producers still need to be able to prove they are implementing the guidelines and continually monitoring all control points, including employee sanitation. The majority of on-farm food safety programs for fruits and vegetables in Canada are composed solely of these HACCP-based guidelines with little verification of producer implementation.

GAPs include:

- equipment maintenance program
- sanitation program within facilities/packing area
- end of season
- washroom facilities
- employees
- pest control program
- storage maintenance program
- transportation program
- microbiological sampling.

As fresh fruit and vegetable food safety management is in its infancy, interested individuals and groups are hoping to capitalize on the potential for verification schemes, traceability implementation and guideline design – all at the cost of individual producers. Producers need to lead the discussion about on-farm food safety, in a regional manner, to allow flexibility of programs, keep control close and best fit into the needs of buyers.

Verification provides an evaluation of the risk-reduction steps that are being recorded in a producer's documentation, and is continuously undertaken. Verification provides a producer with a record of how well the on-farm food safety program is being implemented, can reveal potential areas of concern and, over time, can provide the data that demonstrate

improvement. Verification can also be provided for a buyer to demonstrate that the program is accomplishing its goals. Audits can provide a snapshot of a producer's facilities and documentation, but many auditors lack the microbiological or chemical testing capabilities, or interest, that can enhance a program's credibility. Openly providing sample testing methodology and results for a buyer can demonstrate that a producer has nothing to hide and that appropriate steps are effectively being taken to produce safe food.

Communication with employees is an integral part of an on-farm food safety program. Poor employee hygiene has been responsible for over 40% of source identified produce-related outbreaks (Bean and Griffin, 1990). Agricultural employees are on the frontlines of food safety, and providing program ownership for them by setting a good hygiene example, providing effective training and making available current food safety information demonstrates to employees that food safety is non-negotiable. Food safety co-ordinators, either as employees or consultants to individual growers or producer groups, may be best suited to accomplish these tasks.

8.4 Adoption of OFFS – grower perceptions, practical solutions, experiences from the field

The philosophy behind auditing is to provide verification. An audit on its own does not promote the culture of food safety, a culture in which management and employees understand what they need to do, why, and how to reduce food safety risks on the farm.

Researchers have identified three types of barriers to successful implementation of HACCP-based programs including: knowledge barriers – knowing about and understanding the program; attitudinal barriers – agreeing with the principles of the program and believing that actions will have an impact on food safety; and, behavioral barriers such as allocating resources including time, money and staff. It is not enough to provide a set of guidelines and expect growers to comply with standards. Industry organizations and their producer members must be provided with ongoing information, a two-way dialogue of learning and support that will promote the adoption of new practices. Recent research has shown that producers prefer to have on-site visits when learning about production practices and will implement procedures correctly when shown them in terms specific to their site. (Maddox *et al*., 2003). On-farm food safety programs should not waste money by putting producers in classrooms; funds need to be invested into effective on-site visits.

Coaching producers through on-site visits provides the program requirements in specific terms on individual farms and encourages participants to

ensure they are actively implementing, monitoring and maintaining their own on-farm food safety program. Visits should be on-going and occur on a schedule, ideally at least once or more per season. During these visits, participants receive materials for their operations such as hand sanitizers and signage, receive training materials for farm workers, have food safety put into terms that are specific to their site and are provided with a forum where potential risk issues can be discussed.

It is has been found that it is not enough simply to provide producers with a manual of food safety guidelines and expect full implementation and documentation (Powell *et al*., 2002, Luedtke *et al*., 2003). Evaluation of on-farm food safety programs found that simple manuals were not effective in overcoming the barriers to implementing the on-farm food safety program. Workable food safety programs must provide individual support for growers. A food safety coordinator can provide the one-on-one support that is needed and evaluation of such programs has indicated that this one-on-one support is one effective tool to overcoming these barriers.

Good on-farm food safety programs have a mechanism to keep records of risk-reduction practices. The documentation provides a quick reference of specific practices for interested buyers and, or also, for regulators in case of an outbreak without an in-depth investigation. Documenting when equipment sanitation occurs, what chlorine levels are in wash water or when an employee is sick, demonstrates that food safety is a priority. The documentation medium does not matter, whether it is a checklist that is posted on the wall, a computer spreadsheet or a notebook, as long as it is accessible, complete and is kept up-to-date.

Communication of program goals and risk reduction practices with employees is an integral part of an on-farm food safety program. Agricultural employees are the front line barrier for food safety, and providing program ownership for them by setting a good hygiene example, providing effective training and passively posting current food safety information shows employees that it is important and can improve an employee's practices. An external communications network is necessary to support the program proactively, as well as reactively. Following food safety issues such as outbreaks or potential contamination incidents, representatives need to be ready to respond to public questions through the media.

8.5 Examples from Food Safety Network on-farm food safety research

After three years of research by the Food Safety Network with the same farming community, it was found that producer understanding of food safety issues was dependent on a personal experience, similar to previously reported agronomic information transfer (Maddox *et al*., 2003).

Anecdotally, it has been observed that if a producer has had incidents of foodborne illness in the past or has witnessed the effects of such, they are more likely to implement a food safety program vigilantly. The use of the verbal narrative in the form of recent food safety media coverage was well received by many producers (Chapman and Powell, 2004).

Remaining up-to-date on the documentation and recording of practices was identified as an implementation barrier because it is time-consuming and the priority of food safety does not appear to be static with many growers. While food safety is recognized as being important, it is not always viewed as important as other farming issues such as selling prices or the costs of inputs. This is not surprising, as a farmer will not receive any additional price premiums for a product that has been produced following a program, or any documented GAPs. The variability of food safety as a priority with producers was realized when dealing with producers who had the same problems in consecutive seasons, such as a poor equipment sanitation record, though reported that they were following procedures.

Conversely, it was found that producers who did not have food safety issues (such as microbial contamination) in prior years were less trusting of researchers' suggestions to change practices, citing that it had never been a problem in the past. Passively providing information to support an on-farm food safety program was not widely utilized. The majority of producers received the information but did not always read the suggestions or make changes on their sites. This supports the need for various communication vehicles when implementing on-farm programs. On-site visits, phone calls, use of a website, newsletters, faxes and meetings should all be available to make the most impact; trust was built up with producers by being available for questions by every means.

Being too accessible and promoting safe food handling and the reasons behind a food safety program were not always seen as positive. One farmer mentioned that the implementation of the on-farm food safety program was a way for researchers to create more work for themselves; that the program was an attempt to increase reliance through fear. He also maintained that food safety was a myth and people have always dealt with the problems with no consequences.

The ability of producers to communicate effectively with their employees is also a significant barrier, implying that there is an inability for producers to convey reasons for on-farm food safety and control measures. Thus, good communication is not a barrier, bad communication is, and can reduce the effectiveness of program implementation. Providing employees with the tools and a training program is not enough. One farmer relayed an anecdote in which new toilets were installed; all the employees had been provided with latex gloves and instructed when to use them. Within a week of the training an employee with gloves on was seen urinating on the outside of a bathroom unit (which had been installed in a greenhouse on a cement pad). The producer felt that he needed to increase his own vigilance

in explaining the consequences of the unhygienic practices, at all times, but could not watch his employees at all times.

8.6 Conclusions: best practices for an ideal OFFS program for fresh fruit and vegetables

On-farm food safety programs should not waste money by putting producers in classrooms; funds need to be invested in effective on-site visits. On-going research and continuous evaluation is required not only to understand sources and pathways of contamination better, but also to, for example, determine the most-effective ways of communicating with employees, to develop more practical documentation and to integrate on-farm food safety programs better, with nutrient management plans, spray records and environmental farm plans to create a farm-specific approach to produce production.

The components of a complete on-farm food safety system include:

- transparency;
- developed with input from both growers and buyers for acceptance;
- based on the best available science;
- flexible and continuously evolving and improving;
- easy to understand for producers, buyers and consumers;
- providing support for individual growers;
- ensuring understanding of the requirements, documentation and principles;
- utilizing multiple strategies to reduce knowledge, attitude and behavioral barriers;
- efficient and inexpensive; and,
- well documented.

There is no single correct way to include all of the items that are components of an on-farm food safety program; rather, programs should be tailored to the needs of different customers with the goal of retaining or even enhancing market share. The components of a program must also be flexible enough to include the smallest of growers while catering for the needs of large growers.

8.7 References

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Appendix 1: A summary of on-farm food safety programs or guidelines for fresh fruit and vegetables worldwide

The programs or guidelines are those intended to be used on the farm, to be implemented by the grower, an association, for government regulation, for certification or for a third party auditor. The programs or guidelines found were organized by place of origin: Canada, USA and internationally. Second, the programs were divided according to the group that developed or administered them: government, university or college, industry association, industry, retail body or individual companies. There may be many other programs or guidelines, especially those offered by individual companies that are not mentioned. The on-farm food safety programs (OFFS) or guidelines that have been initiated in Canada are shown in Table 8.1, in the USA in Table 8.2 and international programs are shown in Table 8.3.

Table 8.1 On-farm food safety programs or guidelines in Canada

FEDERAL Government initiatives					
Program/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Canadian On-Farm Food Safety Program (COFFSP)	Canadian Federation of Agriculture (CFA) and Canadian Food Inspection Agency (CFIA) (Technical Support)	May 1997	Achieve recognition for national commodity associations programs	Agriculture and Agri- Food Canada's Canadian Adaptation and Rural Development Fund	Http://www.cfa-fca.ca/english/programs_and_projects/onfarm_food_safety.html
Code of Practice for Minimally Processed Ready-to-Eat Vegetables	CFIA	Feb. 1999	Voluntary implementation. No audit. Available on website	CFIA	Http://www.inspection.gc.ca/english/plaveg/fresh/read-eat_e.shtml
Code of Practice for the Hygienic Production of Sprouted Seeds	CFIA	Sept. 2001	Voluntary implementation. No audit. Available on website	CFIA	http://www.inspection.gc.ca/english/plaveg/fresh/sprointe.shtml

Provincial Government initiatives

Program/guidelines	Developed by	Start date	Current status	Funded by	Reference
Keeping Berries Safe: A Grower's Guide to Preventing Food-Borne Illness from Berry Crops	Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)	Feb. 2000	Voluntary implementation. No audit. Available on website	OMAFRA	http://www.gov.on.ca/OMAFRA/english/crops/facts/berry_obgabrochure.html

National commodity associations initiatives

Program/guidelines	Developed by	Start date	Current status	Funded by	Reference
On-Farm Food Safety Guidelines for Fresh Fruit and Vegetables (OFFSP)	Canadian Horticultural Council (CHC)	1997	Phase 3 of COFFSP: Implementation	Agriculture and Agri- Food Canada	Http://www.hortcouncil.ca/offs.htm

Table 8.1 *Continued*

Industry associations/board initiatives					
Program/guidelines	Developed by	Start date	Current status	Funded by	Reference
Food Safety	BC Vegetable Marketing Commission		Voluntary implementation. No audit. Available on website	BC Vegetable Marketing Commission	http://www.bcveg.com/prod 03.htm
Partners in Quality – PIQ Ontario: On-Farm Food Safety Guidelines For Fruit and Grape Growers	Ontario Tender Fruit Producers' and Ontario Fresh Grape Growers' Marketing Boards	Feb. 2001	Voluntary implementation. No audit	OMAFRA Healthy Futures Fund	Ontario Tender Fruit Producers' and Ontario Fresh Grape Growers' Marketing Boards. 2001. Partners in Quality – PIQ Ontario: On-Farm Food Safety Guidelines For Fruit and Grape Growers.
Processing Vegetables: On-Farm Food Safety Handbook	The Ontario Processing Vegetable Grower's Marketing Board	May 2001	Voluntary implementation. No audit	The Ontario Processing Vegetable Grower's Marketing Board	Ontario Processing Vegetable Grower's Marketing Board. 2001. Processing Vegetable On Farm Food Safety Handbook
Ontario Greenhouse Vegetable	Ontario Greenhouse Vegetable Growers	July 1999	All members participating, ongoing farm visits,	OMAFRA Healthy Futures Fund	http://www.ontariogreenhouse.com/pdf/manual.pdf

Growers On-Farm Food Safety Program			microbiological testing and program support by employed food safety coordinators, for verification		
Agricultural Integrated Management Services (AIMS) Project P.I.L.O.T	AIMS with participation from the Ontario Fruit and Vegetable Growers Association (OFVGA), the Ontario Potato Board, the Ontario Asparagus Growers and the University of Guelph	Dec. 2001–2003	Food safety coordinators implement pilot project on 20 trial farms with 9 commodities over the 2002 season. On-site visits, microbiological testing of water and produce and program support	OMAFRA Healthy Futures Fund	(in progress)

Company Initiatives

Program/guidelines	Developed by	Start date	Current status	Funded by	Reference
Integrated Food Safety & Quality Systems	Guelph Food Technology Centre (GFTC)*	Spring 2001	Use HACCP principles to train and audit growers. Also, provide training in SQF (Safe Quality Food) 1000/ 2000 Codes[CM]. Logo available	Paid by individual group that requests services	http://www.gftc.ca/

* GFTC is currently working with the Canadian Mushroom Growers Association to implement HACCP programs on producer's farms. http://www.cmga.ca/protected/mushroom.htm.

Table 8.2 US on-farm food safety programs or guidelines

Federal Government initiatives

Program/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables	Food and Drug Administration (FDA), US Department of Agriculture (USDA) and the Center for Food Safety and Applied Nutrition (CFSAN)	Oct. 1998	Voluntary implementation. No audit. Available on website 12-month pilot across the USA,	FDA	http://vm.cfsan.fda.gov/~dms/prodguid.html
Fresh Produce Audit Verification Program	USDA, Federal–State Inspection Service (FSIS)	Oct. 2001	Voluntary implementation. On-farm scored audits by FSIS based on *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. No logo used. Audit form can be found on the website. Independent third-party audits performed by Federal–State Inspection Service staff	Individual requesting services	http://www.ams.usda.gov/fv/fpbgapghp.htm

Microbiological Safety Evaluations and Recommendations on Sprouted Seeds	USDA: National Advisory Committee on Microbiological Criteria for Food (NACMCF)	May 1999	Voluntary implementation, No audit. Available on website	USDA	http://vm.cfsan.fda.gov/~mow/sprouts2.html

National trade associations initiatives

Program/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Food Safety Auditing Guidelines: Core Elements of Good Agricultural Practices for Fresh Fruits and Vegetables	United Fresh Fruit and Vegetable Association (UFFVA)	Sept. 2001	Voluntary implementation. No audit. Available on website	UFFVA	http://www.uffva.org/food_safety_docs.htm
Field Cored Lettuce: Best Practices	UFFVA, National Processors Association and International Fresh-cut Produce Association (IFPA)	April 2001	Voluntary implementation. No audit. Available on website	UFFVA	http://www.uffva.org/news/wklyUpdate_report.cfm?id=1032&wklydate=4/27/01
Food Safety Guidelines for the Fresh-Cut Produce Industry, Fourth Edition	IFPA	2001	Voluntary implementation. No audit. Available on website	IFPA	http://www.fresh-cuts.org/publications1329/publications_show.htm?doc_id=2034
Revised! HACCP Plan for Fresh-Cut Produce	IFPA	2001	Voluntary implementation. No audit. Available on website	IFPA	http://www.fresh-cuts.org/publications1329/publications_show.htm?doc_id=2037

Table 8.2 *Continued*

Federal Government initiatives					
Program/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Food Safety Program for Florida Producers	Florida Fruit and Vegetable Association	2001	Voluntary implementation. Farmers receive tool kit and education on creating food safety program. Florida Fruit & Vegetable Research & Education Foundation was awarded a grant to teach producers about basic GAPs	FFVA	http://www.ffva.com/food_safety.shtml
University/college initiatives					
Programs/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Reducing Food Safety Risks in Apples: A Self-Assessment Workbook for Producers of Apples, Juice and Cider	Washington State University, Cooperative Extension Home * A * Syst-Farm * A * Syst	May 2001	Voluntary implementation. No audit. Available on website	USDA-CSREES Food Safety Grant	http://organic.tfrec.wsu.edu/FoodSafetyWeb/Home.htm

Food Safety Begins on the Farm: A Grower's Guide, Good Agricultural Practices for Fresh Fruits and Vegetables	Cornell University	2001	Voluntary implementation by Cornell GAPs Team: members in 16 different states train growers and packers	USDA-CSREES and US FDA	http://www.gaps.cornell.edu/
Extensive Regional Food Safety Program	North Carolina State University (NCSU)		Voluntary implementation of fresh produce safety guidelines, risk management, recall information etc. in a cooperative effort by 11 states	Initially Funded by USDA-CSREES	
The UVM Apple Program	University of Vermont Extension	April 2000	Voluntary implementation. No audit. Available on website	USDA	http://orchard.uvm.edu/uvmapple/foodsafety/default.html
Food Safety in Vegetable Production (several documents)	University of California, Davis Cooperative Extension	Feb. 2002	Voluntary implementation. No audit. UC Cooperative Extension farm advisors: address issues relating to production practices, resource management, pest management, food safety and worker safety	UC Davis	http://vric.ucdavis.edu/selectnewtopic.foodsafety.htm
Good Management Practices for Safe Production of Fresh Market Apples and Apple Cider	Penn State: College of Agricultural Sciences	July 2001	Voluntary implementation. No audit. Available on website	Penn State	http://tfpg.cas.psu.edu/part7/part79a.htm

Table 8.2 *Continued*

Industry associations/board initiatives

Programs/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Quality Assurance Program	California Strawberry Commission	1996	California growers, shippers and processors voluntarily implement program. Comprehensive voluntary quality assurance program: field sanitation, traceback, soil and water testing, pesticide use, GAPs	California Strawberry Commission	http://www.calstrawberry.com/facts/industry.asp
Hazard Analysis Critical Control Point (HACCP) Check List	International Sprout Growers Association (ISGA)		Voluntary implementation. No audit. Available on website	IFPA	http://www.isga-sprouts.org/haccp.htm

Industry company initiatives

Programs/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Good Agricultural Practices in the Field	Dole Fresh Vegetables Inc.	1998	Dole's growers only supply them and are required to follow guidelines. Monitored in the field. Facilities are ISO 9000, 14000 and AIB certified (http://www.aibinternational.com/consolidatedstandards/Packinghouses/)	Dole Fresh Vegetables Inc.	http://www.dole.com/industrial/safety/Index.ghtml

Individual company initiatives

Programs/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
ProSafe Certified program	Davis Fresh Technologies (based in the USA)		Three step process. Auditors offer certification internationally. No microbiological testing. ProSafe Certified Logo	Individual Group Requesting Services	http://www.davisfreshtech.com/prosafe/index.html
Food Safety Management and HACCP Programs	Scientific Certification Systems		Bilingual staff develop and implement food safety programs internationally. Third party audits. HACCP-based certification: teach, evaluate and verify.	Individual Group Requesting Services	http://www.scs1.com/
Food Safety Services	Primuslabs.com[a,b]		Affiliated auditors (i.e. AAC Consulting, www.aacgroup.com) and Primus Auditors Worldwide offer certification through manual development, residue testing, microbiological testing and documentation.	Individual Group Requesting Services	http://www.primuslabs.com/index.html

Table 8.2 *Continued*

Industry associations/board initiatives					
Programs/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Good Agricultural Practices (GAPs)	AAC Consulting Group		Primuslab.com logo Provide third party auditing services (Primuslabs Affiliated http://www.primuslabs.com/index.html). Food Safety and HACCP services. No logo used	Individual Group Requesting Services	www.aacgroup.com
Retailer initiatives					
Programs/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
H-E-B Fresh Produce Code of Best Practices	H-E-B	1999	Audits conducted by H-E-B Quality Assurance Partners. Approval is by facility and/or process not by suppliers (not on the farm). Microbiological testing of water mandatory	H-E-B	H-E-B Fresh Produce Code of Practice, 2000

[a] Subway contracted Primus Labs as the third party auditor of all their suppliers of lettuce, tomatoes and green peppers in North America. Subway mandated that all suppliers be in compliance by Feb. 2001 http://www.primuslabs.com/ap/fc_022001.html.
[b] Safeway, Albertson's and Publix Supermarkets Inc. have also requested third party audits of produce suppliers through Primus Labs http://www.primuslabs.com/pb/index.html.

Table 8.3 On-farm food safety programs or guidelines worldwide

Initiatives					
Program/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Code of Practice For Food Safety in the Fresh Produce Supply Chain in Ireland	Food Safety Authority of Ireland (FSAI)	Sept. 2001	Voluntary implementation. No audit	FSAI	Code of Practice for Food Safety in the Fresh Produce Supply Chain in Ireland. 2001. Food Safety Authority of Ireland
Fresh Safe Food: Food Safety Guidelines for the Australian Fresh-Cut Produce Industry Hygiene Code for Fruit and Vegetable Growers	Cooperative Research Centre for International Food Manufacture & Packaging Science (CRS for IFMPS) and The Fresh-Cut Industry Netherlands, Product Board for Horticulture	Dec. 1998	National Food Safety Guidelines. Training for industry. Fresh Safe Food™ Certification logo Voluntary implementation. No audit.		http://www.foodpack.crc.org.au/frames.htm
EUREPGAP Fruits and Vegetables	Euro-Retailer Produce Working Group (EUREP)	Oct. 1997	Use guidelines to certify international certification bodies (i.e. Primuslabs). Use of EurepGap logo. Growers contact certification body.	Individual Requesting Services	http://www.eurep.org/sites/index_e.html

Table 8.3 *Continued*

Initiatives					
Program/guidelines	Developed/ administered by	Start date	Current status	Funded by	Reference
Assured Produce Scheme (APS)	Checkmate International plc (CMi)	June 1997	Certification of individuals internationally	Individual Requesting Services	http://www.cmi-plc.com/
AIB Consolidated Standards for Fresh Produce and Fruit Packinghouses	AIB International (AIBI)		Certification by AIB auditors. AIBI Gold Standard logo	Individual Requesting Services	http://www.aibinternational.com/consolidatedstandards/Packinghouses/
Global Food Safety Initiative Guidance Document	CIES – The Food Business Forum	May 2000	Certification – CIES Logo not on product, only on business communications System not ready until Spring 2002.	Individual Requesting Services	http://www.ciesnet.com/
*SQF Program**	WA Department of Agriculture and SQF Institute (based In Lausanne, Switzerland)	1995 (SQF2000) 1999 (SQF1000)	Accredited companies can certify individuals. Both programs use logos	Individual Requesting Services	http://www.sqf.wa.gov.au/index.html

* Société Générale de Surveillance S.A. (SGS) http://www.sgs.com/sgs/psc/psc_serv.nsf/pages/SQF+2000+-+Safe+Quality+Food+Certification and as previously mentioned the GFTC, are examples of groups qualified to use the SQF program and logo.

9

Alternatives to pesticides in fruit and vegetable cultivation

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9.1 Introduction

Alternative tactics for crop protection reduce pest numbers to a level that does not cause economic damage in the crop. They include cultural and agronomic tactics, mechanical, physical and biological tactics, behavioural control using semiochemicals, and the principle of the sterile insect release method. In conventional high-input agriculture, the most convincing reason, from the grower's point of view, for replacing or complementing pesticides with alternatives is to avoid or delay pesticide resistance and to prevent the resurgence of primary pests and/or upsurges of secondary pests. Other important reasons are food, worker and environmental safety. Alternative tactics are not universal; rather, they are location and time specific. The cornerstone alternatives, cultural tactics being the most essential, work by the principle of preventing pest colonization or multiplication. Some alternative approaches lend themselves to remedial use, but the emphasis is always on prevention rather than intervention.

In this chapter, the basic features of alternative plant protection tactics, their integration, and their food, worker and environmental safety, are outlined. Two production systems for fresh food – apples and greenhouse vegetables – are used to exemplify the possibilities, efficiency and current status of application and integration of alternative methods. The scope of the case studies is mainly European. Lastly, the basic future trends and factors that drive or hinder the adoption of emerging alternative tools are outlined.

9.2 Alternative tactics for pest management

9.2.1 Cultural control

Cultural control is the deliberate alteration of the production system or its individual practices with the goal of avoiding or reducing pest injury to crops by targeting the pest itself through agronomic practices. Preventive cultural tactics, particularly crop rotation, vegetational biodiversity and pest-resistant crop plants, form the baseline for designing an alternative pest management system. On this baseline, other tactics, that is, those of a remedial nature, can be added if necessary. The numerous individual tactics of cultural control tend to be pest and crop specific (Ferro, 2003). The types of cultural tactics below are grouped in three categories based on what aspect of the pest population they influence.

Tactics that prevent, reduce or delay pest colonization of the crop

Site selection

Site selection aims to locate the crop field so that pests cannot easily find their way there from the site of the previous year's crop or from natural overwintering sites, or the site has such abiotic and biotic characteristics that affect pests adversely (e.g. suppressive soils). Pest-free plant material, equipment and soil play a crucial role in ensuring the site does not become 'deliberately' infested with pests.

Increased vegetational biodiversity

Vegetational biodiversity within and around the crop field creates the ecological infrastructure needed to enhance natural pest control in agroecosystems. Traditionally, vegetational biodiversity of an agroecosystem is supposed to alleviate pest problems at least for four main reasons: by making the resources of pests less concentrated, that is, replacing monocultures with more diversified vegetation (the resource concentration hypothesis); by increasing the diversity and/or abundance of natural enemies (the enemies hypothesis); by associational resistance (Root, 1973); and through the decrease of plant apparency to herbivores (Feeny, 1976) (for review, see Andow, 1991). Diverse vegetation is assumed to decrease the probability of pests colonizing the crop, because crop plants are camouflaged among non-crop plants, and because non-crop plants change the crop background in comparison to bare soil, mask and dilute olfactory and visual attractant stimuli, and expose pests to repellent chemical stimuli. The enemies hypothesis assumes that diverse vegetation increases the diversity of natural enemies by providing alternative or supplementary food sources, shelter and beneficial microclimate and by affecting foraging efficiency and/or the nutritional quality of the herbivore prey (Price *et al*., 1980; Strong *et al*., 1984; Andow and Prokym, 1990). Risch *et al*. (1983) concluded that in diverse annual systems herbivore movement patterns are more important than natural enemies in explaining the reduction of monophagous pest pop-

ulations. Further studies have shown that both mechanisms can play a role in reducing pest abundance in diversified vegetation (Risch, 1981; Andow, 1983; Kemp and Barrett, 1989; Altieri *et al*., 1990; Andow, 1991; Ogol *et al*., 1999). Besides the seasonality aspects, other important factors are the biological and ecological traits of the pests, such as voltinism, the degree of polyphagy, dispersal ability, and efficiency and mechanism of locating host plants (Risch *et al*., 1983; Altieri, 1994). Increased vegetational within-field biodiversity can also be used to suppress weeds (Liebman and Altieri, 1988).

Finch and Collier (2000), working especially with cabbage root flies, proposed a theory that emphasizes the positive stimuli that characterize host plants of pest insects. This theory rejects the principle of visual and olfactory masking claimed to result from increased vegetational biodiversity. The basic assumption is that a pest needs to make a certain number of sequential appropriate landings on a host plant before accepting it as an egg-laying site. Non-host plants intefere with this process by interrupting the preprogrammed host finding and acceptance behaviour of the pest. Every time the pest lands on a non-host plant before it has accumulated a required number of landings on a host plant, it has to start the behavioural sequence leading to host-finding from zero. Consequently, the pests do not accumulate in the crop plants to such an extent as they would in a monoculture.

The characteristics of non-crop vegetation within and around fields, the soil type and the type and intensity of management must also be considered when aiming to increase natural pest control by vegetation management (Altieri, 1999; Norris and Kogan, 2000; Östman *et al*., 2001). The key is to identify functional biodiversity and then to determine the best practices that will encourage the desired biodiversity component at the field, farm and even landscape levels (Altieri, 1999). However, it is often difficult to identify the key interactions that need to be manipulated to achieve a desired goal owing to the complexity of interactions in an ecosystem. Consequently, exactly what the grower needs to do in terms of increasing biodiversity of the cropping system to enhance pest suppression may not be clear, although diversification within agricultural fields is relatively easily achieved by different forms of intercropping (see Table 9.1), changing planting strategies and tolerating weedy culture (Helenius, 1998).

Mulches

Artificial and living mulches (defined in Table 9.1) are used primarily for soil conservation and weed control, but the latter also reduce arthropod pest populations owing to increased vegetational diversity (e.g. Brandsaeter *et al*., 1998; Weber *et al*., 1999; Hilje, 2000). Film mulches come in such materials as black, clear and coloured polyethylene, weed-block fabric, aluminium foil, roofing paper and biodegradable paper. UV-reflective aluminium films repel and disorient such insects as aphids, thrips, whiteflies

Table 9.1 Methods of 'farmscaping': some tactics that increase the vegetational diversity and modify landscape composition at the field and farm level

Type of intercropping	Explanation
1 Companion planting	Mix of species of plants within a row or bed. The companion plants are supposed to repel pests.
2 Multiple cropping	Practice of producing more than one crop on the same land in one year either simultaneously or in temporal sequence.
2a Intercropping	Practice of growing two or more crop species in the same field simultaneously in the same, alternate or paired rows.
– mixed intercropping	Practice of growing two or more crop species simultaneously in the same field with no distinct row arrangement.
– strip intercropping	Growing two or more crops simultaneously in the same field in strips wide enough to permit independent cultivation, but narrow enough for the crops to interact agronomically.
– row intercropping	Growing two or more crops simultaneously in the same field where two or more crops are planted in rows in a fixed pattern of spacing and rows.
– relay intercropping	Growing two or more crops in the same field simultaneously during part of the lifecycle of each. A second crop is planted after the first crop has reached its reproductive stage, but before it is ready for harvest.
2b Sequential intercropping	Practice of growing two or more crops in the same field sequentially. Diversification of the agroecosystem is in time only (cf. intercropping).
3 Cover crop	Any living ground cover, usually of close-growing grasses, legumes or cereals, which is planted into or after a main crop and then commonly killed before the next crop is planted. Thus, a cover crop can be grown either simultaneously with the main crop, or without it between crop cycles (cf. living mulch). Benefits are reduced soil erosion, stabilization of soil organic matter layers, improved soil structure, reduced weed abundance and competition, and diminished soil compaction.

Table 9.1 *Continued*

Type of intercropping	Explanation
4 Living mulch	Living mulches are cover crops planted either before or with a main crop and maintained as a living ground cover throughout the growing season. If the living crop is a perennial, it may be possible to maintain it from year to year without the need for reseeding. The purpose of a living mulch is to minimize the occurrence of bare soil and weed seed germination. In many cases, living mulches provide some measure of plant protection against pests owing to the effects of diversified vegetation on the biology, ecology and/or behaviour of pest organisms.
5 Windbreaks, hedgerows, shelterbelts	Linear barriers of trees, shrubs, perennial forbs and grasses that are planted along field edges or other unused areas to provide favourable microclimate for beneficial species and the crops, to protect against wind erosion species of soil, and to serve as shelter and winter refugia for natural enemies of pests.
6 Permanent borders	Strip of permanent vegetation bordering a field, e.g. to attract beneficial organisms.
7 Trap crops	Non-crop plant species grown within or around the cash crop field to attract pests away from the cash crop.

Sources: Dufour, 2000; Geno and Geno, 2001; Hartwig and Ammon, 2002.

and leafhoppers (Matthieu *et al*., 1977; Cardona *et al*., 1981; Brust, 2000; Al-Khatib *et al*., 2001; Reitz *et al*., 2003; Summers and Stapleton, 2002).

Trap crops

Virtually all pests show a distinct preference for certain plant species, cultivars or crop stage. Trap crops refer to the spatial and temporal manipulation of crop stands at a critical time in the pest's and/or the crop's phenology with the objective of concentrating the pest within a preferred trap crop instead of the main crop. A trap crop can be an early or a late crop of the same cultivar as the main crop, or a different plant species. The attraction of the trap crop can be complemented with semiochemicals or insect-food supplements. Pests concentrated in the trap crop should be destroyed with pesticides, natural enemies or cultural methods to prevent them from migrating to the main crop at a later stage (Hokkanen, 1991).

Spatial and temporal planting and harvesting patterns

Alterations in planting and harvest date can result in plants escaping from damaging pest infestations. Early planting ensures that seedlings have reached a non-susceptible or tolerant stage when the pest appears. Planting only after the emergence or immigration of the pests leaves them without hosts. Early harvest date works by preventing pests from reaching damaging population densities or overwintering stage by harvest. The change of harvesting pattern, for example partial or strip harvesting, can be used in some crops to prevent the pests residing in the crop from moving to other high-value crops after harvesting, or to concentrate natural enemies in the remaining crop strips, which then serve as natural enemy foci for the remaining crop (Ferro, 2003).

Tactics that reduce survival of pests by creating adverse biotic and abiotic conditions

Crop rotation

Crop rotation interrupts the normal life cycle of pests by placing them in a non-host habitat. It is the most important preventive control method of many weeds, soilborne plant pathogens and root-living arthropods. With arthropods, rotation is generally most successful against species with long generation cycles and with limited dispersal capabilities. The constrain on this tactic of limited land availability on any particular farm can, in best cases, be overcome by areawide crop rotation (Helenius, 1997).

Plant and row spacing

Sufficiently sparse plant and row spacings are important in preventing conditions from becoming conducive to plant pathogens that usually require a certain moisture threshold to germinate and grow. In contrast, by increasing plant density it is possible to 'dilute' the damage caused by pests to individual plants.

Tillage and destruction of breeding or overwintering refugia

Tillage (soil-turning and residue-burying practices) and seed bed preparation reduce the number of soil-living pest stages (but also that of soil-inhabiting natural enemies!). Some forms of tillage can reduce pest populations indirectly by destroying weeds and volunteer crop plants in and around crop-production habitats. Many pests can breed on alternate host plants and migrate from there to crop plants. The removal of the alternative host stands from the vicinity of the crop site thus helps in alleviating pest problems (for examples, see Norris and Kogan, 2000).

Sanitation

Sanitation includes residue removal to reduce pest inoculum in the subsequent crop in the same or adjacent field; burning and flaming to destroy pests; pruning of infected host tissue to remove infested plant parts or to

change the microclimate of the crop canopy to suppress pest breeding; roguing of infested crop or alternate host plants to prevent pests from spreading to healthy plants; and removing harbourage sites and water sources that contribute to pest multiplication. Soil solarization (Section 9.2.2) is also used for sanitation purposes.

Tactics that reduce injury caused by pests to crop plants

Plant resistance to pests

Resistant plants may be less preferred by the pest (antixenosis that has an impact on pests upon their arrival/first attack on the plant by colour, palatability, hairiness, waxiness, morphology, gummosis and necrosis), adversely affect the pest's normal development and survival (antibiosis that has an impact on pests when they first attack and/or, subsequently, consume the plant; here, tissue hardness, phenology, toxins and deterrents, and nutritional resistance are important factors) or the plant may tolerate the damage without an economic loss in yield or quality (compensation and reduced symptom expression) (van Emden, 2002). Constitutive plant resistance is easy to use, cheap and compatible with other pest management tactics. Even partial resistance can be of help owing to the increase in the development time of pests and, consequently, the duration of their availability to natural enemies. Means of breaking the resistance mechanism can, however, frequently evolve in both herbivores and pathogens (Teetes, 2003). The resistance traits of plants can in some cases interfere with the activity of natural enemies (Bottrell and Barbosa, 1998).

Induced resistance to herbivores or pathogens is activated in the plant upon herbivore or pathogen attack (Karban and Myers, 1989; Haukioja, 1991). It reduces plant exposure to autotoxic effects of secondary compounds and increases herbivore movement on plants; the latter, in turn, results in increased visibility of herbivores to natural enemies, as well as in dispersed damage within the plant or plant stand, which is associated with higher fitness than in the case of concentrated damage (Agrawal and Karban, 1999). Despite a large body of fundamental knowledge on induced resistance that has accumulated, practical applications are still few. They include the exposure of plants to herbivores or pathogens that do not injure the plants strongly while inducing a strong resistance (Karban and Kuc, 1999) and the use of commerical elicitors. These are either synthetic or natural substances in sprays that activate the metabolic pathways resulting in induced resistance (Tally *et al.*, 1999).

Genetic modification with recombinant DNA techniques is the newest way of generating pest-resistant plants. The genetic material of a genetically modified organism (GMO) is altered in a way that does not occur naturally by mating and/or natural recombination (EU, 2001); plant breeding is thus no longer limited by the intrinsic genetic variability within a species. The most successful commercial transgenic crops resistant to insects include cotton, maize and potato, all with transgenes from the insectical bacterium

Bacillus thuringiensis (Bt) (Shelton *et al*., 2002) and herbicide-resistant soybean (Owen, 2000). The use of Bt-crops has significantly decreased the use of pesticides, particularly in cotton (see Shelton *et al*., 2002 for a review). Other means of generating insect resistant crops is with transgenes that code for protease inhibitors that interfere with protein digestion in pest insects; α-amylase inhibitors that interfere with the carbohydrate metabolism of insects; plant lectins that produce chronic effects on survival and development of insects; or certain enzymes such as chitinase (Sharma *et al*., 2000). Resistance against plant pathogens has been achieved by transferring genes from viruses into plants (Gonsalves *et al*., 1998), bacteria, fungi, other plants and insects (Ko, 2000). Herbicide-resistant transgenic crops, for their part, allow chemical weed control when the weeds are most susceptible to treatments without harming the crop plant (see Kuiper *et al*., 2000).

9.2.2 Mechanical and physical control

Mechanical and physical tactics are numerous and usually crop and pest specific. Pests are controlled mechanically by trapping, preventing access to the crop, removal of pests from the crop, and so on. Methods include barriers around tree trunks to prevent pests from descending to the ground for pupation; plastic-lined trenches or fences around the perimeter of crop fields to prevent pests from colonizing fields from outside; suction devices to remove insects from the crop; coloured sticky traps for flying insects; crop covers to deny pests access to the crop; and even hand-picking. Plowing, mowing, disking or chopping of crop debris or soil aims to change the spatial distribution of insects, which exposes them to lethal conditions (Hoy, 1998).

One important means of physical control is exposure of pests to lethal temperatures to disrupt the pest lifecycle. Direct fire by flaming with propane burners is an effective means of killing weeds, relatively immobile insects or insects and pathogens residing in crop residues (Hoy, 1998). Soil solarization to kill soil-living pests is employed in climates with intense sunlight or in greenhouses (Stapleton, 2000). Steam sterilization can be used instead of solarization. Water (flooding, overhead irrigation, misting) can also be used to create adverse conditions for pests or to dislocate them from the canopy.

9.2.3 Biological control

Biological control is the use of living organisms (predators, parasites and pathogens; examples are listed in Table 9.2) to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg *et al*., 2001). Microbial control is the use of micro-organisms and/or their metabolites as

Table 9.2 Examples of different types of natural enemies successfully used for crop protection employing different strategies of biological control: examples mostly chosen from edible crops consumed fresh

Natural enemy	Principal target pest	Target crops
Classical biological control		
Rodolia cardinalis (vedalia beetle) (Coleoptera: Coccinellidae). A predatory beetle	*Icerya purchasi* (cottony cushion scale) (Homoptera: Margarodidae)	Citrus groves in several countries in the world. Introduced originally from Australia
Eretmocerus spp. (Hymenoptera: Aphelinidae). Parasitoids	*Bemisia tabaci* (sweet potato whitefly) (Homoptera: Aleyrodidae)	Introduced into the USA from Eurasia to control cotton whitefly in several crops, including field vegetables
Longitarsus jacobaeae, a ragwort flea beetle (Coleoptera: Chrysomelidae). A herbivore	*Senecio jacobea* (tansy ragwort) (Asteraceae)	Introduced from Europe to North America and Australia to control tansy ragwort, a poisonous adventive weed colonizing open areas such as pastures
Puccinia chondrillina (Uredinales). Phytopathogenic rust fungus	*Chondrilla juncea* (skeletonweed) (Asteraceae)	Introduced from Eurasia to Australia to control skeletonweed in dryland wheat areas
Inoculation biological control		
Typhlodromus pyri (Acari: Phytoseiidae). Predatory mite	*Panonychus ulmi* (fruit tree red spider mite) (Acari: Tetranychidae)	Apple orchards
Phytoseiulus persimilis (Acari: Phytoseiidae). A predatory mite	*Tetranychus urticae* (two-spotted spider mite) (Acari: Tetranychidae).	Greenhouse crops, strawberry, raspberry
Orius spp. (Hemiptera: Anthocoridae) A predatory bug	Thrips	Greenhouse vegetables
Dacnusa sibirica (Hymenoptera: Braconidae). A parasitoid	*Liriomyza* leafminers (Diptera: Agromyzidae)	Greenhouse vegetables
Streptomyces griseovirides (Bacteria). A soil bacterium	Plant pathogenic fungi that cause wilt and seed, root and stem rots (*Fusarium, Alternaria, Rhizoctonia, Phomopsis, Pythium, Phytophthora, Botrytis*)	Soil application in various crops, including vegetables. Acts by competition (depriving pathogens of space and nourishment) and antibiosis

Table 9.2 *Continued*

Natural enemy	Principal target pest	Target crops
Pseudomonas fluorescens strain A506. A bacterium	*Erwinia amylovora* (a bacterium causing fire blight)	Sprayed on apple blossoms to ensure that the antagonist occupies the flowers before the pathogen
Candida oleophila (a yeast fungus) *Pseudomonas syringae* (a bacterium)	Pathogenic fungi causing fruit spoilage during storage phase	*Candida*: fruits, vegetables. Colonizes fruit surfaces and inhibits other microbial colonization *Pseudomonas*: apple, pear, citrus. Works by both chemical antagonism and competition
Inundation biological control		
Trichogramma spp. (Hymenoptera: Trichogrammatidae). Egg parasitoid	Several species of Lepidoptera (moths)	Corn, greenhouse and outdoor vegetables (e.g. tomato and cabbage)
Adalia bipuncatata (Coleoptera: Coccinellidae). A ladybird predatory beetle	Aphids	Greenhouse vegetables. Applied to aphid colonies as a rapid corrective means if aphids escape the control of other biocontrol agents
Steinernema spp., Heterorhabditis spp. (Nematoda: Rhabditidae). Insect pathogenic nematodes	Fungus gnats (Diptera: Sciaridae), root weevils (Coleoptera: Curculionidae) and other beetles, caterpillars (Lepidoptera: Noctuidae)	Principally applied in the soil environment to control pests of various vegetables (root and cole vegetables, artichokes, cucumbers, herbs) and of mushroom
Phasmarhabditis hermaphrodita (Nematoda: Rhabditidae). A nematode parasite of slugs and snails	Several species of slugs and snails	Lettuce, strawberries. Mainly used in domestic gardens

Table 9.2 *Continued*

Natural enemy	Principal target pest	Target crops
Paecilomyces fumosoroseus (Fungi Imperfecti: Deuteromycotina). An insect pathogenic fungus	*Trialeurodes vaporariorum* (greenhouse whitefly), *Bemisia tabaci* (sweet potato whitefly) (Homoptera: Aleyrodidae)	Greenhouse vegetables
Bacillus thuringiensis. An insect pathogenic bacterium. Also available as endotoxin encapsulated in killed *Pseudomonas fluorescens* cells (which protect the toxin from adverse environmental conditions)	Caterpillars, beetles	Vegetables, fruits, nuts
Codling moth nuclear granulosis virus (Baculoviridae). An insect pathogenic virus	*Cydia pomonella* (Lepidoptera: Tortricidae)	Apple
Conservation biological control		
Anagrus epos (Hymenoptera: Mymaridae). An egg parasitoid	*Erythroneura elegantula* (grape leafhopper) (Homoptera: Cicadellidae)	Vineyards in California. Overwintering of the parasitoid is facilitated by planting blackberries in the vicinity of vineyards. The blackberries harbour eggs of an alternative host, the blackberry leafhopper, which overwinters as eggs (the target pest overwinters as adults)
Flowering mini-meadows between rows of vines to provide alternative food for natural enemies	Various pests	Vineyards in Europe

biocontrol agents formulated into biopesticides (van Driesche and Bellows, 1996). The most commonly used microbial is based on the bacterium *Bacillus thuringiensis*, currently comprising about 90% of the total biopesticide sales in the world (Neale, 1997). Biocontrol of plant pathogens employs antagonists, that is, microbes that suppress the plant pathogen by antibiosis, competition, parasitization, induction of host resistance or even predation (Cook, 1983).

Four strategies of biological control are distinguished: classical, inoculative, inundative and conservation biological control (Eilenberg *et al*., 2001):

Classical biological control

Classical biological control (CBC) refers to the intentional introduction of an exotic biological control agent for permanent establishment and long-term pest control to an area that the pest has invaded. The aim is to restore the balance between pest and natural enemy populations that was lost when the pest moved to the new geographical area without its enemies (Eilenberg *et al*., 2001). The majority of the introduced agents have been arthropod parasitoids and predators, including herbivores for weed control (see van Driesche and Bellows, 1996 for examples). The introduced natural enemies have been either those that have coevolved with the pest in its native home (the old associations approach), or, in the case of neoclassical biological control, the introduction is based on a novel association of the pest and a natural enemy species (no previous coevolutionary interaction). The latter strategy enables the control of native pests with introduced natural enemies (Hokkanen and Pimentel, 1984). CBC tends to be most effective in permanent or semi-permanent habitats, such as forests, pastures, rangelands and orchards. The relative success rate of this strategy has been highest for the orders Homoptera and Lepidoptera (Greathead, 1995) and rangeland and farmland weeds (e.g. Markin and Gardner, 1993). CBC has not been used against plant pathogens.

Inoculation biological control

Inoculation biological control is the intentional release of living natural enemies, usually in small numbers, which will subsequently multiply and control the pest for an extended period, but not permanently. It is thus the progeny of the released organisms that control the pest. This strategy is common and effective in crops that are destroyed at the end of the growing cycle, necessitating new inoculation at the beginning of a new cropping cycle. Dozens of different natural enemy species are available commercially worldwide (Anonymous, 1999), particularly for release in greenhouse crops, but also in such outdoor crops as strawberry (Tuovinen, 2000; Petrova *et al*., 2002; Waite, 2002), raspberry (Gordon *et al*., 2002), fruit orchards (e.g. Blommers, 1994) and vineyards (several articles in Lozzia, 2003).

Inundation biological control

Inundation biological control is the use of living organisms to control pests exclusively by the released organisms themselves and not by their progeny, although the released agents can multiply to some extent after application and show some residual effects (Eilenberg *et al*., 2001). Inundation biocontrol aims at rapid control or at constant and maximal exposure of pests to natural enemies by overloading the crop habitat with biocontrol agents. It is particularly suitable in crops of high value and with zero or near zero tolerance of pest damage.

Conservation biological control

Conservation biological control refers to modification of the environment or existing practices to protect and enhance naturally occurring natural enemies or other organisms to reduce the effect of pests (Eilenberg *et al*., 2001). The tactics used to execute this strategy are various and many can be regarded as cultural control with the specific aim of protecting and enhancing natural enemies. Habitat management at the field, farm and landscape level is a crucial form of conservation biological control with the aim of creating suitable ecological infrastructure within the agricultural landscape to provide resources for natural enemies (Landis *et al*., 2000). The resources include alternative food (e.g. nectar and pollen provided by flowering plants, artificial food sprays); shelter and appropriate microclimate created by specifically designed harvesting patterns or intercropping; selection of windbreak and hedgerow species beneficial to natural enemies; and overwintering refugia (e.g. beetlebanks on the margins or within cereal fields for epigeal predators). van Emden (2003) gives examples on putting these principles into practice. In this context, the recognition of the elements of functional biodiversity, the optimal spatial scale of such elements to achieve the desired effect, and their effect on behavioural mechanisms of the natural enemies is of utmost importance (Landis *et al*., 2000; Rossing *et al*., 2003).

Habitat management can have some negative aspects. Pests may benefit from the management tactics by taking advantage of flowering plants in field margins. Some land will be taken out of production. Within-crop diversification can result in yield reductions owing to competition between plant species (Landis *et al*., 2000). Lastly, if the tactics are too complicated, they will not be adopted in practice (Helenius, 1998).

Transgenic biocontrol agents

Traditional breeding methods have been employed since the 1970s to produce predatory mites and parasitoids resistant to pesticides and thus compatible with chemicals, or natural enemies that lack diapause or have increased environmental tolerance (see Hoy, 1992 and van Driesche and Bellows, 1996 for details). Since about 1995, recombinant

DNA techniques have been used to generate microbial biocontrol agents with traits that increase their effectiveness. The traits include increased host killing speed or expanded host range of insect pathogenic viruses, bacteria, fungi and/or nematodes (Segal and Glazer, 2000; Cory, 2000; St. Leger and Screen, 2001); improved tolerance to abiotic factors in entomopathogenic nematodes (Segal and Glazer, 2000); and improved antagonistic capabilities of antagonists of plant pathogens (Migheli, 2001 and references therein). Some transgenic microbials have already been registered or are undergoing the registration process (Migheli, 2001). The first experimental release of a transgenic arthropod natural enemy, a predatory mite, took place in 1996, although in this case the transgene served only as a molecular marker and was not expressed in the mite strain (Hoy, 2000).

9.2.4 Semiochemicals

Olfaction plays an important role in many pest and beneficial species' orientation toward hosts and conspecifics for the purpose of feeding, mating, reproducing and aggregation, or turning away from danger. Semiochemicals (from the Greek semeon, a signal) are behaviour-modifying chemicals that mediate interactions between conspecific arthropods, host plants and herbivores, or host plants, herbivores and their natural enemies (tritrophic interactions) (Flint and Doane, 2003). These mechanisms can be used for the benefit of crop plants by manipulating the behaviour of either the pest, its natural enemies, or both, with the help of organism-derived or synthetic pheromones and allelochemicals. Pheromones mediate interactions, such as mating, trail marking and aggregration, between conspecifics, while allelochemicals mediate interspecific interactions and are grouped into allomones, kairomones and synomones (Vet and Dicke, 1992; Ruther *et al*., 2002). Allelochemical terms are context-specific, not chemical-specific, for example a particular chemical can serve as a kairomone to one species, but to another species the same chemical is an allomone. Allelopathy (influence of plants upon each other through the release of products of metabolism that inhibit the growth of adjacent plants) can be used for weed control, for example by incorporating into soil plant residues that possess allelopathic weed-suppressing substances (Brown and Morra, 1995; Qasem and Foy, 2001).

Semiochemicals are used to lure pests to traps for early pest detection, mapping pest distribution, monitoring pests for action thresholds and decision support, and even for mapping insecticide resistance frequency (Suckling, 2000). Sex pheromones of Lepidoptera and aggregation pheromones of Coleoptera have the longest tradition of being used for the purposes of insect management. Pheromones are used for the detection and monitoring of temporal distribution of pests, mass trapping and, increasingly, for mating disruption. Traps can be combined with a lethal dose of

insecticide or microbial pesticide applied to the trap or its vicinity to kill males (lure and kill tactic) (Suckling, 2000).

Mating disruption is accomplished by placing the pheromone as high-dose point sources in the crop in different kinds of dispensers (Suckling, 2000). Lepidopteran sex pheromones are produced and emitted by female moths to attract males for the purpose of mating. A number of factors related to the behavioural ecology of the pest determine how well pheromones attract males (McNeil, 1991).

When sex pheromones are applied in traps designed to reproduce the ratio of chemical components and emission rate of calling females, males spend their mating energy in pursuit of artificial pheromone sources. This is false trail-following, resulting principally from competition between calling females and point-sources of synthetic pheromones. Male confusion, on the other hand, is supposed to result from ambient pheromone concentrations being so high that they hide the trails of calling females completely. The pheromone receptors in the male's antennae become habituated to the pheromone owing to sensory overload, resulting in diminution in responsiveness via either sensory adaptation or habituation (see Carde and Minks, 1995 for a more detailed treatise). Formulation of the synthetic pheromone determines, in part, the length and constancy of the release period and the level of protection of the pheromone from degradation. High pest population densities, multivoltinism and high longevity of the pest are obstacles for successful mating disruption. Ultimately, the migration behaviour of mated females determines whether mating disruption works in a given location. Despite these limitations, sex pheromones are used successfully for mating disruption of several moth pests (Carde and Minks, 1995).

The general theory of ecology of infochemical use by natural enemies in the tritrophic context was formulated by Vet and Dicke (1992). One candidate for applying the principles of tritrophic interactions for pest management is the push-pull or stimulo-deterrent diversionary strategy. The crop is protected from pests by repellants, antifeedants and oviposition deterrents. Simultaneously, aggregative semiochemicals such as host plant attractants and sex pheromones stimulate colonization of pests on trap crops or entry into traps where pathogens can be deployed (Pickett *et al.*, 1997). Kairomones and synomones can, in principle, be utilized in several ways to manipulate natural enemies for the benefit of crop plants. The response of natural enemies to semiochemicals can be manipulated by selection of natural enemy populations that react most strongly to infochemicals; conditioning to relevant semiochemicals before release in a given crop; and treating of crop with infochemicals to arrest the natural enemies in the crop. An alternative ways is breeding plants with high production of relevant semiochemicals (Verkerk *et al.*, 1998). Control means based on chemical ecology are seldom effective on their own, but must be integrated with other methods.

9.2.5 Sterile insect release (autocidal tactics)

Here, the pest insects are principally put in to control by their own populations by inundating the insect population with mass-reared sterile males which compete for mating with native females in the target environment, causing them to produce sterile eggs. The usual way of producing sterile males is exoposure to radiation (Robinson, 2002). Autocidal methods aim at eradication and can be very effective, however, several stringent restrictions connected with the sterile insect release method have limited the use of this technique to relatively few cases (Hendrichs *et al*., 2002; Kaspi and Parrella, 2002; Loosjes and Tan, 2002; Bartlett and Staten, 2003).

9.3 Integration of alternative pest management tactics

9.3.1 Levels of integration

Three basic systems of plant protection can be distinguished on a continuum that describes the extent of using alternative methods: conventional prophylactic methods relying solely on chemical pesticides; integrated pest management (IPM) (for definition, see Table 9.3); and pest management in organic agriculture that excludes synthetic chemical pesticides. IPM includes essentially the following aspects: appropriate selection of pest control methods, the decision rules that guide the selection of the control action plus the methods of gathering information needed for decision-making; the economic benefits to growers and society; the benefits to the environment; and the need to consider the impact of multiple pests (Kogan, 1998). The control methods, coupled with the sampling and monitoring procedures and economic injury levels (Pedigo *et al*., 1986; Pedigo and Higley, 1992) constitute the tactics of IPM.

Within IPM, a continuum of adoption level emerges from no adoption through adoption of transitional systems (i.e. the use of pesticides combined with scouting and application of economic damage thresholds, or, basically, supervised control), to systems incorporating crop rotations, resistant varieties and habitat management to enhance natural control, and ending with systems that rely primarily on biological control with minimal pesticidal interventions. In many cases, pest control programs are categorized as IPM even if based solely on supervised control, but strictly speaking true IPM starts only after reaching a certain threshold that includes a minimum set of tactical components combined within a basic strategy (Kogan, 1998). The ultimate stage of true IPM has been named biointensive IPM (BIPM) *sensu* Benbrook *et al*. (1996) (but see Kogan, 1998 for critique on the appropriateness of this term).

As integration is central for IPM, what exactly is integrated must be defined. Kogan (1998) defines the three basic hierarchical ecological scales of IPM integration: Level I integrates methods for the control of single species or species complexes (species/population level integration), Level

Table 9.3 Some definitions of integrated pest management (IPM) in chronological order (for further definitions, see Bajwa and Kogan, 1998)

Definition	Source
Pest management is the reduction of pest problems by actions selected after the life systems of the pests are understood and the ecological, as well as economic, consequences of these actions have been predicted, as accurately as possible, to be in the best interest of mankind. In developing a pest management program, priority is given to understanding the role of intrinsic and extrinsic factors in causing seasonal and annual change in pest populations.	Rabb and Guthrie (1970)
IPM is the coordinated use of pest and environmental information along with available pest control methods, including cultural, biological, genetic and chemical methods, to prevent unacceptable levels of pest damage by the most economical means, and with the least possible hazard to people, property and the environment.	Anonymous (1992)
IPM is the use of all economically, ecologically and toxicologically justifiable means to keep pests below the economic threshold, with the emphasis on the deliberate use of natural forms of control and preventive measures (definition by the European Plant Protection Organization EPPO).	Dehne and Schonbeck (1994)
IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impact on producers, society and the environment.	Kogan (1998)

II includes impacts on multiple pest categories (insects, pathogens, weeds) and the methods for their control (community level integration) and Level III integrates multiple pest impact and the methods for their control within the context of the total cropping system (ecosystem level integration). Currently, most IPM systems are at Level I integration. Some programs, such as apple and greenhouse vegetable IPM, are advancing to higher levels. In Europe, where IPM has been advanced in the context of creating regional guidelines for integrated production (IP) according to the standards of the International Organization for Biological Control (IOBC), the most

advanced IPM programs include greenhouse vegetables (30% of the production area, on average), grapes (20%) and fruit production (7%) (Besri, 2003). Not all countries have developed a national IPM policy, which, however, should exist to provide incentives to encourage IPM adoption and define the minimum set of tactical components to be included in the pest management program to qualify it as an IPM program (Besri, 2003).

9.3.2 Apple

Current apple IPM programs vary largely from one country to another and even in different regions within a country owing to differences in the number of major and minor pests, available technology, commitment to IPM approach, and financial and personnel support for IPM research, demonstration and implementation (MacHardy, 2000).

The codling moth, as well as a number of other important lepidopteran pests of apple, are satisfactorily controlled by mating disruption using female sex pheromones. In some important apple production areas, area-wide management programs have been implemented to manage codling moth with mating disruption (e.g. Brunner *et al*., 2002). Species-specific temperature-driven degree-day phenology models have been developed for several important insect pests of apple, including codling moth, to aid in determining their appearance and to target control treatments better (Tiso and Butturini, 1999; Graf *et al*., 2003).

All strategies of biological control are employed to some extent to control apple pests (Solomon *et al*., 2001). Introduction of *Aphelinus mali* into the Netherlands to control the woolly apple aphid exemplifies classical biological control (Mols *et al*., 1999). Inoculation of a new apple plantation with pesticide-resistant predatory mites, such as *Typhlodromus pyri*, is usually the first and prevalent step of IPM in European apple orchards (Blommers, 1994). Baculoviruses and bacterial biopesticides against lepidopteran pests represent the use of inundation biological control in apple (Cross *et al*., 1999b). Parasitoids can have a significant effect on some pests if conservation tactics are employed (Cross *et al*., 1999a). Orchards with living mulches (e.g. flowering strips, grass, bean) usually have lower infestation levels of pestiferous arthropods and more species and more individuals of soil-dwelling predaceous arthropods (e.g. Yu and Yan, 1998; Rieux *et al*., 1999; Brown, 2000). However, it is not always clear to which extent the benefical fauna in the cover crop – or in trees surrounding the orchard (Tuovinen, 1994) – translate into reduction of herbivore numbers in the fruit trees (Rieux *et al*., 1999). Such lack of information has slowed down the wider adoption of this tactic of conservation biocontrol. Living mulches are, however, a practice used in apple orchards to control weeds (Gut, 1993).

The use of apple varieties resistant to apple scab, the most important disease of apple worldwide, has not gained wider adoption except in some

European countries (Carisse and Dewdney, 2002). None of the several microbial antagonists tested against apple scab has reached the commercial stage because of insufficient control (Carisse and Dewdney, 2002). Therefore, the control of the apple scab is still based on fungicide treatments. Timing of treatments is improved and their number reduced by forecasting models that predict the risk of leaf infestation by ascospores (Bugiani *et al*., 2000; Raudonis *et al*., 2003). Fungicide applications can be reduced further if the number of ascospores is reduced in the autumn by leaf shredding and the application of urea to the soil to enhance decomposition of leaf debris and to favour microbial scab antagonists (Carisse and Dewdney, 2002). In north eastern America, efforts have been made to include autumn scab-risk action and sanitation action thresholds systematically as elements in apple IPM programs. The autumn scab-risk action thresholds are employed to distinguish high-risk from low-risk orchards and, subsequently, sanitation action thresholds are used to recommend that the grower use tactics developed for low-risk orchards to control primary infections in the following spring (MacHardy, 2000).

The conventional control of fire blight, another globally important disease of apple, with antibiotic sprays can now be complemented by a bacterial preparation (BlightBan *Pseudomonas fluorescens* A506). The bacteria are sprayed onto blossoms preventively so they colonize the blossoms before the pathogen and slow down its population development. Treatments can be combined with phenology models of fire blight for increased precision in timing the treatments (Johnson and Stockwell, 1998).

Alternative methods of controlling plant pathogens during the post-harvest period include physical methods such as manipulation of temperature conditions and the gas composition of the storage facilities to delay fruit ageing and the disappearance of preformed antimicrobial substances. Antagonistic yeasts, fungi and bacteria can be applied to the surface of the ripe fruit, where they prevent the target pathogens from multiplying by producing metabolites, or by competition or direct parasitism (Mari and Guizzardi, 1998). Some microbes are commercially available for use against post-harvest diseases of apple (see Table 9.1), but until now, they occupy only a very small niche in post-harvest control of pathogens. Recently, the benefits of pre-harvest treatments against post-harvest disease problems have been addressed (Ippolito and Nigro, 2000). Improvements in both pre- and post-harvest biocontrol of pathogens are believed to follow from the use of a combination of antagonist species applied before harvest (Ippolito and Nigro, 2000).

Several apple varieties have been modified genetically to incorporate traits that are beneficial in terms of pest management. Transgenic apples possessing resistance to crown gall (Viss *et al*., 2003), apple scab (Norelli *et al*., 2000), fire blight (Norelli *et al*., 1999), and herbicides (Dolgov, 2001) have been produced and tested in the field. Although the development of transgenetic apples makes good progress in several countries, there is no

approval for marketing expected at the moment (The European Food Information Council Biotechnology Database, 2005a).

Most current apple IPM programs appear to be at the first level of integration *sensu* Kogan (1998), centring around tactics intended to improve pesticide efficiency and targeting (for status reports see Müller *et al*., 2000; Way and van Emden, 2000). Nevertheless, in some countries conventional apple production based solely on chemical plant protection has practically disappeared and IPM is now the prevailing crop protection strategy (e.g. Zürcher *et al*., 2003). The few available results of long-term experiments show that it is possible to reduce pesticide interventions to a very low level when bottom-up, ecologically-based approaches are employed in apple production (Prokopy, 2003). Research in apple IPM in some countries in its most advanced form is now moving to the third level of integration *sensu* Kogan (1998) (Prokopy *et al*., 1994; Cross *et al*., 2003).

9.3.3 Greenhouse vegetables

On a worldwide basis, less than 10% of the production area of greenhouse vegetables is estimated to use IPM. In individual countries and crops, however, the proportion is much higher, for example the over 90% of greenhouse vegetable production area under IPM in Scandinavia and the Netherlands is due to climatic conditions that slow down pest development and highly advanced greenhouse technology. IPM tends to work most reliably in tomato crops. In cucumber, sweet pepper and eggplant it is more complicated owing to a richer pest and disease spectrum. The use of biological control is most advanced against insect and mite pests; here, seasonal inoculation biological control is the usual strategy. About 100 different species of beneficial insects, mites and nematodes are produced and sold worldwide for the greenhouse industry. Fungal, bacterial and viral preparations are also available. Developments in the area of mass production, quality control, storage, shipment and release of natural enemies have decreased production costs and led to better product quality (van Lenteren, 2000).

Disease control by biological means is not as advanced as arthropod control. Commercial products based on microbial antagonists isolated from suppressive soils and targeted against soilborne pathogens are available, as are a couple of microbial products for foliar pathogens, but the latter have not gained wider acceptance yet. Recent successes in biological disease control include the control of grey moulds with yeasts, filamentous fungi and bacteria, and that of powdery mildews with hyperparasitic fungi (van Lenteren, 2000; Ravensberg and Elad, 2002).

Biocontrol is complemented by pest and disease resistant cultivars. For example, breeding of virus-resistant cultivars has greatly alleviated problems caused by whitefly vectored viruses of tomato (e.g. Lapidot *et al*., 2001). Some crop plants have been specifically bred to enhance the action

of biocontrol agents by manipulating the morphological plant traits (van Lenteren *et al*., 1995).

Elements of conservation biological control and habitat management are found in greenhouse IPM. In the Mediterranean region, the immigration of indigenous natural enemies into greenhouses is encouraged by the selective use of pesticides (Castane, 2002). For aphid control, banker plants are used to rear natural enemies in large numbers in the greenhouse. Special delivery methods have been developed to ensure long-term inundation biocontrol of thrips. The method employs controlled-release sachets that are hung in the crop and contain alternative prey of predatory mites to sustain prolonged production of predators in the sachets (Sampson, 1998).

Physical screening of vents is used to exclude immigration of pests from outside (Murphy and Ferguson, 2000). UV-absorbing plastics are used as photoselective covers in some areas where viruses vectored by whiteflies and thrips are a commonly occurring problem (Antignus *et al*., 1996); this tactic utilizes knowledge of the visual ecology of pest insects that require UV-light for orientation to host plants. The closed environment of greenhouses allows the use of extreme temperatures to kill pest arthropods and plant pathogens between crops (Lindquist, 1998). During the cropping cycles, the temperature in the greenhouse can be manipulated in combination with relative humidity/vapour deficits to control plant pathogens and, to some extent, to enhance the action of natural enemies (Lindquist, 1998). Semiochemicals are used to monitor the presence and density of moths and woolly aphids, and sticky traps and roller traps are used to monitor and mass-trap flying insects. For a complete treatise of alternative methods in greenhouse crops, see Albajes *et al*. (2000).

Cucumbers are being engineered genetically, particularly in the USA, to achieve resistance towards different viruses and bacteria, but no marketing of transgenic cucumber is to be expected at the moment (The European Food Information Council Biotechnology Database, 2005b). Other than in the USA and Canada, genetically modified tomatoes are not currently approved for cultivation, but ketchup, purée and preserves from transgenic tomatoes modified for extended shelf-life are about to be approved in Europe. In terms of pest management, interest in tomato has focused on resistance towards pest insects and viruses (The European Food Information Council Biotechnology Database, 2005c).

9.4 Safety of alternative methods

9.4.1 Biocontrol concerns

Alternative methods of pest management are generally considered safe to the users, consumers and the environment. However, potential risks are associated with the use of certain alternative tactics. Risk, by definition, is

a combination of the magnitude of unwanted consequences, or hazard (i.e. any imaginable adverse effects which can be named and measured) and the likelihood (probability) with which the hazards may occur (Migheli, 2001). In respect of biological control, some consider that the hazards associated with its use are not as explicit and easy to identify as those caused by non-living entities (e.g. chemical pesticides) (Migheli, 2001). This is because natural enemies are self-perpetuating and self-dispersing; these qualities are, in fact, essential for the success of classical biological control. In effect, then, biological control can be irreversible. Furthermore, no list of imaginable hazards of any control method can contain unexpected hazards.

There are at least four types of potential adverse effects of biocontrol agents: displacement of non-target organisms in the target ecosystem owing to competition; direct risks to non-targets owing to predation, toxicity and pathogenicity, which may result in unwanted population level effects; allergenicity and/or pathogenicity to humans and other animals; and factors related to the potential of the biocontrol agent to hybridize with other organisms in the ecosystem (Cook *et al*., 1996; van Lenteren *et al*., 2003; Hokkanen *et al*., 2003). All these risk aspects are relevant for microbial control agents (e.g. Goettel *et al*., 2001 on the safety of insect pathogenic fungi). Therefore, national regulations require a detailed analysis of the health risks and environmental impact of microbials in order to obtain registration. Registration requirements for predators and parasitoids are more variable, partly because of fewer direct health risks related to their production and use. Some countries require registration of exotic agents, but exempt indigenous agents from regulation, or regulation concerns all agents irrespective of their origin (Hokkanen *et al*., 2003). In any case, additional requirements are in place if the biocontrol agent is a genetically modified organism (Zadoks, 1998).

Regardless of which pest control method is used, there will always be at least some impact on the overall biological community of the target habitat caused by the reduction in the pest population. Despite criticisms that classical biological control can pose risks to non-target species in their new environment (Howarth, 1991), in actuality negative environmental effects of the releases of arthropods have rarely been reported (Lynch *et al.*, 2001; for a list of reported and suspected cases of unintended consequences, see Hoddle, 2002). The majority of the introduced arthropod species are specific or relatively specific natural enemies, possessing little, if any, danger to non-target species via host shifts. Nevertheless, release of exotics should not be considered totally risk free. The risk evaluation process of the introduction is particularly stringent with herbivores and pathogens of weeds, which must not become pests of cultivated plants. This is ensured either by monophagy or by ecological separation of the agent from its potential alternative host plants (but see Louda *et al.*, 2003).

Both permanent establishment and reproduction of the biocontrol agent in the target environment are possible consequences of inundation biocontrol, depending on the characteristic of the agents and the conditions of their target habitats. This can lead to a range of potential direct and indirect consequences to the flora and fauna of the release environment. A growing number of countries now apply risk assessment procedures for importation and release of new natural enemies (see OECD, 2004). Here, two basic elements are central: identification of potential hazards and a summary of the risks and benefits of the release in comparison to other relevant control methods (van Lenteren *et al.*, 2003). The most crucial aspects in the biology of the inundation biocontrol agents in terms of risk assessment are capability of establishment in the target area, dispersal capacity and host range (see van Lenteren *et al.*, 2003 for details). A risk assessment methodology employing the risk factors related to establishment, dispersal, host range and non-target effects was proposed by van Lenteren *et al.* (2003) and applied to categorize the risk levels of the biocontrol agents already commonly used in Europe. The methodology generates risk indices for biocontrol agents and classifies them into safe, risky and intermediate, in terms of risk. The indices can then be used in decision making on acceptable releases of new biocontrol agents, developing risk management plans and justifying the need and extent of cost/benefit comparisons with other control methods. These methods are currently being tested and refined by an IOBC/WPRS working group (H. Hokkanen, personal communication).

Microbial control agents have the potential to cause occupational health risks to those involved in either their production or application (Goettel *et al.*, 2001), as well as to consumers who eat the produce treated with microbials. During the production and application process, worker exposure to fungal spores or bacteria may lead to respiratory health effects unless appropriate protective measures are applied (Baelum *et al.*, 2003). The risks of microbials to consumers after ingestion of the treated produce are mitigated by imposed post-treatment safety periods, the high specificity of the microbial control agents (insect viruses, Bt) and the fact that conditions in the alimentary tract of vertebrates often are detrimental or non-conducive to the agent (fungi and nematodes) (e.g. Jensen *et al.*, 2002). Despite this, some areas are still insufficiently charted. For example, it is known that vegetative cells of some Bt strains have the capacity to produce a variety of non-specific enterotoxins and beta-exotoxins, which could possibly cause food poisoning and/or diarrhoea in humans, as such toxins can survive normal food preparation regimes (Bishop *et al.*, 1999). Therefore, these aspects are currently under further study in some countries (Licht *et al.*, 2003). The safety of microbial control agents is of utmost importance in the context of pre- or post-harvest treatment of fruits and vegetables with the purpose of controlling pathogens during the storage phase, as this can

involve the emergence of fungi previously not considered harmful to humans (Marasas and Vismer, 2003).

9.4.2 Concerns related to genetically modified crops and biocontrol agents

The principal food safety risks related to the consumption of transgenic crops or genetically modified (GM) foods include potential toxicity of the new gene products to humans; alterations in levels of useful nutrients and potential toxicants; gene transfer between plants and the human and animal gut microflora (this concerns the safety of antibiotic resistance-markers included in the transgenes); and lastly, allergenicity caused by either intended or unintended introduction of a new protein (see WHO, 2000 for review). The safety of transgenic food crops is assessed according to the principle of substantial equivalency, or a comparative approach to conventional foods. If, after critical investigation of possible toxicants and allergenics, essential nutrients and other relevant characteristics, a new food is found to be substantially equivalent to an existing traditional food, the new product is considered to be as safe as the conventional food. If the substantial equivalency of the new food with traditional ones is only partial, the identified differences should be evaluated further. In case the new food has no traditional equivalents, an entirely new procedure to evaluate its safety must be developed (OECD, 1993). Substantial equivalency is not a safety assessment as such (Novak and Halsberger, 2000). Shelton *et al.* (2002) provide a synthesis of critics of the substantial equivalency principle, for example the claim that there is no unanimous articulation on the degree of acceptable difference between new and traditional foods (Millstone *et al.*, 1999). Transgenic crops intended solely for animal consumption or for production of pharmaceuticals can pose additional problems as it can be difficult to keep them completely separated from plants or plant products intended for human consumption (see Sampson, 2000).

According to the Precautionary Principle, which is applied to the safety evaluation of GM foods in Europe, any new GM food intended to be commercialized must be assessed on a case-by-case basis of the level of risks it may pose to the environment and to human health and safety (Kinderleder, 2000; Saeglitz and Bartsch, 2003). Risk evaluation does not preclude the use of a production technology if risks of acceptable and manageable levels are identified. The new European authorization procedures for GMOs and GM food and feed include the new principles stated by the EU (2001, 2003). The intergovernmental organizations OECD (1993) and FAO/WHO (2001) have also designed strategies for the safety evaluation of GM foods.

Based on the absence of any adverse effects related to the consumption of GM foods commercialized so far, currently available GM foods are considered safe to eat (International Council for Science, 2003). It should be borne in mind, though, that the long-term effects of GM foods are unknown.

In the future, assessing the safety of GM foods may become more difficult owing to more substantial and complex changes in foods. Progress in assessing the safety of GM foods also requires further developments in methods for identifying and characterizing potential allergens (Society of Toxicology, no year). Despite the conclusions concerning the safety of current GM foods (International Council for Science, 2003) and some results showing that transgenic crops can even have health benefits (Munkvold *et al.*, 1999), no unanimous general consensus on the food safety issue, the general methodology of assessing food safety of transgenic crops, and the proper intepretation and communication of the safety assessment results to the scientific community, the regulatory bodies and the general public has been achieved so far, not even within the scientific community itself (see Shelton, 2003; and http://www.plab.ku.dk/tcbh/Pusztaitcbh.htm for the evolution of and links to the Pusztai case concerning the effects of lectin-coding transgenic potato diet on the gastrointestinal structure of rats).

The ecological risks of transgenic crops are less well known than their safety as food, and depend on both the specific genetic application and the agricultural system and environment in which the crops are grown. The main categories of ecological risk are the following: outcrossing (a cross to a strain with a different genotype) of the transgenic plant with its wild relatives; horizontal transfer of the transgenes to other organisms such as bacteria; transgenic plants becoming weeds and replacing native plants; effects of Bt protein in the soil; harmful effects on non-target species exposed to the new proteins and evolution of resistance by the target pest (Krimsky and Wrubel, 1996; Rissler and Mellon, 1996; Shelton *et al.*, 2002). Current evidence shows that outcrossing of some transgenic crop species with land races and related wild species is possible via pollen (Halfhill *et al.*, 2002; International Council for Science, 2003). However, the recent developments in genetic engineering include technologies that deliver the transgenes to maternally inherited chloroplasts; consequently, pollen does not contain the transgene and thus gene flow of the transgene to other plant species via pollen becomes impossible (Daniell, 1999).

Although the registration process of transgenic crops currently includes requirements for proof of environmental safety and guidelines for resistance management (see e.g. Dutton *et al.*, 2003; MacDonald and Yarrow, 2003), the general protocols and conceptual framework for testing and monitoring non-target effects of transgenic crops as well as for monitoring the resistance of pests to transgenic crops in the field are only at the developmental stage (Groot and Dicke, 2002; Shelton *et al.*, 2002; Dutton *et al.*, 2003; Levidov, 2003; Losey *et al.*, 2003; Poppy, 2003; Schmitz *et al.*, 2003). The long-term ecological effects are particularly difficult to predict and expensive and complex to test. Ultimately, the environmental impact of transgenic crops needs to be compared to the degree of risk posed by alternative cultivation methods, including pesticide use. Such comparison, however, may not satisfy all parties because of differing opinions about what constitutes

an adverse environmental impact and differences regarding the risk level that is acceptable (International Council for Science, 2003).

9.5 Future developments

The integration and implementation of alternative pest management methods with each other, combined with reduced use of pesticides, requires new approaches in education and training as well as national support infrastructure (Jeger, 1997). In outdoor crops, significant changes in IPM are to be expected in computer communications, reliability of weather-driven computer models and delivery of information in near-real time, and applications of GIS (geographic information system) and GPS (geographical positioning system) in precision farming (Cuperus *et al.*, 2003). In greenhouses, it is likely that indirect use of temperature and light for pest management purposes will increase as more sophisticated environmental controls, monitoring equipment and building structures become available.

Genetically modified crops and biocontrol agents continue to be developed, but their fate in the market depends on the legislative approach taken by different countries. Plant breeding programs that aim to use allelochemicals to target herbivorous pests for natural enemy attack are an anticipated step in the application of chemical ecology (Verkerk *et al.*, 1998). Other anticipated applications of chemical ecology include lure and kill traps using microbial pesticides, plume masking of oviposition cues using non-host odours, the use of oviposition deterrents, the advancement of the push-pull strategy, and manipulation of beneficials by means of semiochemicals and plant volatiles (Suckling, 2000). Further applications of induced resistance in plants await discovery (Kuc, 2000). In the area of biological control, increasing emphasis will be on biocontrol of foliar plant diseases (van Lenteren, 2000; Ravensberg and Elad, 2002). New diagnostic methods for identification and characterization of pests and pathogens continue to be developed and methods combining high levels of sophistication, exactness and simplicity will also become available for field use (Kennedy and Button, 2000). Thus, there is a plethora of areas in which alternative and usually very specific tactics of pest management can be advanced. The greatest challenge will consist in incorporating the new technologies into a whole to advance the integration level of current IPM systems.

On a wider scale, the systems approach to achieving ecological modernization of horticultural and agricultural IPM and IP will increase in emphasis (Lewis *et al.*, 1997; Rabbinge and Rossing, 2000). This means a shift towards strengthening ecosystem components that minimize the need for remedial treatments and minimizing their disruptive effects. The ultimate aim is total ecosystem management (see also Brown, 1999; Hill *et al.*, 1999). The currently intensive research on functional biodiversity and landscape connectivity demonstrates that new options for pest control arise when

systems management is approached from the farm and regional scales. Here, the eventual adoption of the widescale strategies is likely to be hampered by the fact that the systems become too complicated and large to manage in detail and their effectivity and economic feasibility is difficult to show experimentally. The already available comparison of conventional, IP and organic production systems on the farm scale prove, however, that such comparisons are possible.

9.6 Sources of further information and advice

Principles of insect pest management are treated in Metcalf and Luckmann (1994) and those of IPM in Dent (1995), Norris *et al.* (2002) and Walter (2003). A new book relating IPM and ecological theory is expected by Kogan (2005). The general principles, practice and success factors of biocontrol are treated in van Driesche and Bellows (1996); Gurr and Wratten (2000) and Gurr *et al.* (2004), and the risks and benefits in Hokkanen and Lynch (1995) and Hokkanen and Hajek (2003). The journal *Environmental Biosafety Research* publishes the most recent developments in the safety issues of transgenic organisms (http:// www.edpsciences.org/ebr). Useful sources of term definitions for pest management, IPM and biocontrol are Coombs and Coombs (2003), www.pestmanagement.co.uk/lib/glossary/glossary_a.shtml and Pimentel (2002). A CD-rom (Anonymous, 1999) based on an extensive international listing of suppliers of beneficial biologicals is available at www.cplpress.com. The website of the Oregon State University (http://ippc.orst.edu/biocontrol/biopesticides/) lists commercialized microbial biopesticides. An updated list of lepidopteran sex pheromones can be found at http://www-pherolist.slu.se/pherolist/pherolist.cgi. The European Plant Protection Organization (EPPO) maintains a list of biocontrol agents widely used in the EPPO region (http://www.eppo.org/STANDARDS/biocontrol/bio_list.htm). The EPPO (www.eppo.org/STANDARDS/biocontrol.htm) and OECD (2004) both have produced standards or guidances for import, release and registration of exotic biocontrol agents.

A plethora of research institutes deal with alternative pest management. The FAO's Global IPM pages at www.fao.org/ag/AGP/AGPP/IPM and those of the University of Cornell in the USA (www.nysaes.cornell.edu/ent/biocontrol/) provide links to biocontrol sites of research institutions. The Commonwealth Agricultural Bureau International (CABI) has a worldwide network of research centres under CABI Bioscience dealing with several aspects of IPM (www.cabi.org). The IOBC has its global site at www.oilb.agropolis.fr, with links to regional sections and their publications, as well as to events related to IPM. The IOBC publishes bulletins of several working groups dealing with integrated pest management of different crops, including fresh produce. The website of the Consortium for

International Crop Protection at www.ipmnet.org contains a database of IPM resources, IPMnet News, Radcliffe's IPM World Textbook and IPMnet event calendar.

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10

Improving the safety of organic vegetables

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10.1 Introduction

In recent years there has been an increased demand for and sale of organically grown food, which can be explained by several factors. The consumers that select organic vegetables think that the products are chemical free, safer and healthier (Bruhn, 2004). Some consumers are also sceptical of technology and believe that a natural, low technological approach is better for health and the environment. Other reasons might be curiosity or that the products 'looked good' or 'tasted better'. The recent food scandals in both North America and Europe (for example BSE/Creutzfeldt-Jacob's disease, dioxins) may also partly explain why there is an increase in the demand for organic foods.

'Organically grown vegetables' denote vegetables that have been produced in accordance with the principles and practices of organic agriculture (Bourn and Prescott, 2002). Little scientific evidence has until recently existed on the issue of food safety of organic vegetables, presumably because of the low production and sale of these vegetables. However, since the organic industry is now growing, questions have been raised and researchers have started to look into the status of the microbiological safety of organic vegetables. In this chapter, we will focus on the microbiological risks from bacteria and fungi.

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10.2 Organic agriculture

The term 'organic agriculture' is not only limited to certified organic farms and products, but covers a whole range of agricultural systems that rely more upon natural resources and recycling in the local environment than external input. According to Codex Alimentarius (Anonymous, 2001): 'Organic agriculture is a holistic production management system which promotes and enhances ecosystem health, including biological cycles and soil biological activity. Organic agriculture is based on minimizing the use of external inputs, avoiding the use of synthetic fertilizers and pesticides. Organic agriculture practices cannot ensure that products are completely free of residues, due to general environmental pollution. However, methods are used to minimize the pollution of air, soil and water. Organic food handlers, processors and retailers adhere to standards to maintain the integrity of organic agriculture products. The primary goal of organic agriculture is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people.'

This definition describes the main technical differences between organic and conventional agriculture in the absence of use of synthetic pesticides and fertilizers where low input and recycling of local resources are important factors. Other branches of organic agriculture are biodynamic agriculture and permaculture. In some cases traditional agriculture may be considered as almost organic (non-certified organic) owing to the development of management systems that are adapted to local environment and cultural resources.

10.2.1 Some statistics

In February 2004, approximately 24 million hectares were managed organically worldwide (Willer and Yussefi, 2004). Australia harbours the largest parts of the land area under organic management, whereas Argentina and Italy come second and third. In Australia and Argentina most of the organic land is extensive grazing land for cattle. The largest percentage of organic land is in Europe and the areas under organic management are increasing. In European countries like the United Kingdom and Norway areas used for organic plant production are increasing (Organic Statistics United Kingdom, http://statistics.defra.gov.uk/esg; Debio Samlet Statistikk 2003, http://debio.no). In the UK the area used for organic vegetable production increased, with 12% in 2003, whereas in Norway there was a 17% increase in the land area used for plant production.

10.3 Standards and regulations

The International Federation of Organic Agricultural Movements (IFOAM) made the first basic standards in the 1980s. After this, worldwide standards

have been set down and most of these are based on IFOAM with their own national additions. EU-regulation 2092/91 is based on national legislation and the IFOAM standard. Codex Alimentarius has made voluntary guidelines for organic agriculture for the UN organizations FAO and WHO. In the USA, the National Organic Program (NOP) is based on the Organic Food Production Act and the national Organic Standards Board regulation. The standards are largely consistent, but there are some differences. The goals of protecting soil, water and biodiversity are common for the standards.

The NOP is the only set of standards which regulates the use of raw manure. The use of manures from 'factory' farming is prohibited by the EU and Codex. There are also strict regulations on the use of manures containing human excrement and sewage. According to the IFOAM, the use of human excrement is strictly prohibited unless detailed sanitation requirements are described by the standard-setting organization. There are no standards for the use of green manures. Household wastes are permitted as long as they are treated either by composting or fermentation, or from a source-separated origin that is regularly checked for contamination. National standards for compost exist and these deal with sanitization issues as well as other issues. These standards vary among countries. Most of these standards have temperature–time requirements and testing criteria for the content of pathogens (Hogg *et al*., 2002). Pathogen testing usually involves testing for specific microorganisms, like *Salmonella*, and enumeration of faecal coliforms or *Escherichia coli*.

Synthetic pesticides are prohibited in organic agriculture. In order to control pests and weeds, the system depends upon crop rotation, selecting varieties with regard to local conditions. Pest management is described in the standards and regulations mentioned above. The management practices depend on plant or animal preparations, traps and natural repellents and so on.

10.4 Safety risks from vegetables

10.4.1 Bacteriological risks

Microbiological food safety risks are, as far as bacteria are concerned, connected to the occurrence of pathogenic bacteria causing human infections (invasive bacteria, e.g. *Salmonella* spp, *Escherichia coli* (EIEC), *Shigella* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter* spp. and some *Aeromonas* spp.) or food poisoning caused by toxin formation. These toxins can be preformed in the food during preparation or storage (e.g. *Bacillus cereus* (some types), *Clostridium botulinum*, *Staphylococcus aureus*). Toxins can also be produced in the intestine after ingestion (e.g. *Clostridium perfringens*, *Bacillus cereus* (some types), *Vibrio cholerae*, *E. coli* (ETEC) and some *Aeromonas* spp). When it comes to vegetables,

organic as well as conventional, the invasive bacteria and the toxin producers after ingestion are of interest.

Salmonella spp., Shiga-toxin producing *E. coli* (STEC), *Campylobacter* spp. and *Y. enterocolitica* are occasionally isolated from the intestines of both domestic and wild warm-blooded animals. *C. perfringens* and *L. monocytogenes* may also be isolated from animal faeces, whereas *V. cholerae* and *Shigella* spp. have mainly been isolated from humans. Studies have shown that *Salmonella* and STEC survive relatively well on vegetables (Beuchat, 1999; Brandl and Mandrell, 2002; Ercolani, 1979; Weissinger *et al*., 2000). However, STEC seems to be more resistant to different environmental conditions than *Salmonella* spp. (Baloda *et al*., 2001; Fenlon *et al*., 2000; Jiang *et al*., 2002; Kudva *et al*., 1998; Ogden *et al*., 2002; Wang *et al*., 1996; Wang and Doyle, 1998). Winfield and Groisman (2003) discussed whether *Salmonella* spp. is more capable of surviving in non-host environments than *E. coli* and concluded that the ability of *Salmonella* to survive outside its hosts provides the bacterium with an increased probability of infecting new hosts.

Contamination with *Campylobacter* spp. has traditionally been associated with poultry products, raw milk and untreated drinking water, but *Campylobacter* spp. have also infrequently been isolated from vegetables (Kumar *et al*., 2001; Park and Sanders, 1992). More recent research has identified *Campylobacter* spp. as potential contaminants for vegetables (Brandl *et al*., 2004; Evans *et al*., 2003; Kärenlampi and Hänninen, 2004) and fresh produce may thus be associated with sporadic outbreaks of campylobacteriosis.

Y. enterocolitica is mostly linked to pork products, but untreated drinking water may also be associated with disease (Ostroff *et al*., 1994). Use of untreated water or surface water may then be a source of contamination for vegetables since it has been shown that *Y. enterocolitica* may be detected in vegetable products (Fredriksson-Ahomaa *et al*., 2001; Johannessen *et al*., 2002). The use of pig manure may contaminate produce with the pathogen. However, to the authors' knowledge, no experiments have been conducted in order to investigate the potential of *Y. enterocolitica* to contaminate fresh produce.

L. monocytogenes is a ubiquitous bacterium that is commonly isolated from the environment, but may also be isolated from animal faeces (Unnerstad *et al*., 2000). *L. monocytogenes* has been isolated from commercially available vegetables (Beuchat, 1996; Farber *et al*., 1989; Heisick *et al*., 1989; Johannessen *et al*., 2002; Prazak *et al*., 2002) and has also been shown to survive well on vegetables under experimental conditions (Beuchat *et al*., 1986; Beuchat and Brackett, 1990, 1991; Prazak *et al*., 2002). Studies have shown that *L. monocytogenes* from faecal sources persists for three to six weeks after artificial contamination of soils (Al-Ghazali and Al-Azawi, 1990; Jiang *et al*., 2004; Van Renterghem *et al*., 1991).

There have been outbreaks of shigellosis associated with vegetables (Crowe, 1999; Molbak and Neiman, 1998) and irrigation water contaminated with sewage was probably the source of contamination for an outbreak associated with imported lettuce in Norway (Kapperud *et al*., 1995).

Clostridia and *Bacillus* spp. are ubiquitous in the natural environment and are commonly isolated from soil, dust and the intestinal tracts of humans and domestic animals. Spores may be isolated from vegetables and may lead to disease when the spores sporulate in the intestines of humans.

The focus on animal manure in connection with vegetables often eaten raw is therefore inevitable, and it is a reasonable assumption that the extended use of raw manure as fertilizer in organic agriculture may be a safety risk compared to conventionally grown vegetables. Manure may also harbour other pathogenic microorganisms like fungi, parasites and viruses, but viruses and parasites will not be discussed in this chapter.

10.4.2 Mycological risks

Very few foodborne fungi cause infections in man and from a food safety point of view, it is mainly the mycotoxins produced by fungi contaminating food, among them vegetables, rather than the fungi themselves that are of importance. Various mycotoxin producers (e.g. *Fusarium* spp., *Alternaria* spp., *Penicillium* spp., *Aspergillus* spp.) may colonize both organic and conventional vegetables in the field or during storage. The variation of species present depends on the vegetable as well as climatic and storage conditions. The presence of potential toxin producers, however, does not necessarily mean that there are mycotoxins present in the vegetables since environmental factors play an important role. None of the potential mycotoxin producers have the intestine of warm blooded animals as their natural reservoir and therefore the impact of raw manure as fertilizer is not as important as for bacteria.

Factors like the absence of use of synthetic pesticides, including fungicides and other agricultural measures like crop rotation, tillage system and mineral nutrition status might be more important than manure management, even if reports on such factors are inconclusive. In conventional agriculture, fungicides are used to prevent yield loss caused by a range of plant pathogenic fungi. Results from studies in grain fields in Norway indicate, however, increased *Fusarium* infection in grain treated with fungicide (Elen *et al*., 1999, 2000, 2002).

10.5 What is known about the safety of organic vegetables?

In order to study nutritional quality and pesticide residues in organic versus conventionally grown fruits and vegetables, several studies have been

conducted (reviewed by Bourn and Prescott, 2002; Woese *et al*., 1997), but relatively few surveys have been performed to investigate the bacteriological and mycological/mycotoxicological safety of organic vegetables (Loncarevic *et al*., 2005; McMahon and Wilson, 2001; Mukherjee *et al*., 2004; Sagoo *et al*., 2001).

10.5.1 Bacteriological surveys

Mukherjee *et al*. (2004) found a significantly higher prevalence of *E. coli* in organic compared with conventional produce in Minnesota, USA. When comparing certified organic produce to conventional produce, however, the difference was not statistically different. They also found that the type of manure used was related to the bacteriological quality, but that age of the manure did not seem to play any role. In a large study in the United Kingdom where ready-to-eat organic vegetables were examined, 99.5% of the samples were found to be of acceptable hygienic quality (Sagoo *et al*., 2001). The unacceptable products had too high levels of *E. coli* and *Listeria* spp. (not *L. monocytogenes*). McMahon and Wilson (2001) conducted a smaller study where neither *E. coli* nor common human pathogenic bacteria were detected. Loncarevic *et al*., (2005) found a slightly higher incidence of presumptive *E. coli* in domestically produced organic lettuce than in imported organic lettuce. None of these studies concluded that organic vegetables were of poorer bacteriological quality than conventional ones.

As far as the authors know, only one outbreak of foodborne disease has been associated with organic produce. In Germany several children became seriously ill after consumption of sandwiches (Tschäpe *et al*., 1995). Shigatoxin producing *Citrobacter freundii* were found to be the cause of the disease. The conclusion from the epidemiological study was that green butter made with organic parsley fertilized with pig manure most likely was the reason for disease. Another outbreak linked to manure constituted four cases of *E. coli* O157:H7 infection associated with vegetables that were only fertilized with manure (Cieslak *et al*., 1993). These two outbreaks alongside other vegetable associated outbreaks (Ackers *et al*., 1998; Hilborn *et al*., 1999; Kapperud *et al*., 1995) have caused concern about the origin of contamination and the use of manure as fertilizer has been hypothesized as one source of contamination of vegetables.

10.5.2 Fungi and mycotoxin surveys

For many vegetables little information concerning the extent of mycotoxin contamination is available. Owing to knowledge of the specific association of fungi to certain foods (Filtenborg *et al*., 2002), assumptions can be made concerning mycotoxin contamination in general. Garlic and other onions are often spoiled by *Penicillium allii* (producer of roquefortin C) (Frisvad and Filtenborg, 1989) and *Petromyces alliaceus* (producer of ochratoxin A)

(Hesseltine *et al*., 1972; El-shayeb *et al*., 1992) but surveys of these toxins in onions are not available. Among potential toxigenic fungi spoiling tomatoes, *Alternaria arborescens* and *Alternaria solani* are quite common (Filtenborg *et al*., 2002). *Alternaria* species produce a range of toxic metabolites, amongst them are alternariol, alternariol monomethyl ether and tenuazonic acid which have been demonstrated to have been found in infected tomatoes (Ozcelik *et al*., 1990; Hasan 1995), but also in olives, peppers and fresh and processed fruits (Scott, 2001). Toxigenic *Fusarium* species often involved with dry rot in potato tubers are *F. sambucinum* and *F. solani* var. *coerulum*, while *F. crookwellense* are more often isolated from damaged potatoes. Diacetoxyscirpenol and other trichothecenes have been reported from potato tubers inoculated with *F. sambucinum* (Desjardins and Plattner, 1989; Desjardins *et al*., 1993; Jelen *et al*., 1995), while another study reported deoxynivalenol in potato tubers (El-Banna *et al*., 1984).

Very few studies on mycotoxin content in organically produced vegetables have been conducted. In a survey by Solfrizzo *et al*. (2004) on *Alternaria* toxins in 266 carrot samples produced under organic conditions, none of the *A. alternata* toxins were detected, while the phytotoxin radicinin produced by *Alternaria radicina* was detected in three samples. In another study by Malmauret *et al*. (2002) on contaminants in organic and conventional foodstuffs, only a few samples were included in each category. They found higher levels of deoxynivalenol in organic compared with conventional wheat and higher levels of patulin in organic compared with conventionally produced apples, but no conclusion could be drawn according to production system. More recent surveys have compared the occurrence of mycotoxins in organic and conventional grain and cereal products (Döll *et al*., 2002; Schollenberger *et al*., 1999, 2002). These surveys have found that the results favour organically produced grains with lower levels of zearalenone and deoxynivalenol. However, the opposite was found for ochratoxin A in cereal from conventional and organic farms in Poland (Czerwiecki *et al*., 2002).

From current knowledge the conclusion is that in some commodities the presence of mycotoxins can be expected irrespective of production system, but more surveys on mycotoxin levels are needed as far as vegetables are concerned.

10.6 Managing bacteriological risks

In all the organic standards, protection of soil, water and crops are important issues. In order to achieve this, proper manure and waste management is required. Animal manure may harbour human pathogenic bacteria. Different studies have shown that STEC and *Salmonella* survive rather well in manure (Duffy, 2003; Forshell and Ekesbo, 1993; Himathongkham *et al*., 1999; Kudva *et al*., 1998). Green manures and household wastes may also

contain organisms hazardous to humans (Brinton, 2004). Manure management is described in the standards, although not in detail. In the NOP standards (USA), raw manure is only permitted on land used for crops that are not intended for human consumption. If the edible parts of the product are in contact with the soil surface or particles of soil, composted manure must be incorporated into the soil at least 120 days before harvest or 90 days before harvest if the edible parts are not in contact with the soil surface or particles. If regulations like this are implemented in national standards elsewhere, there would be consequences like, for example, having to apply manure in the autumn instead of the spring because of the short growing season.

Experiments have indicated that manure contaminated with pathogenic bacteria, such as STEC and *Salmonella* spp., used as fertilizer may contaminate vegetables that are cultivated in the manure-amended soil (Islam *et al*., 2004a; Islam *et al*., 2004b; Natvig *et al*., 2002; Solomon *et al*., 2002). The results from these experiments indicate that the time of introduction of pathogen, concentration of pathogen and type of vegetable are important factors in the transmission process. Solomon *et al*. (2002) studied the potential uptake of *E. coli* O157:H7 in lettuce and found that this particular bacterium could be internalized in lettuce tissue. In a study by Johannessen *et al*. (2004) where the manure used for fertilization was naturally contaminated with *E. coli* O157:H7, the bacterium was isolated from fertilized soil, but not from the lettuce grown in this soil. Splashes of contaminated soil on to the products may also lead to contamination of vegetables. These findings may have important implications for organic agriculture where manures are commonly used as fertilizer and where recycling of local resources and low external input is an important part of the system's approach.

Since it has been shown that the use of manure as fertilizer may lead to contaminated vegetables, sufficient treatment of manure is needed. Composting is a common practice among farmers for treating manure; it is the aerobic biological decomposition of solid organic waste and it requires moisture and the right proportion of carbon to nitrogen at temperatures of 40–65 °C (Buckley, 2001; Veenhuizen *et al*., 1992). This form of treatment may take place in windrows, piles or in vessels and the process may include turning steps in order to aerate the compost mix or the material can be kept static. Composting is a highly variable process that depends on factors like the activity of the microbes present in the composting material and other physical and chemical factors.

Common to all forms of composting is an increase in temperature that is considered to be the main bactericidal effect. A commonly used temperature–time regime is 55 °C for 14–15 days in windrow composting or 3–5 days if the process takes place in a vessel or in a static aerated pile. In addition to temperature, desiccation and different chemical processes (production of ammonia) during the process are also probably playing a role in

the inactivation of unwanted bacteria present in the compost. Typically, well-managed compost reaches temperatures in a range of 50–65 °C, which is above the thermal death points of mesophilic pathogens (Jones and Martin, 2003); however these temperatures will not kill spores present. In a study of composting cattle manure in piles, temperatures in excess of 70 °C were reached and remained above 55 °C for up to 70 days (Gibbs *et al*., 2002). Christensen *et al*. (2001) showed that turning the windrows resulted in a fall in the temperature of the windrow. Another disadvantage of windrows is that the outer layer of the row is always colder than the core of the windrow and that turning is required to achieve a proper sanitizing effect. Then again, the turning causes a fall in the temperature. In-vessel composting has the advantage of a more even temperature development and may also be easier to control. Static aerated piles have been shown to have good effect on the thermal inactivation of pathogenic bacteria (Mote *et al*., 1988).

Recent research has shown that even though the temperature has not reached the desired or requested minimum, the numbers of faecal coliforms and *E. coli* are greatly reduced (Larney *et al*., 2003). This reduction is thought to be due to the antagonistic effects of the other microflora present or other reactions taking place during the process. Several studies have been conducted in order to investigate the effect of different composting methods on pathogenic bacteria (Droffner and Brinton, 1995; Jiang *et al*., 2003; Krogstad and Gudding, 1975; Larney *et al*., 2003; Lung *et al*., 2001; Mote *et al*., 1988; Turner, 2002). The results from these studies have shown a variable effect upon survival of the pathogens depending on the method used. Brinton (2004) reported finding higher concentrations of faecal coliforms in green composts compared to manure composts indicating that green composts are a likely, if non-suspected source of contamination. This contamination might be due to cross-contamination from dirty equipment, faeces from animals and so on.

Manure may also be treated by storing the manure over a period of time or by anaerobic digestion (Kearney *et al*., 1993a, 1993b). A storage period will in the end act like a passive fermentation of the manure, but is not easy to control and will also depend on the temperature development in the manure. Anaerobic digestion or fermentation may take place in anaerobic lagoons or in digesters. The digesters require more work, but the process takes less time than the other processes mentioned (Veenhuizen *et al*., 1992).

Tillage practices, including the use of cover crops, may also influence the survival of human pathogenic bacteria in soil. This has been studied by Gagliardi and Karns (2000 and 2002) who found that the presence of manure enhances the survival of *E. coli* in no-till soils and that *E. coli* O157:H7 persists for a longer period of time in soil with cover crops.

The organic agricultural system itself, with its absence of synthetic pesticides and mineral fertilizers, may have a favourable effect on the native

microflora in soil. The soil microflora are important competitors for any pathogenic bacteria that are introduced to soil. Soil will provide a hostile non-host environment for most of the human pathogens that are important in this context. The soil microflora are adapted to the soil environment where the temperature may be different from the intestines of animals and humans and nutrients will also be sparse. Interestingly, a study by Höflich *et al*. (2000) has shown that the *Pseudomonas* spp. and other bacterial species from the rhizosphere were more stimulated by organic manure than mineral fertilization. More recent research has shown that bacteria native in soil, among these *Pseudomonas* spp., have antagonistic effects against human pathogenic bacteria such as *Salmonella* spp., *L. monocytogenes* and *E. coli* O157:H7 (Cooley *et al*., 2003; Liao and Fett, 2001; Schuenzel and Harrison, 2002).

In conclusion, the largest technical difference when discussing the hygienic quality of organic and conventionally produced vegetables is the increased use of manure-based fertilizers. It must be emphasized that the risks of using manure as fertilizer is the same for both conventional and organically grown vegetables. It is important to keep in mind that there are several other pre- and post-harvest factors such as clean equipment, preventing animals from having access to vegetable fields, satisfactory sanitary facilities for workers and, probably most important, clean water, which play a role when discussing the microbiological safety of organic vegetables.

Since food legislation applies to organic foods as well as conventional foods there is no reason to believe that certified organic vegetables are of any poorer hygienic quality than conventionally produced fresh produce or will be in the future. However, it is important that producers of vegetables, organic as well as conventional, are aware of the potential risks of using manure-based fertilizers and take precautions in order to avoid contamination of soil, water and products.

10.7 Managing risks from mycotoxins

Various studies have identified agricultural practices which have some impact on fungal diseases in crops and also to some extent the mycotoxin contamination. This is by far more-investigated for cereal crops like wheat and maize than for vegetables. Since many toxin producing fungi like *Fusarium* survive in the soil from one season to the next, crop residues are important sources of inoculum. Miller *et al*. (1998) demonstrated, for instance, that crop debris was an important source of inoculum of *F. graminearum* in a crop rotation system with wheat–maize–soybean.

A review article by Champeil *et al*. (2004) on *Fusarium* head blight and mycotoxin production in wheat grains concluded that the crop succession history of the field was very important. Head blight contamination was

more severe if the preceding crop was maize, durum wheat or oats rather than wheat or barley (see Champeil *et al*., 2004 for references). This might be due to the fact that some crops leave less residues than others, and different crops are hosts for different toxin producers. Also Peters *et al*. (2003) showed that potato tubers harvested from three-year rotational soils with barley, red clover and potato were significantly less diseased with dry rot (*Fusarium* spp.) than tubers from two-year rotation with spring barley and potato. These results indicate that for all vegetable commodities one should consider very carefully crop rotation, taking into account that different crops may harbour different fungal species which may serve as inoculum for the next crop.

The organic management system itself with absence of synthetic pesticides and fungicides might have a beneficial impact on the soil microflora. Studies have demonstrated a wider range of fungal species and a greater occurrence of fungi potentially antagonistic to plant pathogens in soil in an organic conversion area compared with a conventionally cultivated area (Sivapalan *et al*., 1993). Investigations by Knudsen *et al*. (1995) indicate that pathogenic fusaria may be suppressed by antagonistic fusaria to a larger extent in organically cultivated fields than in the conventionally cultivated barley fields, even if the authors (Knudsen *et al*., 1999) also found that the high microbial biomass and activity of the soil under organic farming were not always correlated with high disease suppression. In another study Slanina (1995) concludes that the growing system does not have a significant effect on mould or mycotoxin contamination.

In conclusion, when it comes to managing risks from mycotoxins, agricultural practices with emphasis on crop rotation might be the most important factors. The handling and storage of vegetables after harvest is, of course, also of importance, but is not an issue for this chapter.

10.8 Future trends

There is no reason to believe that the market for organic vegetables will decrease in the next few years and thus work to keep organic vegetables safe for the consumers needs to be continued. More work is needed to survey the hygienic status of organic vegetables with regard to both pathogenic bacteria and potential mycotoxin-producing fungi. More research is also needed in the field of management practices in order to ensure safe organic vegetables for consumers.

10.9 Sources of further information and advice

There are several useful websites for information and advice on organic agriculture and the addresses to some of these are listed below. The national

standards and certification bodies will also probably have websites that can be visited for information. There are several universities and farmer's association that will provide information on the subject. The IFOAM publishes yearly a report on *The world of organic agriculture. Statistics and emerging trends* which is useful for an overview of the statistics of organic agriculture throughout the world. In this report are listed the addresses of certification bodies, and so on worldwide.

10.9.1 General information

www.ifoam.org
www.fao.org
www.soilassociation.org
www.soel.de
www.wrap.org.uk
www.ams.usda.gov
www.fibl.org
europa.eu.int/pol/agr/index_en.htm
www.organic-europe.net
www.ofrf.org
www.epa.gov

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11

Alternatives to hypochlorite washing systems for the decontamination of fresh fruit and vegetables

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11.1 Introduction

Chlorine-based washing systems are currently used by a majority (76%) of fresh produce manufacturers for decontamination purposes (Seymour, 1999). Typically, washing with 100 ppm total hypochlorite solution will achieve a 1–2 log (90–99%) reduction in levels of general microbial groups, i.e. aerobic plate count (APC) or Enterobacteriaceae. In many cases the level of microbial reduction achieved by hypochlorite systems in vegetable and salad crops is only marginally better than that achieved by using potable water alone, however, hypochlorite does have the advantage that it reduces microbial contamination in the wash waters and thus prevents re-introduction of undesirable microorganisms onto washed produce.

Despite the fact that hypochlorite systems have been used for many years, there still appears to be a lack of knowledge throughout the industry about how fully to optimise these systems. Seymour (1999) found that many users did not adequately control the levels of hypochlorite present nor the pH of the solution. These factors can have a great impact on the efficacy of the washing systems in terms of level of microbial reduction achieved. Before considering alternatives to hypochlorite systems, it is pertinent to provide a brief overview of the limitations of hypochlorite systems.

11.1.1 Inadequate control/optimisation of chlorinated systems

In order to achieve the maximum effects from chlorinated wash systems, the activity of the chlorine needs to be optimised and controlled. Chlorine

can exist in many forms when used to wash fresh produce. The common form of hypochlorite used in the fresh produce industry is sodium hypochlorite (NaOCl). This produces hypochlorous acid (HOCl) and sodium hydroxide (NaOH) when added to water (Equation 11.1). In turn the HOCl dissociates to form the hypochlorite ion (OCl^-, Equation 11.2):

$$NaOCl + H_2O \rightarrow NaOH + HOCl \quad [11.1]$$

$$HOCl \rightarrow H^+ + OCl^- \quad [11.2]$$

Owing to the presence of NaOH, the hypochlorite solution has a tendency to be alkaline, which is not optimum for achieving maximum decontamination. Hypochlorous acid (HOCl) is a more effective disinfectant than the hypochlorite ion (OCl^-) yet under alkaline conditions the majority of the chlorine exists in the form of OCl^- (Table 11.1).

For the fresh produce industry, a target pH for optimal efficacy would be around pH 7.0, as a high proportion of the chlorine is in the active form (HOCl) and at lower pH values the solution may become corrosive to factory equipment. Yet it has been reported (Seymour, 1999) that only 20% of the fresh produce industry routinely controlled the pH of the solution and therefore the majority were using non-optimised solutions for produce washing.

Another aspect of hypochlorite systems, which is often misunderstood, is the availability of the chlorine for disinfection purposes. The chlorine in washing systems can exist in different states of availability or activity. Hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) are termed 'free chlorine' and are the active forms of the chemical. Chlorine can also bind with other compounds from organic material, for example ammonia or other nitrogenous compounds to form 'bound' or 'combined' chlorine, for example NH_2Cl. Combined chlorine is much less effective as a biocide than 'free' chlorine. In the majority of cases, it is the levels of total chlorine ('free' plus 'combined') which are controlled by the food industry (Seymour, 1999). This does not give an accurate measure of the efficacy of the system

Table 11.1 Effect of pH on ratio of HOCl and OCl^- in chlorine solutions

pH	HOCl (%)	OCl^- (%)
5	100	0
6	97	3
7	78	22
8	23	77
9	4	96
10	0	100

Source: Dawson (2002).

and it is more effective to control the 'free' chlorine levels, as this is the active form of the chemical.

The amount of free chlorine in a system can be affected by many different factors, for example produce type. Dawson (2002) showed that in a system containing 20 kg produce in 300 litres of 100 ppm chlorine solution, approximately 40% of the chlorine was 'free' when peppers were present and over 90% was 'free' when lettuce was present. The varying chlorine demands of different batches of produce may be one of the causes of inconsistent results from hypochlorite washing systems (see Section 11.1.2). For more information on optimisation of washing systems see Dawson (2002).

11.1.2 Inconsistent results

There is a tendency to consider 'chlorine washing' as a single treatment, however, many factors can alter the efficacy of hypochlorite-based systems, such as the pH of the solution, amount of organic material present and the washing time used. Therefore the microbial reduction achieved using chlorine washes can vary considerably between different treatments. Table 11.2 shows the microbial efficacy of hypochlorite treatments on the APC obtained from fresh produce. It can be seen that hypochlorite treatments achieved reductions in APC ranging from zero to a 3 log reduction (Garg *et al*., 1990) dependent on product type. Similar variation is also seen in inactivation of pathogens on fresh produce. It may be expected that inconsistent results are obtained when comparing studies from different laboratories, as the strength of hypochlorite solution, content time and produce type varied. However, such differences can also be found from work done in a single laboratory using a standardised washing protocol.

Campden & Chorleywood Food Research Association (CCFRA) has done many studies on the inactivation of *Salmonella* and *Listeria* on produce. The levels of inactivation varied between treatments as shown in Table 11.3. Here lettuce samples were inoculated with a level of 10^6 pathogens per gram and stored at 5 °C to allow the organisms to attach. They were washed using hypochlorite treatments using a range of times and concentrations of hypochlorite.

The results demonstrate that there can be between a 0.5–1 log difference in log reductions achieved using any single washing procedure. The results also show that there can be differences in the log reduction achieved dependent on chlorine concentration and wash time. The use of 5 min seemed to achieve a slightly higher level of inactivation than 2 min, whereas there did not appear to be much difference between either 50 or 100 ppm in these trials.

11.1.3 Formation of harmful by-products from hypochlorite

Over recent years there have been increasing concerns over the potentially harmful by-products which can be formed when chlorine reacts with

Table 11.2 Studies using hypochlorite as a decontamination method

Organism	Product	Findings	Reference
Aerobic organisms	Lettuce	Reduction in level with 50 ppm No further reduction at 200 ppm	Mazollier (1988)
Total count	Lettuce	92% reduction in microflora when washed in potable water, 100 ppm for 5 min gave a reduction of 97.8%. Increased contact time of 30 min gave no further increase. +100 ppm TWEEN 80 gave further reductions but decreased sensory quality	Adams *et al.* (1989)
Total count	Lettuce	5 ppm of chlorine in water gave a 90% reduction in level (initial 10^4–10^6 cfu g^{-1}).	Somers (1963)
Total count	Lettuce (shredded) Carrots Red cabbage	300 ppm chlorine gave a 1000-fold reduction. 300 ppm gave no reduction	Garg *et al.* (1990)
Total count Enterobacteriaceae	Tomatoes	114 ppm chlorine treatment gave higher levels than controls, 226 ppm decreased levels. Recontamination was occurring	Senter *et al.* (1985)
Total count	Oranges	20 to 50 ppm free chlorine reduced levels by 92–99%, wate reduced levels by 74%	Murdock and Brokaw (1958)
Total count	Oranges	Dipping in 1000 ppm hypochlorite for 15 s gave a 90% reduction in surface contamination compared with a 60% reduction with water	Winniczuk (1994)
Total loading	Cantaloupe	<1 log reduction was observed after 1000 ppm chlorine for 1 min immersion treatment	Sapers *et al.* (2001)
Salmonella *Escherichia coli* O157:H7 *Listeria monocytogenes* Yeast and moulds Total count	Whole apples Tomato Lettuce leaves	200 and 2000 ppm chlorine spray reduced organisms by 0.35 to 2.30 logs, 2000 ppm was more effective and most inactivation occurred within 1 min	Beuchat *et al.* (1998)

Table 11.3 Comparison of hypochlorite wash treatments when used to wash inoculated iceberg lettuce

Chlorine concentration (ppm)	Wash time (min)		Log reduction	
			Salmonella	*Listeria*
50	2	A*	0.62	1.05
		B	0.56	0.73
50	5	A	1.22	1.37
		B	0.91	0.64
100	2	A	0.53	1.15
		B	0.95	0.71
100	5	A	0.91	1.04
		B	1.59	2.12
100	10	A	1.19	1.41
		B	1.87	2.44

* A and B are replicate treatments done at different times.

organic compounds present on fruits and vegetables and other foods. The organochlorine compounds formed are considered to be potential carcinogens and hence there is a concern that chlorine may eventually be banned for use in washing fresh produce (see Section 11.4).

Studies have been carried out to assess the organochlorine compounds formed when chlorinated water is used to wash chicken or is used in drinking water. The compounds formed when chloride reacts with organic compounds include chloropropanols such as monochloropropanediol (3MCPD), chlorohydroxyfuranones (Maki-Paakkanen *et al.*, 2004) and trihalomethanes (Villaneuva *et al.*, 2004). These compounds have been shown to increase the risk of a range of cancers including bladder, kidney and liver. These compounds could potentially be formed when chlorinated water is used to wash fresh produce.

11.1.4 Future use of hypochlorite in fresh produce washing

The use of hypochlorite-based systems for fresh produce washing is already prohibited in various European Union countries, for example Denmark and Germany. In addition, the UK Register of Organic Farmers (UKROF) (Soil Association) does not include hypochlorite on the list of permitted biocides for fresh produce decontamination, although other chemicals such as Antibac, Drywhite, Citrox and Aqua-a-live are allowed.

There is a feeling amongst the UK manufacturers and retailers of fresh produce that a ban on the use of hypochlorite systems is likely in the future. If this occurs then there will be a requirement for alternative treatments that are cost effective and achieve a similar level of inactivation as

hypochlorite. In order to assess whether alternative biocides are suitable as a replacement for hypochlorite, there is a requirement for a standard approach to assess the efficacy of biocide systems.

11.2 Standardised approach to biocide testing

In order to assess the usefulness of alternatives to chlorine, there is a requirement to compare data on the microbial reduction achieved using hypochlorite and the alternate biocide. Currently, if such a comparison was attempted based on published data, it would be likely to be difficult to do or may achieve misleading results because the methodologies used in different studies are not consistent. Beuchat *et al*. (2001a, b) called for the standardisation of a method to determine the efficacy of sanitisers in inactivation of pathogens on fruits and vegetables. They raised a number of areas of the washing procedure, which could impact on the inactivation achieved and should be controlled. In particular, they were concerned with studies where a known amount of human pathogen, such as *Salmonella* or *Escherichia coli* is inoculated onto the surface of the produce and then the amount of removal is assessed.

Parts of the washing protocol which should be standardised are:

- Type of produce to be used in trial – whether it should be whole or cut and whether it should be previously washed or unwashed. This is important as the surface area of the produce used can affect the efficacy of the treatment.
- Organism of interest – should a single strain or mixed culture be used? What procedure should be used for preparation of inoculum, i.e. what laboratory media and what time and temperature for growth of the organisms? What inoculation procedure (spray, dip, spot inoculation, level of inoculum) should be used as this can affect the attachment and localised concentration of the organism?
- Test conditions – including time of washing treatment, concentration of biocide and hypochlorite control, application method (spray, dip, fogging), static wash or agitation and ratio of product to sanitiser.
- Analysis of results – i.e. number of replicates to be used and choice of appropriate statistical analysis.

Many studies use a high (10^6 cfu g^{-1}) level of pathogenic organisms for biocide efficacy studies and this may not always be realistic in terms of the likely contamination level seen on fresh produce. Therefore a standard protocol should also consider how effective a biocide is at inactivation of low levels of human pathogens. In addition, the effect of the biocides on levels of natural spoilage organisms (APC, Enterobacteriaceae or coliforms) as well as inoculated pathogens is important, the naturally present organisms affect the shelf-life of the product during storage.

Studies at the CCFRA have considered important aspects of the biocide efficacy testing protocol, and in particular optimising the control treatment using hypochlorite, to ensure consistent results could be achieved. This is important as one of the criteria against which the success of alternative treatments is measured is that it achieves a similar level of inactivation as hypochlorite. If the inactivation seen with hypochlorite varies between repeated treatments, then such a comparison would be hard to make.

11.2.1 Standardisation of 'drying time' or attachment of pathogens after inoculation

In the different literature studies on the efficacy of biocides, the time for which the inoculated organisms are allowed to 'dry' or 'attach' to the produce is as short as 30 min (Bari *et al.*, 2003) to 24 h (Beuchat *et al.*, 2001b). Table 11.4 illustrates that when the inoculum (10^5 cfu g^{-1}) was left to dry/attach on iceberg lettuce for various amounts of time prior to washing, a difference in log reduction achieved was observed. It appeared that the lowest log reduction was achieved when the inoculum was dried for 4 h.

11.2.2 Time of washing treatment/level of hypochlorite used

Many reported studies use wash times of up to 10 min or longer, however, the UK fresh product industry routinely wash fresh produce for much shorter times than this, typically 1 min. Some studies have shown that increased washing time has no greater effect than short washing time, as much of the reduction in numbers is achieved in the first minute. Table 11.5 shows that there was a slight increase in the level of log reductions seen when the wash time was increased from 1 to 5 min, although the increase was unlikely to be sufficient to be of practical significance.

The results in Table 11.5 also show that there was only a slight increase in the efficacy of hypochlorite with concentrations of 25–100 ppm.

Table 11.4 Results of hypochlorite washing (100 ppm/5 min) inoculated iceberg lettuce (10^5 cfu g^{-1})

Organisms	Log reduction cfu g^{-1} (h)				
	Attachment time at 5 °C (time between inoculation and washing)				
	0	0.5	1	4	24
Salmonella	1.17	1.40	0.95	0.86	1.14
Listeria	1.28	1.36	1.54	1.12	1.84

Table 11.5 Effect of wash time and chlorine concentration on log reduction (cfu g^{-1}) of pathogens inoculated onto iceberg lettuce

Chlorine level (ppm)	Wash time (min)	Log reduction (cfu g^{-1})	
		Salmonella	*Listeria*
25	5	0.80	0.85
50	5	1.22	1.0
100	5	1.1	1.25
100	1	0.92	1.33
	2	0.68	1.15
	5	1.05	1.79

11.2.3 Level of pathogens inoculated onto the produce

In biocide efficacy trials, the level of inactivation seen is often given as the number of 'log reductions' achieved but often the levels of organisms inoculated onto the produce are much higher than would be expected to occur naturally. It was important to determine whether the log reductions observed with any treatment were affected by the initial level of microorganisms present. Iceberg lettuce was inoculated at levels of 10^3 or 10^5 cfu g^{-1} and washed with 100 ppm of hypochlorite for 5 min. The log reductions achieved were 0.90 and 1.05, respectively, indicating that there are minor differences in log reduction, depending on inoculum level.

The results of these experiments demonstrate that in order to test the efficacy of different produce decontamination systems, a standardised protocol defining inoculum level, time and a standard hypochlorite treatment (concentration/times) would be useful.

11.3 The use of alternative compounds to hypochlorite

There are a number of alternative decontamination treatments which have been suggested for use in washing fresh produce. An overview of the range of potential alternatives is given in this section. Two of these (ultrasound and hydrogen peroxide) are discussed in further detail in Section 11.4.

11.3.1 Chlorine dioxide

Chlorine dioxide has received attention as a disinfectant for fruits and vegetables, mainly because it is not affected by pH and organic matter to the same degree as chlorine (Simons and Sanguansri, 1997). One disadvantage of chlorine dioxide is that it is unstable, it must be generated on-site and can be explosive when concentrated. The oxidising power of chlorine dioxide is reported to be about 2.5 times that of chlorine and its activity is

not affected by pH (Beuchat, 1998). This is because, unlike chlorine, chlorine dioxide does not hydrolyse in water to form hypochlorous acid. Instead, it remains dissolved as a gas and may decompose to its chlorite and chlorate ionic forms (Simons and Sanguansri, 1997). Costilow *et al*. (1984) found that 2.5 ppm chlorine dioxide was effective in killing microorganisms in wash water, but at concentrations up to 105 ppm, failed to reduce the level of microorganisms present on fresh cucumber. Zhang and Farber (1996) also reported similar efficacy of chlorine and chlorine dioxide on fresh produce inoculated with *Listeria monocytogenes*. The effectiveness of chlorine dioxide in killing pathogenic microorganisms on specific types of fruits and vegetables may require further research.

11.3.2 Organic acids

Organic acids can occur naturally in many fruits and vegetables and may retard the growth of some microorganisms and prevent the growth of others (Beuchat, 1998). Most of these acids behave primarily as fungistatic agents, while others are more effective at inhibiting bacterial growth. These include acetic, citric, succinic, malic, tartaric, benzoic, propanoic and sorbic acids. Their mode of action is attributed to reduction of the intracellular pH of microbial cells by ionisation of the undissociated acid molecule. These acids do not generally kill the microorganisms but affect the cell's ability to maintain pH homeostasis, disrupting substrate transport and inhibiting metabolic pathways (Seymour, 1998). Washes and sprays containing organic acids, particularly lactate, have been successfully used to disinfect animal and poultry carcasses. Karapinar and Ganul (1992) demonstrated a 6 log reduction in *Yersinia enterocolitica* inoculated onto parsley by washing in a solution containing 2% acetic acid or 40% vinegar for 15 min. However, the sensory quality of the parsley after disinfection was not specified. Zhang and Farber (1996) also found that 1% lactate in combination with 100 ppm chlorine was more effective than either lactate or chlorine alone at reducing *L. monocytogenes* inoculated onto lettuce.

11.3.3 Hydrogen peroxide

Hydrogen peroxide can have a lethal or inhibitory effect on microorganisms depending on the pH, temperature and other environmental factors (Beuchat, 1998). Hydrogen peroxide is classified as generally recognised as safe (GRAS) for use in food products as a bleaching, oxidising, reducing and antimicrobial agent (Sapers and Simmons, 1998). Sapers (1996) demonstrated that hydrogen peroxide vapour treatments were highly effective in reducing microbial numbers on whole cantaloupes, grapes, prunes, raisins and nuts. Dipping freshly cut green bell pepper, cucumber, courgette, cantaloupe and honeydew melon in hydrogen peroxide solution had no adverse effect on appearance, flavour or texture, but H_2O_2 induced

severe browning of shredded lettuce. Treatment significantly reduced the levels of *Pseudomonas* spp. on these products but had no effect on yeasts and moulds. Results from a number of studies suggest that hydrogen peroxide has potential as a sanitising agent for fruits and vegetables (Beuchat, 1998).

11.3.4 Acidified sodium chlorite

Acidified sodium chlorite can be used on certain meat and seafood products and raw fruit and vegetables in the USA (USDA, 2001). Dips or sprays can be used from 500 to 1200 ppm. Park and Beuchat (1999) demonstrated a 3 log reduction in *Escherichia coli* O157 and *Salmonella* levels when inoculated on cantaloupe and honeydew melons. Concentrations of 850 and 1200 ppm with a 3-min contact time were used. Taormina and Beuchat (1999) also used acidified sodium chlorite. They demonstrated that 500 ppm could reduce the population of *E. coli* O157 on alfalfa seeds by more than one log. There has been little research on the use of acidified sodium chlorite for use with fruit and vegetables, but it does have potential.

11.3.5 Trisodium phosphate

Trisodium phosphate has been tested as a disinfectant for fresh produce by a few authors. Zhuang and Beuchat (1996) demonstrated complete inactivation of *Salmonella* Montevideo. Liao and Sapers (2000) observed a 1 log reduction on the surface when tomatoes were dipped in 15% trisodium phosphate for 15 s.

Zhang and Farber (1996) found that use of 2% trisodium phosphate did not reduce *L. monocytogenes* levels on lettuce. *E. coli* O157:H7 and *Campylobacter* were shown by Somers *et al*. (1994) to be sensitive to low levels of trisodium phosphate; 10^5–10^6 cells were killed within 30 s when 1% trisodium phosphate was used.

Pao and Davis (1999) found that an 8-min treatment with 2% trisodium phosphate did not reduce the level of *E. coli* on orange surfaces any more than water alone. Pao *et al.* (1999, 2000) demonstrated that alkaline washes with sodium and potassium hydroxide reduced *E. coli* on the surface of oranges, and suggested that high pH waxes used on the surface of fruits can protect against *E. coli*. Sapers *et al*. (2001) found that a 1.33 log reduction in total count was achieved on cantaloupe melons washed for 10 min in 4% trisodium phosphate and a 1.13 reduction when 8% trisodium phosphate was used.

Despite the antimicrobial potential of trisodium phosphate, Beuchat (1998) suggests that its action may be limited owing to the increase in pH of the wash water to 11–12 when trisodium phosphate is used, which may limit applicability to produce. There are also concerns regarding disposal of waste phosphates into the environment (USDA, 2001).

11.3.6 Ozone

Ozone has been used to inactivate microbial contamination in drinking water for a long time. Xu (1999) thoroughly reviewed the use of ozone with fresh produce. Ozone is an oxidising agent that is 1.5 times stronger than chlorine and is an effective antimicrobial agent. It is a high-energy molecule that has a half-life of only 20 min in water at room temperature. It decomposes into oxygen and, therefore, ozone does not form any residues on the product being washed.

Ozone is a naturally occurring triatomic oxygen molecule:

$$3O_2 \rightleftharpoons 2O_3 + \text{heat and light} \qquad [11.3]$$

It is generated naturally by UV irradiation from the sun and lightning. It is generated commercially by UV lights (185 nm wavelength) or corona discharge. Two types of gas can be used to generate ozone in wash water: air 1–3% (w/w) and oxygen at 2–12% (w/w). The antimicrobial action is due to oxidation of cell membranes. Ozone can be used in fruit and vegetable process systems to remove decontamination and then the wash water can be re-ozonated and recycled with no chemical residues left over.

Shelf-life extension of pears, apples, grapes, oranges, raspberries and strawberries has been achieved after ozone treatment (Beuchat, 1998). Ozone can be a useful disinfectant for product decontamination and prevention of contamination of wash water. However, it does need to be generated on site and can cause degradation of metal equipment. It must also be noted that toxic vapours can be produced and to safeguard employee health, sufficient ventilation is required.

11.3.7 'Other' compounds

Nguyen-the and Lund (1992) found that carrot juice had an antimicrobial effect on *L. monocytogenes*.

Lin *et al*. (2000) studied the effect of allyl and methyl isothiocyanate (AITC/MITC) (key components of Japanese green mustard) on *L. monocytogenes*, *E. coli* O157:H7 and *S. Montevideo* inoculated onto lettuce and tomato surfaces. AITC was more effective against *Salmonella* and *E. coli*: an 8 log reduction was achieved after 4 and 2 days, respectively on lettuce. An 8 log reduction of *S. Montevideo* was achieved on tomato skin and a 5 log reduction was achieved on stem scars. Apples were also tested and AITC only achieved a 3 log reduction on apple stem scars.

Dawson (personal communication) found that MS-2 bacteriophage, polio virus and feline calicivirus were, in some instances, inhibited by carrot extract.

11.3.8 Hot water

Fleischman *et al*. (2001) found that a 15-s water treatment at 80 or 95 °C was sufficient to produce a 5 log reduction in *E. coli* O157:H7 level on apple surfaces. Treatment at 40 or 60 °C for 60 s did not produce a reduction.

11.3.9 Alkali

Work by Takeuchi and Frank (2001) studied the efficacy of 1% $NaCl–NaHCO_3$ and an alkaline-based washing agent (pH 10). *E. coli* O157:H7 was inoculated onto lettuce, which was washed with each of the solutions for 3 min. The alkaline wash produced a 0.7–1.1 log reduction compared with 0.2–0.4 for the $NaCl–NaHCO_3$ wash. They also found that more viable cells were present 30–40 μm from the cut surface and that stomata protected cells from the disinfectants.

11.3.10 Ultrasound

Ultrasound decontamination of fruit and vegetables has been reported in Seymour and Burfoot (2000) and Seymour *et al*. (2002). Power ultrasound is used at frequencies of 20–100 kHz and requires the presence of a liquid medium. Waves are produced at a high amplitude which forms cavitation bubbles. These bubbles then generate mechanical energy by oscillation and collapse and can remove particles from surfaces (Seymour *et al*., 2002).

S. Typhimurium was inoculated onto the surface of various produce types, which were then treated for 10 min at a frequency of 32–40 kHz in an ultrasound tank. Log reductions of 1, 1.3 and 1.5 were achieved for cabbage, strawberry and pepper, respectively. If produce was cut there was less reduction observed.

Trials were carried out to assess the efficacy of ultrasound, chlorine and a combination of both treatments. *S. Typhimurium* was reduced by 1.7 and 1.6 logs, respectively but a combination of both gave a 2.7 log reduction. Use of ultrasound requires an input of electricity, so may be more expensive than chemical sanitisers. It also generates significant heat.

11.3.11 Photodynamic inactivation

Seymour and Burfoot (2000) studied photodynamic inactivation as a disinfection method for use with fresh produce. Singlet oxygen ($^1O_2$) is a high energy form of oxygen and is toxic to all living organisms. It can be produced by the interaction of light of a specific wavelength, photosensitisers and molecular oxygen (O_2). Photosensitisers include Rose Bengal (a xanthene dye) and Methylene Blue (a thiazine dye). In the dark the dyes exist in a ground state (S) which then becomes activated (^1S) by light; a triplet, excited state (^3S) can be produced by excess energy from the ^1S molecule. The ^3S molecule can then follow one of two routes, a Type 1 route is direct inactivation of the triplet state to produce highly reactive free radicals (hydrogen peroxide, superoxide and hydroxyl) through auto-oxidation. The Type 2 route produces singlet oxygen by transfer of energy to O_2. It is thought that the antimicrobial action is due to inhibition of DNA and RNA synthesis and interference with the membrane enzymes and transport processes.

Seymour and Burfoot (2000) found that there was a difference in sensitivity of Gram positive and Gram negative organisms to singlet oxygen: the Gram positive *L. monocytogenes* was reduced by 10 to 100-fold, while the Gram negative *S. Typhimurium* and *E. coli* demonstrated no reduction. The authors conclude that it is unlikely that this technology will be feasible on a large scale owing to inadequate light penetration and the high organic loading present on produce.

11.3.12 High pressure

This technology is only really used with liquid products such as juices. Teo *et al*. (2001) demonstrated a 4–8 log reduction of *Salmonella* and *E. coli* O157:H7 after processing at 615 MPa. However, more research is required in this area, which could be promising for fresh liquid products.

11.3.13 Ionisation

A ionisation system, Tarn-pure™, can be used to decontaminate fresh produce and wash water. The system passes an electric current through a silver electrode which releases Ag^{2+} into the water, which at a level of 1 ppm can reduce bacterial loading on fresh produce (Batemann, personal communication). However, no independent peer reviewed information is available for this system.

11.3.14 Biocontrol

Biocontrol can be defined as an inhibitory effect of one organism on the growth and survival of another. Janisiewicz *et al*. (1999) found that *Pseudomonas syringiae* prevented *E. coli* O157:H7 growth in wounds on the surface of apples; a 2 log increase in *E. coli* O157:H7 occurred in wounds containing no *P. syringiae*, but no growth occurred when this organism was present.

Bennik *et al*. (1999) found that *Enterococcus mundtii* did not prevent the growth of *L. monocytogenes* in produce but did prevent growth on a vegetable agar. A bacteriocin, mundticin, produced by *E. mundtii* acted as a biopreservative on modified atmosphere packed (MAP) mungbean sprouts. Further studies by Carlin *et al*. (1996) demonstrated that the natural flora present on endives inhibited *L. monocytogenes* growth; Vescovo *et al*. (1996) found that lactic acid bacteria strains could inhibit various pathogens (*S. Typhimurium*, *Staphlococcus aureus*, *Aeromas hydrophila* and *L. monocytogenes*) on vegetable salads. Liao and Fett (2001) tested 120 natural bacterial isolates from produce for their efficacy against pathogens. *P. fluorescens* and a yeast strain gave a 1–2 log reduction in *S. Chester* and *L. monocytogenes* levels.

Biocontrol methods have been studied to prevent post-harvest spoilage organism growth and the USDA (2001) suggest that non-pathogenic organisms out-compete pathogens for space and nutrients, and may produce bacteriocins. More research on use of biocontrol to prevent human pathogens is required.

Leverentz *et al*. (2001) inoculated *Salmonella* specific bacteriophage onto honeydew melon previously inoculated with *Salmonella*. A reduction of 3.5 logs was achieved for melon stored at 5 and 10 °C and a 2.5 log reduction was achieved at 20 °C. The trials were repeated using apple; however, on this produce type the phage was inactivated, possibly owing to the low pH, and was thus not an effective decontaminant for apple. Use of phages may be a useful decontamination method, which should be investigated further.

11.3.15 Combination treatments

Sequential washing techniques have been assessed by Singh *et al*. (2002). They evaluated the efficacy of chlorine dioxide, ozone and thyme essential oil, alone and with sequential washing. Shredded lettuce and carrots were used. A 3 log or more reduction was achieved using sequential washing techniques. Water washing for 10 min gave a 1 log reduction compared with a 1, 4.8 and 1.97 log reduction when using aqueous chlorine dioxide (10 mg l^{-1} for 10 min), ozonated water (9.7 mg l^{-1} for 10 min) or thyme oil (1.0 ml l^{-1} for 5 min), respectively.

Sequential washing techniques gave greater log reductions; thyme oil followed by aqueous chlorine dioxide/oxonated water or ozonated water/aqueous chlorine dioxide gave log reductions of 3.75 and 3.83 on lettuce and 3.99 and 4.34 on baby carrots. The authors conclude that sequential decontamination treatments can achieve greater log reductions of *E. coli* O157 on lettuce and carrots than single treatments. Other authors, such as Seymour and Burfoot (2000), demonstrated that combining ultrasound and chlorine washing was more effective than either treatment alone.

11.4 Strengths and weaknesses of alternative treatments

In order for an alternative to be effective it must:

- be easy to use
- be cost effective
- require minimal changes to current factory equipment
- achieve a minimum of a 2 log decrease in target microorganisms (or at least equivalent to hypochlorite when tested in comparative studies)
- have been 'approved' for use in a food environment
- have no concerns over safety issues associated with its use, i.e. no production of by-products.

Alternatives, which will be discussed in greater detail, are ultrasound, photodynamic inactivation, hydrogen peroxide, UV and ozone.

11.4.1 Ultrasound

Ultrasound (US) is known to disrupt biological structures and has the potential to cause cell death when applied with sufficient intensity. US consists of sound waves with a frequency (pitch) in excess of that in the audible range (20 Hz to 18 kHz). Several theories have been proposed about the precise mechanism of US cell damage. Sound waves transfer vibrational energy from one place to another through a series of alternate compression and rarefaction waves in the propagating aqueous medium. Most of the applications for power US use frequencies in the range of 20–100 kHz and require only the presence of a liquid medium for power transmission (reviewed by Roberts, 1991; Mason, 1993). At sufficiently high power, bubbles or cavities are formed, a process known as cavitation (Scherba *et al*., 1991). US leads to cavitation at surfaces which is caused by the vapour pressure falling locally below the saturated vapour pressure. Many changes can occur to the bubbles during their growth and collapse and it is these changes which lead to a 'cleaning' action on surfaces.

Cavitation can be either stable or transient, producing one of two different effects (Roberts, 1991; Scherba *et al*., 1991). In stable cavitation, small bubbles oscillate during the compression and rarefaction cycles as the ultrasound passes through the liquid. This causes strong eddies to be formed in the surrounding liquid, attracting other small bubbles into the sonic field. These bubbles can form microcurrents around themselves, which then spread into the liquid, a process known as microstreaming. This streaming effect provides a large force which effectively 'rubs' or 'shears' membrane surfaces, causing the cell membrane to break down. In contrast, transient cavitation occurs when the bubble size changes much quicker (within a few oscillatory cycles) causing the bubble to collapse at different intensities (Sala *et al*., 1995). The collapse is thought to generate very high local temperatures (up to 5000 °C), pressures (in excess of 1000 atm) and electrical potential (Raso *et al*., 1998). It has been proposed that these extreme conditions are responsible for the majority of the antimicrobial effects of US treatment. The localised high temperatures and pressures bombard the cell membranes and may be strong enough to disrupt cell wall structures or to remove particles from surfaces (Schuett-Abraham *et al*., 1992).

Several workers have examined the effects of US on the inactivation of various bacteria (Ordonez et *al*., 1987; Scherba *et al.*, 1991; Raso *et al*., 1998; Pagan *et al*., 1999), spore formers (Sanz *et al*., 1985) and moulds (Idrissi *et al*., 1996). However, these results are difficult to compare owing to variation in the physical parameters, such as US frequency, power level, the size and shape of the ultrasonic bath, the depth, volume, temperature and nature

of the liquid, and treatment time (Jeng *et al*., 1990). Essentially, there are no reliable means of quantifying the cavitation activity and microbiological laboratories must rely on empirical evaluations to evaluate the performance of each individual ultrasonic treatment system (O'Donoghue, 1984). US has recently been proposed for use in food preservation but this purpose has not been readily adopted. This is probably due to the perceived adverse effects on food quality brought about by the high-intensity treatments required to inactivate the most resistant microorganisms.

Seymour and Burfoot (2000) evaluated the use of various ultrasound treatments for fresh produce inactivation. Treatment times of up to 10 min were assessed using ultrasound frequencies of 25 kHz, 32–40 kHz and 62–70 kHz. The work concentrated on the visual quality of a range of product types and the reduction achieved in levels of *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes*. The types of fresh produce examined were baton carrots, cabbage, cucumber, mint, iceberg lettuce, parsley, pepper, spring onions and strawberries.

In terms of the sensory effects of the ultrasound, treatments were dependent on the type of ultrasound equipment used. Produce treated in an ultrasonic tank (15 W l^{-1}) for 5 min showed no damage with the exception of mint, which had slight loss of green colour at the edge. When an ultrasonic bar was used (225 W l^{-1}), many produce types showed signs of colour defects, that is, loss of colour or blackening and there were some signs of textual changes. With respect to the level of reduction in the numbers of attached pathogens, on average, there was a 1.5 log reduction in levels of microorganisms using chlorine alone or the ultrasound tank alone. If the two treatments were combined then a 2–5 log reduction was achieved.

Seymour and Burfoot (2000) proposed some likely capital costs, which would be associated with the industrial use of ultrasound treatments (Table 11.6).

Table 11.6 Estimated capital costs of ultrasound decontamination for fresh produce

Throughput of product (tonnes/day)	Total throughput (approx.) (product + liquid) (kg h^{-1})	Power (W l^{-1})	Generator costs (£)	Transducer costs (£)	Tank costs (£)	Delivery installation costs (£)	Total capital cost (£)
1 (8-h day)	320	15 (for 15 min)	12 528	6 720	960	–	20 000
10 (16-h day)	1 600	15 (for 15 min)	62 640	31 360	4800	–	99 000
100 (16-h day)	15 625	15 (for 15 min)	10 × 62 640	10 × 31 360	10 × 4800	–	~1 million

Table 11.7 Microbial reduction (log_{10}) achieved by hydrogen peroxide treatment

Organism	Produce	H_2O_2 (50 °C)	H_2O_2 (22 °C)	Water (50 °C)	Water (22 °C)
Enterobacteriaceae	Lettuce	1.48	1.46	1.20	0.10
	Cabbage	3.24	1.63	0.50	0.50
Salmonella	Lettuce	1.58	1.06	0.75	1.27
	Cabbage	2.45	1.90	0.33	1.07
APC	Lettuce	2.56	0.00	0.51	0.21
	Cabbage	1.98	1.20	0.09	0.04

They concluded that the associated costs were too high to justify the limited increase seen in microbial reduction and felt it was unlikely to be used by the fresh produce industry as a chlorine alternative.

11.4.2 Hydrogen peroxide

It has been shown (Lin *et al*., 2002) that 2% hydrogen peroxide in combination with mild heat (50 °C) was able to achieve a 3–4 log reduction in levels of *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 inoculated onto lettuce. The quality of the treated produce was shown to be acceptable to consumers (McWatters *et al*., 2002). Studies at CCFRA evaluated the reduction in levels of *Salmonella* inoculated at a level of 10^6 cfu g^{-1} and levels of naturally present APC and Enterobacteriaceae on lettuce and cabbage after washing for 10 min in 2% hydrogen peroxide at 22 °C and 50 °C. Control treatments were also done using potable water at both temperatures. The log reductions achieved are shown in Table 11.7.

Treatment with hydrogen peroxide at 50 °C was able to achieve up to a 3 log reduction in levels of natural flora and *Salmonella*, which was similar or better than that which may be expected with hypochlorite systems. An important aspect of produce decontamination is not just the initial level of microbial inactivation but also the impact of the treatment on the microflora during subsequent storage.

Lettuce and cabbage, which had been treated with H_2O_2 as described above, were stored at 8 °C for up to 7 days and the levels of the relevant organisms monitored. Figure 11.1 shows the survival of *Salmonella* on cabbage during storage at 8 °C. Whilst the levels of *Salmonella* decreased throughout storage for all treatments, they decreased more rapidly for samples treated with hydrogen peroxide. The growth of Enterobacteriaceae on lettuce stored at 8 °C was slower for samples treated with hydrogen peroxide and remained 100-fold lower than samples treated with water throughout the trial (Fig. 11.2). Costs of treatment with hydrogen peroxide would be similar to hypochlorite and it would be easy to use in current washing systems.

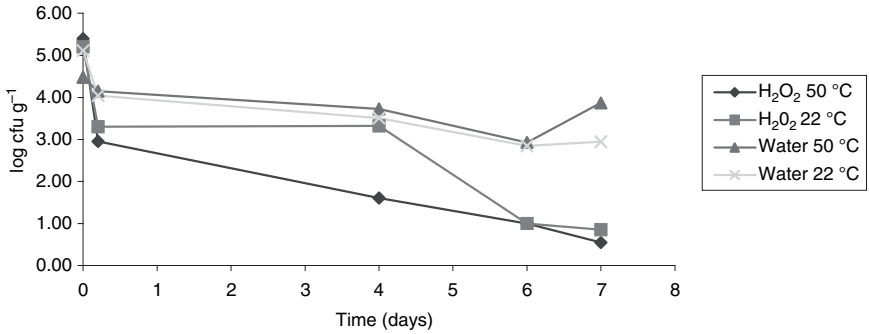

Fig. 11.1 Survival of *Salmonella* on cabbage at 8 °C after washing with hydrogen peroxide or water at 22 °C or 50 °C.

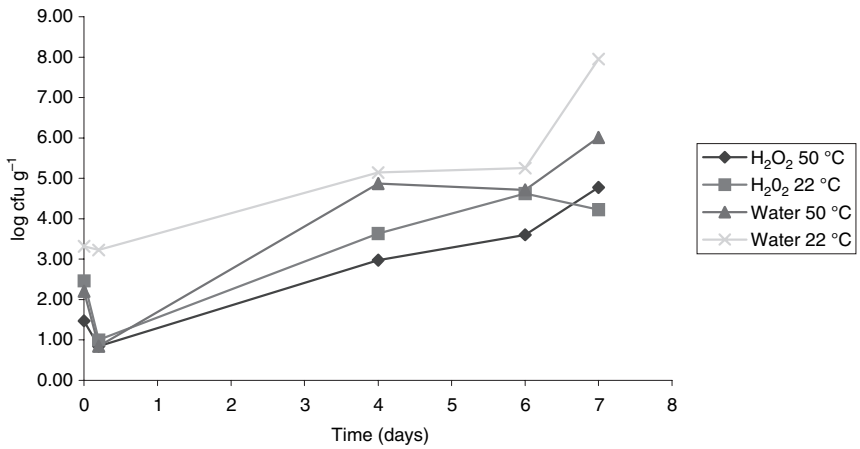

Fig. 11.2 Growth of Enterobacteriaceae on lettuce at 8 °C after washing with hydrogen peroxide or water at 22 °C or 50 °C.

11.5 Future trends

Over the past few years the market for freshly prepared produce has risen sharply and is likely to continue to increase in future as there is a demand for convenient, ready prepared salads and vegetables. Effective decontamination treatments need to be in place to maintain product safety and quality. For the foreseeable future, the UK fresh produce industry is likely to continue to use hypochlorite as it is cost effective and has been used for many years for fresh produce decontamination. There is no single alternative that is emerging as a potential replacement for hypochlorite. It appears unlikely that any alternative will be used by the majority of the fresh produce industry, which is the case for hypochlorite where 76% of manufacturers routinely used this as a washing solution (Seymour 1999).

The move away from hypochlorite-based systems will require manufacturers to assess the efficacy of the proposed alternatives compared with the current hypochlorite systems. This is important as different alternatives may be more effective for different produce types. In order for such trials to be meaningful, they should be done using a standardised testing protocol as suggested in this chapter.

11.6 Sources of further information and advice

Two particularly useful reference sources are:

- USDA (2001). Analysis and evaluation of preventative control measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce, US food and Drug Administration Center for Food Safety and Applied Nutrition, September 30, 2004. www.jifsan.umd.edu
- Beuchat, L.R. (1998). *Surface Decontamination of Fruits and Vegetables Eaten Raw: A Review.* WHO, Food Safety Unit. WHO/FSF/FaS/98-2.

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12

Ozone decontamination of fresh fruit and vegetables

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12.1 Introduction

Intense consumer interest in nutritious and fresh foods in recent years has brought about changes in traditional eating habits. Fruit and vegetables have become popular and are widely consumed. Food service establishments have added fresh food options in the form of salads to their menus and may offer 30 or more items in the salad bars (Sawyer *et al*., 1985). Though nutritious, these fresh fruits and vegetables have been reported to harbor high levels of contaminants such as soil and dust, animal manure, moisture and spoilage microorganisms. Some of the spoilage microorganisms are pathogenic and have resulted in a worldwide increase in foodborne disease outbreaks (Beuchat, 1998; Burnett and Beuchat, 2001). Current disinfection treatments used for removing disease-causing pathogens from the surface of whole and ready to eat fruit and vegetables have been found to be partially effective (Graham, 1997). Although good manufacturing practices (GMPs) and a hazard analysis and critical control point (HACCP) approach can reduce the risk of contamination, there is a need to find effective methods to prevent diseases associated with raw produce. Ozonation has been used to purify water, in Europe, since 1906 (Graham, 2000). It is a novel technology whose potential can be exploited for decontamination of fresh produce. This chapter looks into the currently used fruit and vegetable decontamination methods and the potential of ozone use as an alternative owing to its enhanced disinfection mechanism.

12.2 Decontamination of fruit and vegetables

Microbial contamination of fruit and vegetables can occur at various stages from the farm to the table. Propagation of microorganisms occurs during growth in the field, harvesting, post-harvest handling and transportation, storage, and processing and marketing for human consumption (Cherry, 1999; Deza *et al*., 2003). The efficacy of disinfectants varies with the type of produce, surface morphology, infecting microorganism, microbial attachment and infiltration (Burnett and Beuchat, 2001).

Researchers have tried several methods including chemicals like chlorine, hydrogen peroxide, sodium hypochlorite, calcium hypochlorite and ethanol to control microbes on fresh or cut fruit and vegetables (Hoover, 1997; NACMCF, 1999; Fett, 2002). The use of organic acids, essential oils and natural preservatives extracted from plants has also been investigated (Wan *et al*., 1998; Rodgers *et al*., 2004). Marquenie *et al*. (2003) examined fungal development in strawberries treated with pulsed white light and UV-C illumination in conjunction with thermal treatment. *Escherichia coli* O157:H7 inoculated alfalfa seeds, at various seed layer thicknesses, treated with pulsed UV-light exhibited complete elimination of the pathogen though the viability of seeds treated up to 90 s was reduced (Sharma and Demirci, 2003b).

Deza *et al*. (2003) reported that rinsing fresh tomatoes inoculated with *E. coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* with neutral electrolyzed water (NEW) reduced an initial population of 5 $\log_{10}$ cfu cm^{-2} to less than 1 $\log_{10}$ cfu cm^{-2} irrespective of the strain and time of treatment. Izumi (1999) treated fresh-cut vegetables like carrots, bell peppers and potato with electrolyzed water at 15–50 ppm available chlorine and obtained a microbial reduction up to 2.6 $\log_{10}$ cfu g^{-1}. It was found that the extent of disinfection varied significantly with the type of product and method of treatment, such as rinsing, dipping and dipping/blowing. No discoloration of the treated samples was observed. In a study by Sharma and Demirci (2003a), soaking *E. coli* O157:H7 contaminated alfalfa seeds and sprouts in electrolyzed oxidizing (EO) water, at pH 2.6, resulted in reductions up to 1.6 and 2.72 $\log_{10}$ cfu g^{-1}, respectively. However, germination of the treated seeds reduced significantly with increase in free chlorine concentration and soaking time.

Chlorine concentrations of 50–200 ppm, currently used by the food industry to wash fresh produce, and 20 000 ppm, used for decontaminating seed sprouts, are also in many cases unable to prevent regrowth of pathogens such as *E. coli* O157:H7 and *Salmonella* (Cherry, 1999; NACMCF, 1999). Apart from low effectiveness, these interventions tend to form carcinogenic by-products such as trihalomethanes (Pontius, 1996) and cause taste and odor defects in treated products. This may be attributed to the inability of aqueous solutions to surpass the surface tension barrier and reach microbial cells in the deep crevices of rough fruit and vegetable sur-

faces, which in turn can contaminate the edible portion (Jaquette *et al*., 1996; Beuchat, 1998; Scouten and Beuchat, 2002). Establishing the best method for preparation and decontamination of a particular fruit or vegetable and achieving the Food and Drug Administration (FDA) mandated 5 log reduction in the microbial count of certain fresh fruits and vegetables, without affecting the sensory attributes (Cherry, 1999), can thus be challenging. It is therefore necessary to understand the mode of action of the various disinfectants and investigate alternative treatments such as ozone.

12.3 Ozone as a sanitizer

The increasing need for more sanitizers to control infection and disease concurrent with the need to reduce the accumulation of chemical residues to maintain safe air, water and food supplies, has created pressure on researchers to develop new methods, which are more effective and safe for food decontamination. Investigations on disinfection of foods, including the use of gaseous ozone to increase shelf-life and the use of ozone dissolved in water to sanitize surfaces of vegetables, fruit and other agricultural commodities, support the disinfection power of ozone (Graham, 1997, Kowalski *et al*., 1998; Kim *et al*., 1999a, b; Kim and Yousef, 2000). Compared to chlorine and hypochlorous acid (HOCl) the oxidation potential of ozone is higher by 1.5 and 3000 times, respectively (Suslow, 1998). High oxidation potential, ability to produce hydroxyl free radicals and decompose to oxygen, a non-toxic product, imparts strong antimicrobial characteristics to ozone (Rice, 1997). Ozone decays quickly in air and water (Horvath *et al*., 1985), thus, its use may be considered as a process rather than a food additive, with no safety concerns about consumption of residual ozone in food products (Graham, 2001).

Ozonation involves the on-site production of low concentrations of ozone gas from ambient air, by means of an ozone generator. The gas so produced is injected immediately into a water or gas stream where it dissolves (Pryor and Rice, 1999). Susceptibility of microorganisms to ozone varies with physiological state of the culture, pH of the medium, temperature, humidity and presence of additives like acids, surfactants and sugars (Hoigne and Bader, 1975; Castillo *et al*., 2003). Byun *et al*. (1998) exposed *E. coli* O157:H7 cultures on tryptic soy agar and in phosphate buffer to ozone concentrations between 3 and 18 ppm for up to 50 min. They reported that the microorganisms in buffer were more susceptible to ozone than those on agar. Relatively low concentrations of ozone and short contact times are sufficient to inactivate pure cultures of bacteria, molds, yeast, parasites and viruses in solution (Restaino *et al*., 1995; Kim *et al*., 1999b). However, in food the inactivation of microorganisms by ozone is governed by the nature and composition of food surface, type of microbial contamination, and the degree of attachment to or association of microorganisms

with food (Kim *et al*., 1999b). Presence of food may contribute organic matter, apart from the microbes being disinfected, which competes for ozone. Additionally, food may limit the accessibility of ozone to surface contaminants, such as those in the crevices of fruit and vegetables, thereby varying the inactivation kinetics of ozone with the treated commodity (Kim *et al*., 1999a).

12.3.1 Chemistry of ozone

The word ozone comes from a Greek word 'ozein' which means smell. Ozone is a bluish, slightly water-soluble gas with a distinctive smell (Graham, 2000). It is partially soluble in water and its solubility increases as the water temperature decreases. In nature, ozone is formed in the earth's atmosphere by the action of lightning or high energy ultraviolet rays. Oxygen molecules are ruptured, producing oxygen fragments which unite with other oxygen molecules to produce ozone, O_3 (Graham, 1997). In another process of ozone formation, oxygen floats upward into the atmosphere and in turn is converted into ozone by ultraviolet radiation.

Being an unstable gas, the half-life of ozone is about 20 min, depending on the temperature (Graham, 2000). Increase in temperature reduces the stability and disinfection efficiency of ozone in water (Camel and Bermond, 1998). In water, efficiency depends almost entirely on the amount of ozone-demanding material, such as organic matter. After reaction with organic matter, excess ozone reverts back to oxygen. Thus ozone has to be produced on-site.

12.3.2 Generation of ozone

Two methods most commonly used for the preparation of commercial ozone are corona discharge (CD) and ultraviolet light (UV). Both these methods are inspired by naturally occurring atmospheric phenomenon (Pryor and Rice, 1999).

The maximum concentration of ozone gas generated by UV light (at 185 nm) is 0.1% by weight using dry air as the feed gas. At this concentration, the maximum solubility in water at 25 °C is 0.35 mg/l. The solubility of ozone in water is a function of the partial pressure of ozone gas over the liquid surface. It follows Henry's law (Equation 12.1) and is limited by the equilibrium between the gas and the saturated solution of ozone in water. For increased concentration of ozone, the exposure time required increases:

$$P_{\text{gas}} = k_{\text{H}} c \qquad [12.1]$$

where P_{gas} = partial pressure of the gas, k_{H} = Henry's law constant (units depend on units used for pressure and concentration) and c = concentration of ozone in gas.

The concentration of ozone in water can be determined by the spectrophotometric method through substitution of values in the Beer–Lambert Law:

$$c = A/b * \varepsilon \qquad [12.2]$$

where c = concentration of ozone in water (ppm, $mg\,l^{-1}$), A = absorbance value at UV 258 nm, b = length of path of light = width of quartz cuvet (cm) and ε = molar absorptivity of ozone = $2900\,M^{-1}\,cm^{-1}$

Substituting values and conversion factors (48 000) balances the units to yield Equation 12.3:

$$c = \frac{A * 48\,000}{1 * 2900}\ \text{ppm} \qquad [12.3]$$

Considering the technical and economical factors, the CD method is preferred as it gives a higher ozone concentration of 1.5% by weight, in dry air and its power requirement is only 6–8 kilowatt hour (kWh) (compared to 44 kWh for the UV method) for every 454 g of ozone generated (Pryor and Rice, 1999). A corona is generated for ozone production by applying an electric current across two metallic electrodes separated by a dielectric insulator and an air gap. Ozone is produced by passing oxygen or air through the electric field wherein a certain percentage of the oxygen molecules dissociate and recombine as ozone.

Ozone generators produce greater concentrations of ozone gas with a slower flow rate. Small bubbles produced by a sparger when ozone is introduced into water provide an interface for ozone transfer to the water. However, this transfer occurs only on the surface of the bubble where ozone is in direct contact with water. Therefore large bubbles are less effective and tend to produce off-gas (Rich, 1994)

12.3.3 Mechanism of ozone disinfection

Susceptibility of organisms to biocides is a function of the product of dissolved ozone concentration and exposure time. Ozone owes its biocidal effectiveness to the direct lysing of cellular walls owing to breakdown of sulfydryl groups and increased permeability (Silva *et al.*, 1998). It readily oxidizes organic matter in bacterial membranes, which weakens the cell wall and leads to cell rupture, leading to almost immediate death of the cell caused by release of cellular material into the external environment. This is unlike the effect of chlorine and other oxidizing and non-oxidizing biocides, which must be transferred across the cell membrane where they interfere with either nuclear reproduction or enzymatic activity (Pryor and Rice, 1999). Figure 12.1 gives a schematic diagram of biocidal mechanisms of ozone and chlorine.

Free chlorine is highly effective, owing to its penetration characteristics but it reacts with salts, contaminants and so on in water depending on the

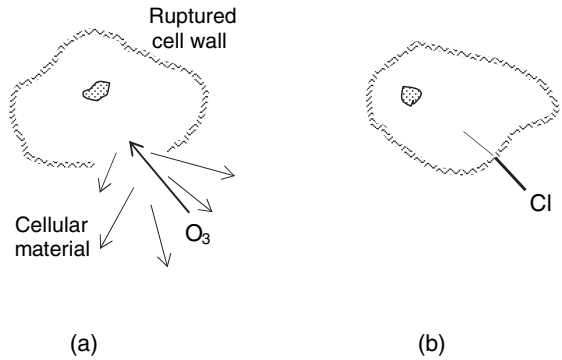

Fig. 12.1 Mechanism of action of ozone and chlorine as biocides (a) ozone and (b) chlorine (adapted from Pryor and Rice, 1999).

pH of water, thereby forming products, which are not so effective. Ozone does not react with water and the free electrical charge of cells does not reduce its effect. The critical concentration of ozone to kill germs is called the 'flash point'.

12.3.4 Use of ozone as a disinfectant for fruit and vegetables

Ozone has been used for the treatment of drinking water since the early 1900s. In the 1940s, ozone was introduced in the chemical industry for oxidation of chemicals. Initially, use of ozone was limited by lack of efficient, reliable and cost effective equipment, but there have been improvements with time. Over the years ozone has been used for treatment of municipal and bottled drinking water, municipal and industrial wastewater treatment allowing reuse, as a replacement for chlorine in bleaching of paper (it helps to give chloride free effluent), for swimming pool treatment, and so on (Camel and Bermond, 1998; Kim *et al*., 1999b; Pryor and Rice, 1999; Rice, 1999).

The declaration of ozone as 'generally recognized as safe' (GRAS) in mid-1997 opened doors for its use in the food industry (Rice, 1999). On June 26, 2001, the FDA approved safe use of ozone in gaseous or aqueous phase for antimicrobial treatment, storage and processing of foods (Federal Register, 2001). Potential applications in the food industry are in meat processing, disinfecting hatcheries and hatched eggs, poultry chiller water and poultry carcass disinfection, mold control, treatment of process water for recycle and re-use, cleansing of cold storage rooms and the extension of shelf-life of fruit, vegetables and fish. Kim *et al*. (1999b) have reported that ozone treatment decreased chemiluminescence, oxygen uptake, catalase and peroxidase activities and had a marked inhibitory effect on the growth of surface microorganisms.

Pseudomonas fluorescens population in a batch reactor reduced by 5 $\log_{10}$ within 30 s of treatment with 1.2 ppm ozone (Kim and Yousef, 2000). In the same study *E. coli* O157:H7 count decreased by 3.8 $\log_{10}$ and *Leuconostoc mesemteroides* count reduced by 3.3 $\log_{10}$ with 1 and 1.5 ppm ozone treatments. Kowalski *et al*. (1998) showed that *E. coli* and *Staphylococcus aureus*, when exposed to high concentrations of airborne ozone (between 300–1500 ppm), demonstrated high degrees of bacterial sterilization. The disinfection action of ozone in air was similar to that of ozone in water, although the threshold concentration at which ozone inactivates bacteria and virus in water is as low as 0.1 and 0.2 ppm.

Horvath *et al*. (1985) enlisted the uses of ozone in the food industry. They state that the use of ozone during ripening of cheese and subsequent storage destroys spores created on the surface, thereby increasing shelf-life. Ozone treatment also speeds up the aging of wine, avoids turbidity and refines its bouquet, which is retained for a longer time. The shelf-life of milk, bottled juices and soft drinks is also improved by ozone through suppression of sour spoilage.

In the treatment of lettuce with gaseous and aqueous ozone, Kim *et al*. (1999a) concluded that the most effective microbial kill for *Pseudomonas fluorescens* was obtained by bubbling gaseous ozone in water containing shredded lettuce. It was observed that high speed stirring increased the effectiveness of the treatment. A reduction of 3.9 and 4.6 $\log_{10}$ cfu g^{-1} was obtained, respectively, for mesophilic and psychrotrophic bacteria when the lettuce in water was bubbled with ozone for 5 min.

Achen and Yousef (2001) sparged apple wash water with ozone and obtained a 3.7 $\log_{10}$ cfu g^{-1} reduction in *E. coli* O157:H7 counts on the apple surface. The reduction was less than 1 $\log_{10}$ cfu g^{-1} in the stem and calyx region. Rodgers *et al*. (2004) compared the efficacy of ozone, chlorine solutions and peroxyacetic acid on produce (apples, strawberries, lettuce and cantaloupe) contaminated with *E. coli* O157:H7 and *L. monocytogenes*. They obtained the highest reduction in population with ozone. A comparison of the effectiveness of sodium hypochlorite solution, acidic electrolyzed water and ozonated water for inactivation of aerobic bacteria on lettuce showed that although ozone did not damage the surface structure of lettuce, its disinfectant effect was less than that of acidic electrolyzed water (Koseki *et al*., 2001).

The use of ozone treatment as an alternative to SO_2 fumigation to reduce post-harvest fungal decay of grapes showed promising results in a study by Sarig *et al*. (1996). Ozone concentration available for decontamination of the grapes, after degradation by organic matter, was approximately 0.1 mg l^{-1}. Populations of fungi, yeast and bacteria, naturally present on fruit surface, were considerably reduced by ozone exposure for 20 min. However, storage of strawberries under an ozone atmosphere for 3 days at 2 °C did not prevent fungal decay but increased the vitamin C content three times (Perez *et al*., 1999).

Apart from microbial decontamination, the use of ozone has also been investigated for removal of pesticides in fruits and vegetables (Hwang *et al*., 2001; Liu *et al*., 2003). Ozonation has been found to be effective in the degradation of pesticides such as malathion and captan in solution and mancozeb (Dithane 75 DF) on fresh apples.

Although several researchers have shown the potential of ozone for decontamination of fruit and vegetables, some studies indicate that improvements in the treatment methods are imperative to ensure food safety. Combination of ozone with other treatments such as thermal processing and hydrostatic pressure, or the use of wetting agents and oxidizing water as a pre- or post-treatment may improve the efficacy of decontamination by ozone.

12.4 Combination of ozone with other decontamination techniques

Sharma *et al*. (2002a) subjected alfalfa seeds, before sprouting, to treatment with ozonated water and continuous ozone sparging with and without subsequent exposure to heat. Heat treatment reduced *E. coli* O157:H7 populations on the seeds by $4.8\log_{10}\text{cfu}\,\text{g}^{-1}$ without significant reduction in percentage germination relative to a control treatment.

Weissinger and Beuchat (2000) found that combining aqueous sanitizers with a surfactant enhanced inactivation of *Salmonella* on alfalfa seeds by promoting access of the lethal agent to the bacterial cells. Pretreatment with a wetting agent prior to sparging with ozone for 3 min reduced the *E. coli* O157:H7 population on apples by $3.3\log_{10}\text{cfu}\,\text{g}^{-1}$ (Achen and Yousef, 2001). However, in a study on the decontamination of *E. coli* O157:H7 contaminated alfalfa seeds, addition of surfactants such as Tween 20 and 80 and SPAN 20 and 80 to the ozone sparged water did not increase the effectiveness of the treatment (Sharma *et al*. 2002b).

Application of pressure facilitates better penetration of the sanitizers into the inaccessible cracks and crevices of foods, thus enhancing microbial decontamination without compromising quality (Mazzoni *et al*., 2001). The main advantage of application of hydrostatic pressure includes uniform transmission of pressure, regardless of the size and shape of sample (Mussa, *et al*., 1999). The relatively limited research in this area, however, shows that improved methods of ozone delivery under pressure to achieve elimination of pathogens in fruit and vegetables are required (Sharma *et al*., 2002b).

The use of ozone in combination with initiators such as UV or H_2O_2 can result in advanced oxidation processes (AOPs) that are highly effective against the most resistant microorganisms; however, applications of AOPs in food are yet to be exploited (Khadre *et al*., 2001).

12.5 Drawbacks of using ozone

Ozone has strong disinfectant characteristics but may not be well suited for all applications. Owing to its short half-life it has to be generated on-site because of problems associated with storage and transportation. This increases the cost of treatment and the equipment complexity. Besides, certain drawbacks that are harmful to humans either directly or through food and cause deterioration of food properties, may limit its use. A few limitations to the use of ozone that have been investigated by researchers include but are not limited to instability and high oxidation power, potential to deteriorate food quality and possible human susceptibility caused by exposure.

12.5.1 Instability and oxidation power

Ozone is a highly unstable gas and can oxidize or ionize the matter being treated or decompose into oxygen and free radicals. It is corrosive and requires the use of corrosion-resistant materials such as stainless steel. The decomposition of ozone involves complex processes that are governed by the types of radicals formed in solution and various types of organic matter present in the medium, which induce, promote or inhibit radical chain reaction. Thus an accurate comparison of the effectiveness of ozone, at a certain concentration from different sources, on the inactivation of microorganisms cannot be generalized (Kim *et al*., 1999b). Besides, low ozone doses, which can inactive pure microbial cultures, may be ineffective against certain viruses, spores and cysts (EPA, 1999).

12.5.2 Deterioration of food quality

In a study of microbial decontamination of black pepper with ozone, Zhao and Cranston (1995) used ozone as a substitute for ethylene oxide. They carried out ozonized air sparging for 10 min at 6 l min^{-1} air flow rate and obtained a 3–4 $\log_{10}$ reduction in microbial population. Increasing the moisture content increased microbial reduction. However, oxidation of certain volatile oil constituents of ground black pepper occurred under the influence of ozone-treatment. The aroma quality of ozone-treated strawberries was reduced during storage owing to changes in sugar and organic acids (Perez et al., 1999). The changes may be attributed to inactivation of the sucrose to glucose and fructose degradation pathway in microbes by ozone induced oxidative stress. Kim *et al*. (1999b) cited changes in surface color of some fruits and vegetables like peaches and carrots; discoloration and undesirable odors in ozone treated meat; decreased ascorbic acid content in broccoli florets and thiamin content in wheat flour; and lipid oxidation affecting sensory quality in grains, ground spices, milk powder and fish cake.

However, the alterations in sensory attributes are based on the type of food, concentration of ozone used and other conditions such as contact time.

12.5.3 Human susceptibility

As per the Occupational Safety and Health Administration (OSHA), ozone in air at concentrations above 0.1 ppm has a very strong odor which causes irritation of the nose, throat and eyes. Exposure to high concentrations of ozone and off-gas may induce mutagenic defects and can even lead to death over longer periods. It is important to exercise caution when using ozone and to establish safe limits for treatment of food. Efficient systems for the detection and destruction of excess ozone are required. Workers in food processing facilities should wear respirators to avoid over exposure to ozone and follow GMPs and HACCP systems for increased safety.

12.6 Future prospects for ozone use in the food industry

Ozone is proving to be a more effective biocide than chemicals owing to its high reactivity and oxidizing power. It is, therefore, becoming popular in the decontamination of food products, especially fresh fruit and vegetables, in spite of its highly unstable nature. The FDA's affirmation of ozone as a GRAS in 1997 and its subsequent approval for use in food processing has led to significant research being directed towards development of efficient disinfection methods and systems. Nevertheless, identification of the most suitable applications and general implementation on the industrial scale has been sluggish (Majchrowicz, 1999). This may be due to the fact that ozone treatments involve relatively high capital investment and operational costs. Capital costs for aqueous ozone systems may vary from US$ 25 000–150 000 depending on size, while those for gaseous systems may be as high as US$ 250 000 (Majchrowicz, 1999). The operation and maintenance cost, including labor and replacement parts, for ozone disinfection has been estimated by the EPA (1999) to be US$ 18 500.

In order for ozone disinfection to become a commercially viable alternative to chemical decontamination of fresh whole or cut produce, it is necessary for investigations to be focused on the most suitable products and treatment combinations with UV light exposure, high hydrostatic pressure and sonication with particular emphasis on scale up of the treatment procedures. An in-depth study of the sensory attributes of ozone-treated fruit and vegetables is also needed. In conclusion, the future of ozone in fruit and vegetable processing may be envisioned to be a complete post-harvest operation that involves ozone for storage, removal of physical, chemical and microbial contaminants and recycling of wash waters for later batches.

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12.8 Useful contacts and information sources

The use of any decontamination technique requires in-depth knowledge and thorough investigation of the behavior of the microorganisms, the product being treated and the antimicrobial, as well as numerous other factors such as contamination sources and treatment conditions. Apart from a number of researchers and scientific institutions involved in investigating the use of ozone as a disinfectant for fruit and vegetables, some resources that are especially handy include the *Bad Bug Book* (FDA, 2003) which provides basic facts regarding foodborne pathogenic microorganisms and natural toxins. It is a comprehensive text with information from the Food & Drug Administration, the Centers for Disease Control & Prevention, the USDA Food Safety Inspection Service and the National Institutes of Health. Beuchat's (1998) WHO report on surface decontamination of fruits and vegetables is an informative review of the pathogen problem, available solutions and their drawbacks. The direct food additive petition by Graham (2000) is an extensive resource on the properties of ozone and its application in the food industry. It compiles results from various studies on the application of ozone for control of pathogens on meat, seafood, fruit and vegetable. It also describes various methods for ozone quantification and safety regulations associated with the use of ozone.

The application of ozone for the disinfection of foods is a relatively novel approach. New ozone disinfection methods are constantly being investigated and communicated to the scientific community as well as the public. Therefore, no single source can possibly gather this vast body of information and present it as a comprehensive resource. This chapter is an effort towards presenting the various aspects of the use of ozone for decontamination of fruit and vegetables, but is in no way ground-breaking.

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13

Irradiation of fresh fruit and vegetables

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13.1 Introduction

Food irradiation is a 'cold' process for preserving food and has been established as a safe and effective method of food processing and preservation after more than five decades of research and development.[1–11] Although for some time a consensus has been reached in the scientific community about the wholesomeness of gamma irradiation of food, the general public remains concerned about this technology even though a number of recent reports show increasing interest in food irradiation from government regulators and industry and decreasing apprehensiveness of the public.[1,12–14] Any food processing technology can be used only when it shows advantages in term of effectiveness, public health, cost, convenience, and so on over competing technologies. The small temperature increase, absence of residue and effectiveness of treatment of pre-packed food are the main advantages; the comparatively high cost and absence of complete consumer acceptance are the most prominent disadvantages of food irradiation technology.[15] The following beneficial results can be achieved without the production of radioactivity or toxic compounds and with no vital loss in the nutritional quality of the foodstuffs:[16,17] extension of the storage life; elimination or inactivation of parasites and the control of insect infestation at all stages of their life cycle; reduction or elimination of food spoilage and pathogenic organisms; prevention of sprouting of root vegetables, onions, garlic and other members of this plant family; delaying of fruit ripening; preservation of the freshness of food beyond the normal shelf-life at ambient temperature; improvement of sensory properties in some foods; reduction of the use of chemical preservatives, pesticides or fumigates and finally, reduction in the energy used for food preservation.

Food irradiation is not a new technology. It was first patented for food preservation in 1905 by two British scientists. Studies on the effects of X-rays on food and food constituents are numerous in the biomedical literature of the 1920s and 1930s. Research in this area was intensified when irradiation was considered as a means of providing safe food and artificial radioisotopes became available in the 1940s and 1950s.[18–23] Food irradiation was approved in 1963 for control of insects in wheat and wheat flour[24] in the United States and in the European Economic Community for irradiation of some specified foods in 1987.[25] Today, food irradiation is approved by more than 40 countries for more than 100 irradiation food items or groups of food for consumption.[16] This chapter will be presented under five different sections. After a brief summary, the action of ionizing radiation, sources of ionizing radiation, and the concepts of dose and different dose levels used in the radiation processing will be presented as part of this introduction; disinfestations, shelf-life extension, decontamination, and advantages and disadvantages of food irradiation with special emphasis on fruit and vegetable applications are included in the scope of irradiation; the current status of analytical methods used in the detection of irradiated fruits and vegetables will be covered under analytical detection methods; applications relative to fruits and vegetable preservation will be summarized in the fourth section and sources of further information and advice will be given in the fifth section.

13.1.1 Action of ionizing radiation

The word radiation refers to the whole electromagnetic spectrum as well as to all atomic and subatomic high-energy particles. Different types of radiation can be grouped in terms of ionizing and non-ionizing radiation. Non-ionizing radiation is electromagnetic radiation with a wavelength λ of about 1.0 nm or longer. Ionizing radiation covers the rest of the electromagnetic spectrum and all the atomic and subatomic high-energy particles. The word ionizing refers to the ability of radiation to ionize the atoms and molecules of the matter through which it passes.

Ionizing radiation penetrates matter to varying degrees depending on the nature of the matter and the characteristics of the radiation. The penetrating powers of the gamma and X-rays are greater than the penetrating power of all types of atomic and subatomic particles. The efficiency of radiation in producing radiation effects, however, depends on its ability to knock electrons out of atoms of the matter exposed to its action. Beta particles generally have a greater ability to produce ionization in matter than gamma rays.

Ionizing radiation interacts with the atoms and molecules of the irradiated material by transferring energy to electrons which gives rise to the creation of positive and negative ions.[26] The effects of radiation on biological materials can be classified as direct and indirect action. In direct action,

chemical events occur as a result of direct energy transfer from radiation to the target molecule, but in the case of indirect action, intermediates, such as the hydroxyl radical (OH^-), hydrated electron (e^-_{aq}), hydrogen atom, or hydrogen peroxide produced from the radiolysis of water are involved in the transfer of energy, and thus in the occurrence of chemical events.[27,28] While hydrogen peroxide and OH^- radical are strong oxidizing agents, the hydrogen radical is a strong reducing agent. The formation of ion pairs, free radicals which are parts of molecules, groups of atoms, or single atoms that possess an unpaired electron, the reaction of free radicals with other molecules, recombination of free radicals, and related physical and chemical phenomena provide the mechanisms by which microorganisms, enzymes and food constituents are altered in an irradiated material.

13.1.2 Sources of ionizing radiation

Ionizing radiation of electromagnetic and particulate origin is used in food irradiation. It includes gamma rays from natural radioactive elements and artificially induced radioactive isotopes produced in nuclear reactors, usually ^{60}Co, less commonly ^{137}Cs, X-rays generated from machine sources operating at or below 5 MeV, and electrons generated from accelerators operating at or below an energy level of 10 MeV.[29,30] Two gamma rays with average energies of 1.17 and 1.33 MeV are produced after the disintegration of ^{60}Co isotope. In food irradiation applications, electron and X-ray energies are restricted to be 10 and 5 MeV, respectively, to limit the extent of nuclear reaction capable of inducing radioactivity in irradiated product. The mean energy of ^{60}Co gamma radiation (1.25 MeV) is also below this limit. It is generally accepted that with a maximum energy of 10 MeV and normal trace heavy metal content, any radioactivity induced in irradiated foods is no greater than the natural radioactivity of the foodstuffs caused by ^{14}C and ^{40}K and, furthermore, the induced activity decays rapidly by a factor of 10 and 20 during the first 24 h following irradiation.[16,18,19,31]

The accelerated electrons may be used directly or can interact with a suitable target to produce a beam of high-energy photons. Such photons are generally termed X-rays. Gamma and X-rays are electromagnetic radiation, high-energy photons of the same nature as visible light but with higher energy. An essential difference between radioactive and machine sources is that a machine can be switched off when not required, while the process of radioactive decay cannot be arrested. The characteristics of three different radiation sources are summarized in Table 13.1.

Although different equipment can be used to produce the different ionizing radiation, the same chemical changes are produced by the different ionizing radiation. The power of penetration of the radiation and hence the dimensions and density of the food product being irradiated are the only practical differences in the evaluation of the radiation used in food processing.[32,33] ^{60}Co radioisotope is used in most commercial gamma

Table 13.1 Characteristics of irradiation sources

Radiation source	Characteristics
Cobalt-60	1. High penetrating power 2. Permanent radioactive source 3. High efficiency 4. Source replenishment needed 5. Low throughput
Electron beams	1. Low penetrating power 2. Switch-on switch-off capability 3. High efficiency 4. High throughput 5. Power and cooling needed 6. Technically complex
X-rays	1. High penetrating power 2. Switch-on switch-off capability 3. Low efficiency 4. High throughput 5. Power and cooling needed 6. Technically complex

Source: Kilcast, 1995.[32]

irradiation plants. ^{137}Ce from nuclear fuel is another radioisotope source although it is not used to any great extent at present.[34] ^{60}Co has a half-life of 5.26 years. To retain the rate of output of the plant, replacement of 12.3% of the activity is required each year. Therefore, continuous processing is desirable for the economic operation of plant with a continuously decaying source.

A beam of electrons at a very high power level can be obtained from commercial electron accelerators at electron energies (10 MeV) acceptable for food irradiation. However, electrons at these energies are only capable of limited penetration into food, so the material must be presented to the beam in a thin layer if it is to be treated in bulk, or all surfaces must be presented if a surface treatment is desired. Successful application of electron beam treatment is, therefore, limited by the thickness of the food to be treated and would be appropriate for topical use especially with fruits. The main advantages of machine sources are:[16]

- they can be switched off
- the electron beams can be directed over the packaged food to ensure an even dose distribution.

Total investment and per unit cost breakdown are approximately the same for electron beam and ^{60}Co and both these two sources require a large plant for economic viability.

An isotope source cannot be switched off and so it must be shielded. In order to make the maximum use of the continuous emission from radioisotope sources, it is necessary to box the source in with target material. Sources are contained within the processing area by the use of thick concrete walls and lead shielding. To prevent leakage of radiation, openings in the shielding for entry of product or personal, must be carefully constructed.[16] The distance of the food from the source which varies as the food moves, the bulkiness of the food and its heterogeneity are the important parameters to take into consideration for an acceptable irradiation process.

In comparison to an electron beam, gamma ray sources provide a relatively low dose rate. Therefore, irradiation generally requires a longer exposure time in order to provide a specified absorbed dose. However, electron beams produced by linear accelerators that are powered by electricity require considerably shorter exposure times, typically seconds or minutes.

13.1.3 Doses used in radiation processing

The biological effects of ionizing radiation depend both on the energy of incident radiation and on the amount of radiant energy transferred to the unit mass or volume of the target material. Energy received by the target material is called absorbed energy and in irradiation processing, it is described as the absorbed dose or simply the dose. Dose is measured in a gray (Gy) Unit, which is equal to the energy absorption of one joule per kilogram. Large doses are expressed in kilograys (kGy). The rad (1 rad = 0.01 Gy) was also widely used in the past. The extent to which irradiation affects the organoleptic quality of foods varies widely and depends on the chemical composition and physical structure of foods. Therefore, the radiation dose administered to a food depends on the resistance of the organisms present and the objective of the treatment. 15 kGy is the maximum recommended dose for food processing with the average dose not exceeding 10 kGy.[35–37]

Food irradiation processes can be categorized depending on the intention of the processing and the dose used. There is a thousand-fold difference between the dose needed to inhibit sprouting and that needed to kill all microorganisms. A survey of useful applications in the dose range up to 50 kGy is given in Table 13.2.

A dose of irradiation less than 0.1 kGy (very low doses) inhibits sprouting in potatoes, onions and garlic.[39,40] Low doses (below 1 kGy) are effective for disinfestation and also extend the shelf-life by delaying ripening, thus appearing to offer an acceptable technical substitute for the use of pesticides which are now banned or restricted in many countries.[41] Microbial and insect contamination of fruits and vegetables, which are highly susceptible to infestation, can also be reduced at these radiation doses. Deterioration of fresh fruits and vegetables can be delayed by treatment with low dose irradiation.[42] Mold growth on fruits such as strawberries and ripening

Table 13.2 Irradiation doses for food processing

Level	Function	Dose range (kGy)
Low	Inhibit sprouting (potato, onion, garlic)	0.05–0.15
	Delay ripening (fruit and vegetables)	0.10–1.00
	Eliminate insect infestation (grain, cereal products, dried and fresh fruit, dried fish)	0.20–1.00
	Prevent trichinosis	0.30–1.00
Medium	Eliminate spoilage organisms	1.00–3.00
	Eliminate parasites and pathogens (except viruses and spore formers)	3.00–8.00
High	Sterilization	25.00–50.00

Sources: adapted from Ehlermann, 1989[15] and Jones, 1992.[38]

of fruits and vegetables can be inhibited if they are irradiated at low doses. Foodborne diseases and food spoilage can be avoided, to a great extent, by killing or reducing populations of microorganisms in foods at still higher doses (medium doses) of irradiation. These doses are referred as pasteurizing doses. Pasteurization by radiation coupled with refrigeration can delay food spoilage of highly perishable meats and fish. Killing or reducing the number of food poisoning bacteria is likely to become one of the most important applications of irradiation. Sterilization, also referred to as radappertization, is performed at high radiation doses (25–50 kGy). It is designed to eliminate most if not all microorganisms in a food. Results are similar to those achieved when canning low acid food.

13.2 Scope of irradiation

Food preservation is imperative for life. Traditional preservation methods comprise smoking and drying (meat, fish), drying (fruits, herbs), protective storage (grain or seeds in jars, granaries or sloes) and salting (vegetables, such as whole green beans, olives, some meats and fish).[42] Heat sterilization, refrigeration, controlled atmosphere storage of fruits, freeze-drying and vacuum packing techniques are added to the traditional preservation methods. Preservation of foods by irradiation was added to these techniques at the beginning of the 20th century. The potential applications of irradiation can be summarized as: control of insects, reduction of microbial load, prolonging shelf-life and improvement of product quality.[43,44]

13.2.1 Control of insects (disinfestation)

Grains and tropical fruits may be infested with insects and larvae. They must be controlled. Chemicals are usually used for this purpose. Low doses,

below 3 kGy are effective in damaging insects, at various stages of development, that might be present on the food, thus irradiation can substitute for chemicals such as ethylene dibromide and methyl bromide, which are used for disinfestation. Irradiation was reported to damage the sexual viability of an insect or its ability to reach adulthood.[44] Doses in the range of 1–3 kGy presently requested by legislation are considered to be sufficient to kill the insects. Prevention of infestation of insects by radiation is the most promising application of food irradiation processing. It is frequently used as an effective quarantine treatment for control of fruit flies.

13.2.2 Reduction of microbial load

Controlling the common health hazards caused by foodborne diseases and reducing the microbial load are the major benefits of sterilization by radiation. The principal targets of the gamma rays, with respect to biochemical function, are nucleic acids and membrane lipids. The reactive intermediates produced after irradiating foods injure or destroy microorganisms by changing the structure of nucleic acids and cell membranes, thus interfering with metabolic enzyme activities and cell division. The reduction in cell numbers depends on the total dose of radiation received. Because of relative sizes of their DNA molecules, organisms differ in their sensitivity to radiation in correlation with their complexity. A simple guide is that the smaller and simpler the organism, the higher the dose of radiation that is needed to destroy it.[45] The most common and troublesome pathogenic bacteria are sensitive to irradiation and can be reliably inactivated by doses smaller than 10 kGy. The sensitivities of various microorganisms to irradiation are summarized in Table 13.3.

13.2.3 Prolonging shelf-life

The shelf-life of some foods such as potatoes, yams and garlic can be extended by inhibition of sprouting using relatively low radiation doses (0.05–0.15 kGy). Delaying the ripening and senescence of some fruits is another method used in the extension of shelf-life. This method is frequently used to extent shelf-life of some tropic fruits such as bananas, litchis, avocados, papayas and mangoes by irradiating these fruits at 0.12–0.75 kGy.[47] The shelf-life of beef, poultry and seafood is also prolonged by destroying spoiling microorganisms. The shelf-life of strawberries and tomatoes can be extended about two to three times when they are irradiated at low radiation doses. However, these foods should be ripe before irradiation because ripening and maturation of fruits and vegetables are impeded upon irradiation owing to inhibition of hormone production and interruption of the biochemical processes of cell division and growth.[45] For prolonged storage, nevertheless, a separate heat treatment is required to prevent the enzymic spoilage of fruits and vegetables entirely.

Table 13.3 Radiation dose (D) required to eliminate 90% of various bacterial populations (one log cycle reduction)

Microorganism	D (kGy)
Pathogenic bacteria	
Aeromonas hydrophila	0.04–3.40
Bacillus cereus (vegetative cells)	0.02–0.58
B. cereus (spores)	1.25–4.00
Campylobacter jejuni	0.08–0.32
Clostridium botulinum (spores)	0.41–3.20
Clostridium perfringens (vegetative cells)	0.29–0.85
Escherichia coli	0.23–0.45
E. coli O157:H7	0.24–0.47
Listeria monocytogenes	0.25–0.77
Salmonella	0.37–0.80
Staphylococcus aureus	0.26–0.45
Yersinia enterocolitica	0.04–0.39
Vibrio	0.08–0.44
Spoilage bacteria	
Clostridium sporogenes	2.30–10.90
Micrococcus radiodurans	12.70–14.10
Moraxella phenylpyruvica	0.63–0.88
Pseudomonas putida	0.08–0.11
Sporolactobacillus inulinus (spores)	2.10–2.58
S. inulinus (vegetative cells)	0.35–0.53
Streptococcus faecalis	0.65–070
Viruses	2.02–8.10

Source: Monk *et al*., 1995[30] and Radomyski *et al*., 1994.[46]

13.2.4 Improvement of product quality

The nutritional adequacy of irradiated foods is one of the most debated aspects of radiation-processed foods. Like other food processing techniques, irradiation creates an alteration in the nutrient composition of the treated food. Composition, radiation dose, processing temperature, presence or absence of air, storage conditions, packaging and so on are the principal factors affecting the nature and extent of these changes. A variety of stone fruits, citrus fruits and papaya irradiated at varying doses were found to preserve the sensory qualities. However, low-dose treatment of some fruits and vegetables caused softening and other problems, as in the case of irradiated grapefruits and oranges.[19] Nutrient losses in irradiated foods are in the same range as those caused by cooking, freezing and even just storage.[39] Thiamine, ascorbic acid and vitamins A and E are the most irradiation-sensitive vitamins.

Irradiation of fruits at a dose level of several kGy has been shown to increase the juice yield, that is, to improve product recovery. The gas pro-

duction factors in soybeans were observed to decrease markedly after a sequence of soaking, germination, irradiation and the subsequent drying process of the beans and this effect was found to be maximum at a dose of 7.5 kGy.[48] In general, at the doses tolerated by fruit tissues, the effects of irradiation on fruit quality are rather slight.

13.3 Advantages and limitations of food irradiation

Food irradiation has clear advantages as well as limitations, like any other method of food preservation. It is neither as wonderful as some backers claim nor as terrible as critics charge. Rahman[49] identified five different special advantages of food irradiation: minimal food losses, improved public health, increased international trade, an alternative to fumigation and increased energy savings.

Disinfestation and shelf-life extension improvements provided by irradiation can be used to control and minimize the post-harvest losses caused by insect infestation in grain, pulses and fruits. This point is more important for the developing countries where the losses during the post-harvest stage are high.[48] One estimate suggested that 30% of all food storage losses could be prevented by irradiation of foods. Food could be transported from the region where it is produced to where it is needed without suffering substantial losses from infestations.[19]

Many foods are contaminated with pathogenic microorganisms or parasites. Food irradiation is particularly effective in controlling foodborne spoilage microorganisms and decontaminates food from pathogenic bacteria, yeasts, molds and insects. This decontamination can improve the hygienic quality of the food and prevent potential health hazards and thus improve greatly public health concerns.

Fresh food crop losses caused by insect infestation, spoilage or limited shelf-life range from 10 to 30% in most subtropical countries and up to 50% in some cases.[50] This prevents the improvement of international trade in fresh foods produced in subtropical and tropical countries. Irradiation can be used to increase or improve international trade of fresh food by providing an effective quarantine procedure for infested or infected foods or prolonging shelf-life.[41,42,44,51]

Irradiation in the dose range 0.2–0.7 kGy has been demonstrated to be an effective alternative to fumigants like ethylene dibromide (EDB), methyl bromide (MB) and ethylene oxide, which are either prohibited or are being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons. Irradiation potentially reduces the contact of many foods and food ingredients with chemicals designed to prevent the growth of infesting microorganisms. Irradiation can replace or drastically reduce the use of food additives, fumigants and food preservatives, which generate potential hazards for the consumer as well as personnel in the food processing industry.[16,52]

The energy needed for irradiation preservation of foods is small compared with the energy required for canning, refrigeration and/or frozen storage. The total energy used for refrigerated raw, cut-up chicken is 17760 kJ kg^{-1}, for frozen raw cut-up chicken is 46600 kJ kg^{-1} (3–5 weeks frozen storage) and for canned chicken meat is 20180 kJ kg^{-1}. However, refrigerated and irradiated raw cut-up chicken requires a total energy of 17860 kJ kg^{-1}.[53] Thus, irradiation together with refrigeration could be used as a substitute for frozen storage for distribution of raw food where feasible with a substantial reduction in energy and cost.[41] Although the irradiation process has a low operating-cost and requires low energy, it demands high capital costs and a critical minimum capacity and product volume for economic operation.[54,55]

The food irradiation process is not suitable for all foods, but there is no preservation method that can be used for all foods. Organoleptic changes and off-flavor development occur above the threshold radiation doses recommended for treating food and elimination of all microorganisms or their toxins is not possible at low doses. However, some of the organoleptic alterations can be offset when the food is irradiated in the frozen state. Variability in effects leads to difficulty in standardizing the radiation processing.[56] Vitamin losses are also often mentioned as a serious disadvantage of irradiation. In fact, the losses caused by irradiation processing are, in most cases, not of nutritional significance and are smaller than the ones caused by various heating processes.[57] A clear disadvantage of irradiation compared to thermal processing is the lack of enzyme inhibition in foods, even at the high radiation doses required for commercial sterilization. One way to overcome these disadvantages and limitations is to use irradiation in combination with other methods of food preservation. Irradiation demonstrates its greatest potential in combination with other treatments.[12] Irradiation can be applied to foods in small packages or in bulk in a frozen state or at room temperature, with great versatility in applications, and it is the only preservation method available for inactivating pathogenic microorganisms such as *Salmonella* in frozen foods.[50,57] The greatest disadvantage of food irradiation is its name. Ionizing radiation evokes unpleasant associations of radioactivity, nuclear threats, high technology, genetic mutation and cancer.[16,57]

13.4 Effects of irradiation on fruits and vegetables

Irradiation may produce changes in physical properties such as electrical impedance, viscosity and wetability; in chemical properties such as changes in proteins, lipids, carbohydrates, nucleic acids, vitamins and volatiles; in histological or morphological characteristics and microflora of foods exposed to radiation. Effects of irradiation on foods vary with the food type and the dose applied.

13.4.1 Changes in physical properties

Radiation may induce changes in the ion transport characteristics of cell membrane, which results in changes in the electrical conductivity of irradiated foods. Thus, measurements of electrical impedance and/or conductivity might serve for integral characterization of unirradiated and irradiated tissues and cells. The electric impedance measurement method has been used in the literature to characterize irradiated potatoes.[58–60] Membrane permeability is another important parameter for controlled solvent transport into the cells. Changes produced by irradiation in the cell membrane permeability are expected to produce changes in the viscosity of homogenates and suspensions of irradiated biological materials. The viscosity measurement method has been used to determine doses received by irradiated spices, condiments[61–64] and onion powder.[65]

13.4.2 Changes in chemical properties

Many factors influence the type and extent of chemical changes that may occur as a result of irradiation. The chemical complexity of foods, in general, makes a precise prediction of changes produced by irradiation difficult. However, irradiation causes chemical changes in foods in amounts directly related to the radiation dose. Based upon extensive studies, several general statements about these effects on foods can be made. The types of reactions induced by radiation are oxidation of metals and ions, oxidation and reduction of carbonyls to and from hydroxyl derivatives, elimination of unsaturation in double bonds, decrease of aromacity of aromatic and heterocyclic compounds and hydroxylation of aromatic and heterocyclic compounds.[66]

Changes in proteins

Molecular uncoiling, coagulation, unfolding and even molecular cleavage and splitting of amino acids may occur at the doses that cause sprout inhibition and inhibition of ripening. The principal effects of radiation on protein molecules have been reported as being concentrated on sulfur linkage and hydrogen bonds rather than peptide linkage.[67] The carbonyl groups of peptide bonds are expected to be far more stable to the presence of free radicals produced after irradiation of foods than are the carbonyl groups in the ester linkage of acryl glycerol or the glycosidic linkages between sugar units in polysaccharides.[19] Thus, more peptide bonds are expected to remain unbroken following irradiation up to at least 10 kGy. However, the side chains of amino acid residues within protein molecules are not necessarily as stable as peptide bonds. Therefore, selective protein damage would be expected to take place in irradiated foods owing to the different susceptibilities of some amino acid residues to irradiation. According to Desrosier and Desrosier,[68] ionizing radiation attacks directly and/or indirectly amino acid side chain bonds in the following sequential order: – S – CH_3, – SH, imidazol, indol, alpha-amino, peptide and proline.

Increases in the levels of glycine, leucine, tyrosine and phenylalanine upon irradiation at 10 kGy were reported to be the cause of the observed increase in the amount of total free amino acids.[67] Protein unfolding produced by radiation is believed to cause the creation of more reactive sites in foods.

Swallow[69] reported the effects of irradiation on food proteins. Interaction of irradiation with food matter gives rise to hydroxyl radicals, hydrated electrons and other species. These, in turn, react with protein producing protein free radicals. Some protein radicals are also formed by direct action and through radiolysis of fat. At higher dose levels, cleavage of the sulfydryl group from sulfur amino acids in proteins causes changes in the aroma and taste of foods. Enzymes in foods are relatively resistant to radiolysis.[69] Destruction of many food enzymes requires five to ten times the irradiation dosage needed to inactivate microorganisms.[70]

Changes in carbohydrates

Irradiation can depolymerize high molecular weight carbohydrates by breaking them into small units.[71] This process is important in the breakdown of cell wall materials such as pectin leading to the softening of fruits and vegetables. Although carbohydrates are hydrolyzed and oxidized to simpler compounds, there is no change in the degree of their utilization and hence no reduction in nutritional value. The physical or chemical changes produced in sugars at an irradiation dose less than 1 kGy were reported to be small and less extensive than changes observed following thermal treatment.[72] Water exerts a protective effect when sugars are irradiated in the solid state. Sugars in many fruits undergo little change upon irradiation owing to the fact that the fruits rich in sugars also contain a large quantity of water. Increases in the initial levels of water-soluble reducing sugars have been observed in irradiated fruits compared to those of untreated ones.[73] Such an overall increase in initial total reducing sugars was reported to result from degradation of starch.

Carbohydrate free radicals are produced in irradiated foods and propagation of free radical reactions is intensified by low moisture content. The carbohydrate free radicals created by irradiation initiate molecular changes in starches. Degradation of starch molecules is higher at high irradiation doses leading to decreased viscosity and increased water solubility of the starch. High-dose irradiation levels did not affect carbohydrate availability in representative fruits and vegetables.[74] Carbohydrate even protected certain labile amino acids such as cysteine that might be present.[75]

Changes in lipids

Nawar[76] reported that the effects of irradiation on the lipid fraction in foods are qualitatively similar to irradiation of natural fats or model systems analogous to fats. The normal process of auto-oxidation of fats that gives rise to rancid off-flavors is initiated and promoted by radiation in irradiated

foods. More unsaturated fats are expected to oxidize more readily than less unsaturated fats. The presence of oxygen plays a crucial role in the formation of this process, thus it can be slowed down by elimination of oxygen by appropriate techniques. Radiolytic decomposition occurs via a preferential break at the level of the carbonyl function of the double bond,[77] a process especially pronounced in unsaturated fatty acids. The decomposition in fatty acid molecules gives rise to the formation of some volatile compounds responsible for off-odors.[78] Lipid peroxide formation and breakdown continue in both unirradiated and irradiated foods during the storage period. The rates of peroxide formation early in this storage period depend on the dose, the dose rate and the temperature.[19] The complete absence of both water and oxygen would be necessary to minimize free radical chain reactions with the double bonds of mono-unsaturated and polyunsaturated fatty acids. Since neither water nor oxygen can be eliminated completely from any food intended for human consumption, it is not possible to suppress the irreversible decreases in unsaturated bonds of fatty acids in any food at any dose and dose rate foreseen for commercial food irradiation. The formation mechanisms of peroxides and volatile compounds and the development of off-flavors have been reported in the literature.[76]

Crude lipid and phospholipid concentrations have been reported to decrease in irradiated potatoes.[74] Changes in glyceride content as well as fatty acid composition of the fruit pulps were found to accompany the ripening of both control and irradiated fruits, although the rate of change was relatively slower in the irradiated fruits. The slower change was attributed to a delay in ripening of irradiated fruits.[79]

Changes in vitamins

There is conflicting evidence regarding the effect of irradiation on vitamins as many studies have been performed in vitamin solutions, which show greater losses than those found in the heterogeneous mixtures of compounds in foods.[45] Vitamins have different sensitivities towards irradiation. Water-soluble thiamine and ascorbic acid and fat-soluble vitamins E and K are the most sensitive.[40] The extent of thiamine losses was reported to depend on the type of food, its physical state and the temperature during irradiation, the degree of exclusion of air and the irradiation dose and dose rate in the dose range 3–10 kGy.

Many studies have shown that irradiating fruits and vegetables causes a substantial or total depletion of their vitamin C content.[2,19,80] Losses may vary from 1 to 95% depending on the fruit and vegetables, specific cultivars, dose, dose rate, exclusion of air, temperature and time elapsed after irradiation. The low doses required for killing or reducing populations of microorganisms and sprout inhibition leave losses of vitamin C in the range of 1–20%, but irradiated potatoes were reported to exhibit lesser losses upon storage than unirradiated controls.[38,81] The losses in vitamin C content

of lemons have been reported to increase with an increase in irradiation dose and still more severely with the time elapsed after irradiation.[82]

13.5 Analytical detection methods for irradiated fruits and vegetables

Detecting irradiated foodstuffs is a fundamental aspect of food irradiation technology. There has been considerable research since the late 1980s in developing and validating a series of reliable detection methods that can be used to distinguish irradiated foods from unirradiated ones. This is essential in order to inform consumers, control domestic and international trade in irradiated food and reassure the public that consumer rights are protected. However, detection of irradiated food is not a trivial problem, because irradiation produces no major chemical, physical or sensory changes in foods at commercial irradiation doses. Furthermore, these changes may be similar to changes produced by other means of food preservation. Therefore, detection methods are focused on minute changes in chemical composition and on physical or biological changes specific to irradiation occurring in irradiated foods. The current status of analytical detection methods for irradiated foods has been discussed in different publications.[53,83–92] Detection methods are based on the determination of the products formed by irradiation, on physical changes such as cell membrane damage or in determining the ratio of live/dead bacteria.[45] At present there is no universal method for identifying all irradiated foodstuffs, but useful detection methods are being developed for specific foods. The following methods are used in the detection of irradiated foods, particularly in the detection of irradiated fruits and vegetables: electron spin resonance (ESR), viscosity, thermoluminescence (TL), lyoluminescence (LL), conductivity, and chemical analysis of volatiles, microflora and DNA molecular composition. Analytical methods used for the detection of irradiated fruit and vegetables and their current status are summarized in Table 13.4. In this section, brief information on the leading analytical methods used in the detection of irradiated fruits and vegetables will be presented.

13.5.1 Electron spin resonance spectroscopy

Electron spin resonance (ESR) is a sensitive and accurate technique used in the detection of species with unpaired electrons. Free radicals, specific for irradiation, are produced in irradiated foods, but these are generally very short-lived species; thus, their use in identification of irradiated foods is difficult. However, radical species trapped in hard and relatively dry components of food are stable enough to be used in the detection of irradiated foods by ESR spectroscopy. Another difficulty is the registration of an ESR signal for non-irradiated food owing to the existence of naturally occurring

Table 13.4 Analytical detection methods and their current status for irradiation treatment of fruits and vegetables

Method	Fruits		Vegetables
	Fresh	Dried	
Electron spin resonance (ESR)	A, B	A	A, B
Thermoluminescence (TL)	A, B	C	A, B
Photostimulated luminescence (PSL)	D, B	D, B	D, B
Hydrocarbons	E, B	C, B	C, B
Cyclobutanones	D	C	C, B
Immunological:			
1. cyclobutanones	D	C	C
2. DNA	C	C	C
3. protein	C, B	C, B	C
DNA-Comet	F	C	F
-MtDNA	F	C	F
Microbiological	G	C	G
Impedance	G	G	H, G
Germination	D	G	D

Source: McMurray *et al*., 1996.[82]
A, research advanced; B, applicable to certain types; C, applicable but not tested;
D, research underway; E, protocol under evaluation; F, preliminary data;
G, not applicable; H, intercomparison in progress.

magnetic species. It has been found, however, that in fruits and vegetables the intensity of native ESR signals increases after irradiation. The growth of ESR signal intensity measured just after irradiation is more significant than that measured after several days of storage. More than 30 varieties of fresh fruit have been tested by Stachowicz *et al*.[93] and they found that only seeds separated from pear, orange and grapefruit irradiated at 3 kGy exhibited single line ESR spectra with an intensity about twice as great as that obtained for non-irradiated samples. Observation of the growth effect of irradiation on the signal intensity even after storage for 3 months was suggested, by these authors, as a potential test to prove the irradiation of the fore-mentioned fruits.[94] It was concluded, however, that radical species produced in the studied fruits giving rise to an ESR singlet are not specific to irradiation, therefore the ESR technique can be only used to confirm the results obtained by other detection techniques not credible enough on their own in relation to the studied foods. An ESR triplet with a strong central and two weak satellite lines was observed for achenes separated from strawberries irradiated in the dose range 1.5–3.0 kGy. Satellite and central lines were attributed to cellulosic component of achenes and to native radical species of unknown origin, respectively.

The most promising results relative to the detection of irradiated foods by ESR spectroscopy have been reported[94] for pressed dates and figs

irradiated in the dose range 0.5–2.0 kGy. Relatively stable multi-component ESR spectra were registered for seeds separated from irradiated dates and figs.

Glidewell *et al.*[95] have reported on irradiated (3 kGy) fresh and frozen strawberries, fresh blackcurrants (various cultivars) and the seeds of strawberries, raspberries and blackcurrants. They observed an ESR triplet with two weak satellites and one intense central line and focused their interest on satellite lines specific for irradiation. These lines were found to decay relatively fast with the decay rates depending on the type of species, but providing the possibility of distinguishing irradiated fruits from non-irradiated ones.

Raffi and his co-workers[96–99] reported on ESR identification of many fresh and dried fruits and vegetables and concluded that ESR spectroscopy was not suitable for identification of irradiated fresh fruits owing to the unstable nature of radicals induced in pulp, but that ESR can be used with dried fruits or dry components such as achenes, pips or stones. The results relative to dried grapes (raisins) and papaya were found to be conclusive. The lowest detectable doses were determined to be 0.12 kGy for grapes and 0.35 kGy for papaya, which were considered to be a guarantee of a high success rate for the detection of these foods.

ESR spectroscopy has been accepted by the Codex Committee on Methods of Analysis and Sampling as general Codex method [EN 13708:2001][100] for the detection of irradiated foods containing crystalline sugar based on two interlaboratory tests, one with dried papayas and raisins[101,102] and another one with dried mangoes and dried figs.[103]

13.5.2 Thermoluminescence method

Thermoluminescence (TL) is the emission of light upon release of radiation-induced charge carriers from their traps in the mineral parts of foods by heating. Thermoluminescence is a radiation-specific phenomenon and it is an established method in radiation dosimetry. After identification of the source of light, it was concluded that irradiation treatment of all foods containing minerals, which can be either as inherent parts of foods or as adhering contaminants might be identified by TL analysis. Although many irradiated foods could be identified by the whole-sample technique, measurement has to be performed on isolated minerals if the food item is contaminated by low amounts of minerals. When the whole sample or isolated minerals are heated in a controlled way, the stored energy is released as light and measured by a sensitive photocounter.

The TL method had been successfully tested in interlaboratory blind trials on spices, aromatic herbs, their mixtures and shrimps up to 1996 and the European Standard [EN 1788:1996][104] has been established. At an early stage, it had already been observed that increased TL could also be measured on the surface of irradiated fruits.[105] Wagner *et al.*[106] reported their

experimental TL results obtained for minerals isolated from irradiated strawberries and mushrooms and concluded that separate TL ratios from non-irradiated and irradiated samples could be clearly achievable. Studies performed by Schreiber *et al*.[107] have shown that potatoes and onions irradiated even at sprout inhibition doses could be detected by TL analysis. Additional interlaboratory trials were initiated to validate thermoluminescence methods for detecting irradiated potatoes,[108] fresh fruits and vegetables.[109] An international comparison based on the study of dehydrated carrots, onions, leek, asparagus and apple was also conducted to extend the applicability of the TL method, as described in the standard [EN 1788: 1996],[104] to dried fruits and vegetables. The result of this collaborative study has indicated that irradiation treatment of the fore-mentioned fruits and vegetables may be identified during the whole shelf-life of the food investigated. Thus, the applicability of the TL method has been extended to cover dried fruits and vegetables. TL detection of irradiated foods can be applied to fruits and vegetables irradiated in the dose range 100 Gy to 2–3 kGy.

TL is a powerful method for detecting irradiated foods, but it is laborious, expensive and strict procedures are needed to prevent contamination by dust in the laboratory. A recent advance in the method is the use of light instead of using heat to release the stored energy. This new technique adopted from TL, called photostimulated luminescence (PSL), does not require isolation of minerals. A small sample of food can be used directly to obtain a result within a few minutes.

13.5.3 Impedance measurement method

Another promising physical method used in the detection of irradiated foods is based on impedance measurement. Irradiation creates changes in the cellular membrane of food, which results in changes in the electrical conductivity or impedance of irradiated foods. Measurements performed on the electrical impedance of non-irradiated and irradiated potatoes indicate an increase in the impedance of irradiated samples.[60] However, the impedance ratio was found to scatter to some extent within one lot of potatoes because of the difficulties encountered in controlling impedance measurement conditions. Investigations taking into consideration all parameters that have the potential to affect the results seem to be needed. An intercomparison study is in progress in this respect.

13.5.4 Chemical and biological methods

2-Alkylcyclobutanones are produced in fatty acids-containing food upon irradiation, but are not found as a result of other degradative processes. They are used as positive markers to indicate that a food has been irradiated. This test has been used, in recent years, to detect irradiated fruits such as mangoes, papayas and avocados.[110] Studies carried out by El-Dien and

Farag[111] on volatile hydrocarbon products extracted from the flesh of avocados, seeds of papayas and mangoes and kernels of apricots have shown that C17:1, C16:2, C15:0 and C14:1 could be used a markers for avocados, papayas and mangoes irradiated at doses of 0.75, 1.5 and 3 kGy, respectively, while C15:0 and C14:1 appeared to be the potential markers for apricots irradiated at a dose of 0.5 kGy.

Detection of irradiation by DNA analysis is a promising and widely applicable method. However, the changes induced in the DNA of irradiated food are not specific and therefore more research is needed to obtain reliable results, especially in the case of irradiated fruits and vegetables. Irradiation induces changes in the microflora of food, but these changes are very much influenced by pre- and post-harvest conditions. Thus, it is not often easy to obtain reliable results from microflora analysis. Kawamura *et al.*[112] reported the results of their studies on the inhibition of seed germination for grapefruit and proposed use of this method as an indication of exposure to radiation for fruits.

13.6 Some specific applications of irradiation in fruits and vegetables

Fruits and vegetables are the parts of plants that are consumed as foods. Fruits are the reproductive organs containing high sugar and acid together with aromatic flavors. On the other hand, vegetables comprise roots, tubers, bulbs, stems and leaves with lower sugar content and aroma. Although, the water content of fruits and vegetables is high (75–95%), their fat and protein contents are low. Therefore, some may need special treatment owing to their delicate nature.[113] Many processing methods can be applied to both fruits and vegetables.

Fruits and vegetables, which are often eaten raw, without the benefit of any microorganism killing step, can be contaminated by various microorganisms including bacteria or parasites. These microorganisms may cause food losses and foodborne diseases. The problem of contamination of fruits and vegetables is more important in developing countries owing to possible use of contaminated water for irrigation or of manure as fertilizer.[114] Contaminated produce is the vehicle for transmission of pathogens. An increase in international trade in these products may, in the future, create serious increases in the number of produce-associated foodborne diseases.

Plant tissues can be damaged quite easily by irradiation. The extent and nature of the damage depend on the type of produce, the condition of the produce and the dosage of irradiation. For some produce, there may be a dosage that will kill some pathogens without causing noticeable damage to the produce itself. For other produce, it may not be possible to use irradiation to promote food safety because the produce would suffer unacceptable damage in the process. Damage in the form of fruit softening, wilting

of leaf vegetables, irregular ripening and increased susceptibility to plant pathogens may occur subsequent to treatment with irradiation.

The purpose of most previous research studies associated with irradiation of fruits and vegetables was to alter rates of ripening, controlling post-harvest pathogens and disinfestation. These studies provided data on the radiation doses that these products could tolerate and also on the doses required to inactivate plant pathogens. Irradiation is an effective method used to achieve partial or complete inactivation of cells of specific pathogens or of potential spoilage microorganisms that may be naturally present on unprocessed foods. However, the use of combination treatment is expected to be more effective and suitable both in eliminating pathogens and in retaining the quality attributes of the product.[115] A summary of the applications relevant to the irradiation preservation of fruits and vegetables will be presented in the next subsection.

13.6.1 Fruit

Temperate fruits

Radiation preservation of cherries, blueberries and cranberries has been reported by Eaton *et al.*[116] who found that the post-harvest shelf-life of these fruits could be extended by low dose irradiation. Miller and McDonald[117] reported the use of a low dose electron beam to treat two blueberry varieties and gamma irradiation to treat another two blueberry varieties. Although, the shelf-life of electron beam-treated blueberries stored at 1 °C increased as the irradiation dose increased from 0.25 to 1.0 kGy, the firmness, flavor and texture decreased, but weight loss, decay, peel color, total soluble solids and titratable acidity remained unchanged. Minimal changes were observed in these quality factors at the dosage levels required for commercial application for quarantine disinfestations, that is, in the dose range 0.5–1.0 kGy, for blueberries irradiated by gamma radiation. Irradiation at or below 0.75 kGy was found to be not detrimental to the post-harvest quality of berries. Thus, treatment by irradiation was proposed as an effective alternative quarantine method to methyl bromide.[117]

The strawberry is one of the most studied fruits with respect to application of gamma irradiation. Couture and Willemot[118] and Brecht *et al.*[119] have reported beneficial effects of low-dose irradiation and storage under a modified atmosphere for strawberries. Radiation doses of 1, 2 and 3 kGy were reported to effectively prolong the shelf-life of strawberries stored at 4 °C by 5, 13 and 16 days, respectively.[120] A dose of 2 kGy seems to be the optimal dose of irradiation in air for pre-cooled, ripe strawberries to reduce fungal infection without significant quality changes.[8] The firmness of the strawberry was reported to decrease at high doses and softening dose limits depended on the cultivar.[121] Pectic substances were found to be changed in irradiated strawberries associated with irradiation-induced texture

changes.[122] Sweetness was reported to be enhanced in irradiated strawberries in comparison with an unirradiated sample owing to a reduction in titratable acidity.[123] The depolymerizations of starch and cellulose were suggested to be the cause of the increase in sugar content.[71]

Tropical fruits

Numerous reports indicated that preservation of tropical fruits would greatly benefit from treatment with ionizing radiation. Gamma irradiation at low dose levels has been shown to improve post-harvest shelf-life of bananas, mangoes and papayas by delaying the ripening process. However, improvement depends on the degree of maturity. According to Akamine and Moy[47] the optimum dose for inhibition of ripening is 0.75 kGy for three-quarter ripe fruits at room temperature. Low dose levels have been found to be effective for insect infestation; thus, irradiation treatment was proposed as a quarantine treatment against fruit flies and mango stone weevil.[73] A delay in ripening of green bananas up to 10 to 12 days has been reported to be possible at an irradiation dose of 0.2 kGy,[124] with minimal changes in pulp texture and vitamin C losses lower than the controls kept under the same storage conditions. Retardation of banana yellowing at a dose of 0.2 kGy has also been reported by Ferguson *et al.*[125] in an earlier work. Thomas *et al.*[126] reported shelf-life extension up to 10 to 12 days for some banana varieties and they showed that fruit maturity at harvest influences the irradiation-induced ripening delay. The maximum effect in terms of ripening delay was found to occur in fruits with low maturities. Grandison[127] reported a reduced rate of respiration and delay of up to three days in ripening of bananas exposed to 0.1–0.3 kGy in an accelerator. Higher doses were found to produce browning of the skin, splitting of fruit and loss of peel texture and vitamin C.

Several studies on irradiation preservation have shown that the shelf-life of mango fruit can be prolonged using a low radiation dose through slowing down rates of ripening and senescence. Although mangoes are considered to be highly tolerant to radiation, the susceptibility to radiation injury varies greatly with the origin of the fruit.[128] Combining irradiation with a mild heat treatment by hot water dip has also been reported to have a synergistic effect on increased shelf-life of mangoes of up to 32 days without affecting nutritional quality.[121,129,130] The effects of irradiation alone and a combination of hot water dip treatment plus irradiation on the storage, sensory, nutritional characteristics and biochemical changes in mangoes have been reported in detail in the literature.[1,131] Based on the results of these studies it was concluded that irradiation alone causes decreases in the respiration rate and color development and increases in fruit softening, but does not significantly affect the sensorial quality of mangoes.[129,132,133]

Irradiated Hawaiian papaya has been shown to soften more uniformly than controls.[134] Softening was observed to take place at a slower rate for fruit irradiated at the 30% yellow stage. Significant changes in skin and pulp

color were not found in fruit exposed to low dose gamma irradiation at the 15–30% yellow stage. A dose of 0.75 kGy was found to delay softening by more than 2 days.[135] The maximum dose that could be tolerated by fruit in the color turning stage was found to be 0.1 kGy above which fruits developed surface darkening. Distribution of flavor and aroma and breakdown in tissue are not seen up to 4 and 5 kGy, respectively. Hot-water treated fruits irradiated at 0.5 and 0.75 kGy doses have been found to show good decay characteristics and extended shelf-life at room temperature by 3 days over the controls owing to delayed ripening and senescence.[136] Akamine and Goo[137] demonstrated that the shelf-life of combination treated papayas can be extended further by storing under a controlled atmosphere.

Subtropical fruits

The data reported up to 1996 relative to the application of gamma radiation to preservation of subtropical fruits such as citrus, grapes and avocados were reviewed by Thomas.[138] Several investigations were undertaken to improve storage conditions of citrus fruits, that is, oranges, lemons and grapefruits. Irradiation studies have pointed out that citrus fruits are fairly sensitive to gamma irradiation. However, the control of the parameters such as dose, dose rate, storage temperature, degree of ripeness, variety of fruit and processing conditions have been reported[139–141] to be very important in optimizing the effectiveness of ionizing treatment. Earlier studies on citrus and grapes were directed towards the possible use of ionizing radiation as a replacement for or supplement to chemical fungicides for controlling storage decay caused by fungal pathogens.[138] However, most of these studies were of limited success owing to the fact that the levels of radiation needed for the satisfactory control of the fungal pathogens were often injurious to the commodity. The treatment of Florida grapefruit with gamma radiation at a dose of 0.3 kGy has been reported[142,143] to delay ripening and increase fruit firmness without damaging fruit quality. Murray[19] reported perceptible changes in aroma and texture, as well as an increase in the number of brown blemishes in the skins of irradiated grapefruits and oranges after 4–6 weeks. Undesirable changes observed in irradiated citrus are mainly tissue softening and enzymatic browning.[144] Tissue softening is expected to result from partial depolymerization of cell wall polysaccharides, mainly cellulose and pectin[71] and from damage of cell membranes.[145] Low dose irradiation of Australian lemon and mandarin has been reported[146] to cause small changes in soluble solids, pH, pulp color, vitamin C, organic acid and sugar. The effects of irradiation, combined with hot water, on the physical, chemical and organoleptic properties of tangerines have been reported by Jobin *et al.*[147] They observed that the appearance, texture, flavor, pH, color and soluble solid content of the pulp were not affected by up to 14 days of storage, but a loss of firmness of the skin was found to occur. O'Mahony *et al.*[148] studied navel oranges irradiated at low post-harvest doses (0.6–0.8 kGy) under controlled atmosphere and compared their results with

those obtained for controls. They concluded that the greatest differences were observed in the degree of blemishing together with smaller differences in flavor, odor color, texture and ease of peeling. Recently, Mahrouz *et al.*[149] reported on the effects of irradiation, cold-water washing and waxing treatments on the shelf-life extension of a late variety of Moroccan *Citrus clementina* (Nour). They found that washing and waxing treatments did not improve the quality of irradiated *C. clementina*, but rather resulted in yellower peels, peel injury and reduction of vitamin C content, acidity and soluble solids. However, irradiation alone was found to enhance significantly the levels of vitamin C and the total phenolic content and to maintain the color of the *C. clementina* during the entire storage period (49 days at 3 °C). Irradiation has also been used[150,151] for disinfestation of citrus fruits and it was concluded that gamma irradiation at dose levels non-injurious to most citrus fruits can be substituted for chemical fumigation as a quarantine treatment against fruit flies.

Shirzad and Langerak[152] reported on the efficiency of gamma irradiation in combination with SO_2 and/or heat treatment on the shelf-life of black Alicante table grapes. Their results showed that irradiation at 0.1 kGy + 0.25% SO_2 controlled molding for a storage period of 40 days, as did a dose of 0.2 kGy. The influence of gamma irradiation and heat-radiation combination treatments on decay and quality of three different seedless grape cultivars has been reported by Thomas *et al.*[153] under different storage regimes. They concluded that gamma irradiation had potential as an alternative to sulfur dioxide fumigation for decay control during shipping and storage. Dimitrov *et al.*[154] reported that doses of 0.2–0.3 kGy did not cause significant changes in the basic composition and physicochemical indexes of a grape variety grown in Bulgaria. They stated that irradiation at 0.2–0.3 kGy did not soften the texture to the extent that it affected the quality of the grapes.

Very many studies relevant to disinfestations of fruit flies and shelf-life extension by delaying the rates of ripening and senescence of avocados have been reported in the literature. Gamma irradiation up to 0.1 kGy was found not to cause immediate visual damage to avocado fruits (Persea Americana Mill.).[155] Although a dose of 0.01 kGy was reported to delay softening, doses of 0.04 and 0.1 kGy were observed to hasten it. No benefit remained from any dose after 3 weeks of storage. Immersing fruits in hot water at 40 °C for 10 min prior to irradiation at 0.025 kGy has been found to improve the shelf-life of Fuerte and Hass avocados grown in Chile.[156] Nevertheless, the avocado is extremely sensitive to radiation and, hence, irradiation is impractical for controlling decay of this fruit.[138]

Tomatoes

The tomato is one of the most popular fruits in the world. Maturity at harvest, rate of ripening and decay caused by microorganisms are the factors determining its post-harvest shelf-life.[157] Ionizing radiation is used

to extend the shelf-life of tomatoes by reducing microbial growth and delaying ripening. However, the information available in the literature is rather confusing and contradictory probably due to the difficulties encountered in controlling parameters like fruit condition at harvest, level of field infection, harvest season, handling procedures during transport, maturity of fruit at the time of irradiation, variety differences, storage temperatures and irradiation doses. The latter are expected to influence the results of preservation processing.

Barkai-Golan *et al.*[158] reported the results of a study concerning fungal development in red tomatoes using a heat–irradiation combination treatment. A hot water dip followed by irradiation at 0.5 kGy was found synergistically to reduce fungal decay from 90 to 100%. Radiation-induced textural changes in tomatoes have been studied by El-Assi *et al.*[159] and they found that green tomatoes were affected more than pink fruit and that the effects were dose dependent. Softening was observed to occur in both irradiated green and pink fruits. Magea *et al.*[160] reported the effect of exogenous calcium salt treatment in inhibiting irradiation-induced softening in diced Roma tomatoes and they found that dipping diced tomatoes in a calcium bath before irradiation maintained the firmness of diced tomatoes and limited irradiation-induced softening. Irradiation alone was not found to influence significantly pectic substances.

13.6.2 Vegetables

Potatoes

The potato is important nutritionally mainly as a source of energy and ascorbic acid[161] and it can be considered as a part of the solution to world hunger.[162] Thus, control and reduction of post-harvest losses of potatoes are important. Various factors such as pre-harvest cultural practices, harvest maturity, harvesting and handling methods and proper curing of the harvested tubers can influence post-harvest losses of the potato tubers. Sprouting is the most obvious manifestation in potatoes.

Radiation processing was reported to provide an effective and safe alternative treatment for improving food security by reducing post-harvest losses of potatoes when coupled with good handling, storage and transport practices.[163] In many countries, irradiation at low doses (30–150 Gy) has been used to suppress sprouting in potatoes.[164] Matsuyama and Umeda[51] reviewed applications relative to sprout inhibition by irradiation and its effect on the quality of potatoes. They concluded that a minimum dose of 30 Gy is required for sprout inhibition in potato tubers, that smaller doses may actually stimulate sprouting and that doses greater than 150 Gy may cause irreversible changes in tubers. A decrease of 15% in the initial ascorbic acid content was observed to occur at sprout inhibiting doses. However, after prolonged storage the difference between irradiated and unirradiated potatoes was not observed to be significant. At an irradiation dose of

150 Gy some changes were also noted in the free amino acid content of potatoes, but these changes disappeared during storage. In an earlier review, an irreversible sprout inhibition was reported by Thomas[165] at a dose of 100 Gy regardless of variety and storage temperature. Irradiation of good quality tubers harvested with minimal injuries gave the best results. The optimum temperature for storage of irradiated potatoes seems to be about 7.5–10 °C, a temperature at which rot development is kept to a minimum. The effect of dose delivery rate on sprout inhibition has also been studied by several researchers. They concluded that a dose delivered at a higher rate is more effective than same dose delivered at a lower rate.[166–168] A most unfortunate effect of irradiation, even at the lowest of the doses used to suppress sprouting, was the induction or enhancement of three types of discoloration: black spot, vascular browning and darkening appearing after cooking. Tatsumi *et al.*[169] found that irradiation-induced browning varied with where the potatoes came from and the time elapse between harvest and irradiation. Inhibition of sprouting was reported to be more effective when potatoes were irradiated soon after harvest and cured when the commodities were still in the dormant state,[51,165,170] although there were considerable variety differences. Larger doses are needed as time after harvest increases.[166]

The objective of treating harvested potato tubers with low-dose ionizing radiation is to prevent the physiological processes leading to sprouting and consequent product deterioration during extended storage.[163] According to Burton and De Jong[171] sprout inhibition by irradiation varies widely with the variety. Losses originating from other causes such as microbiological spoilage can continue to occur during storage. Therefore, aeration, temperature and humidity of the storage environment need to be controlled by appropriate storage management procedures. Storage at cooler temperatures of 10–20 °C is recommended for this purpose.

All of the shoots capable of re-growth are killed and tubers are prone to fungal and bacterial invasion via induced structural damage when potatoes are irradiated at overall doses from 50 Gy to 1 kGy.[19] Irradiation of potato tubers, even at the lowest of the doses used to suppress sprouting, induced selective inhibition of the light-depending development of chloroplasts in the outer cell layers of the tubers, while permitting continued synthesis of solanine which is a toxic alkaloid.[165,172,173] Chips made from irradiated potatoes stored for more than 6 months were observed to be darker than chips made from unirradiated potatoes. Sprout-inhibiting doses of radiation are also effective in destroying tuber mouth, which is a devastating pest of potato. The effect of irradiation at 150 Gy on potato quality for chip production was also reported.[174] It was found that starch and sugar levels of irradiated potatoes were lower and higher, respectively, compared with those of unirradiated potatoes, with some flesh darkening. Quality of chips depended on storage temperature and only slightly on irradiation.

The effects of higher doses, up to 10 kGy, on potatoes were also reported by Cumming.[175] Polyphenol activity was found to be reduced at 2 kGy dose resulting in less darkening on slicing. Some texture softening and minimal starch degradation were also observed at this dose. According to Diehl,[176] industry would save 300 million kW h energy if refrigeration storage following irradiation of potato slices were used instead of adopting freezing and frozen storage. He also reported the results of a test performed among German consumers on potatoes irradiated at 120 Gy and a preference for the treated samples over the control, especially after storage for 3 to 5 months, was found. Similar results were reported for yams irradiated in the dose range 0.5–5 kGy by Cumming and Hajarin.[177] A 100-fold reduction in the surface microbial load was observed at the optimum dose (2 kGy) for color preservation. Nigerian yams irradiated in the sprout inhibition dose range (75–150 Gy) were reported[178] to show excellent sensory results and to reduce rotting. Later work carried out by McGuire and Sharp[179] on sweet potatoes showed significant increases in the sugar concentration even at doses as low as 0.1 kGy.

Onions and garlic

The onion is a universally important vegetable widely used as a spice to improve the gastronomic properties of food. It is consumed in both the cooked and raw states. The onion is one of the more economically important vegetables grown and exported in many countries of the world.[170] Mature onion bulbs can be kept for relatively long periods owing to their natural dormancy mechanisms and low respiration and metabolic activity. However, as in the case of potatoes, pre-harvest cultural practices, harvest maturity, curing and post-harvest storage conditions all can play a role in the losses occurring in stored onions.

Sprout inhibition of onions by irradiation has been studied in the literature by very many workers and divergent results, which were attributed to the multitudinous environmental and physiological conditions, were reported.[180–185] It is now established that the effectiveness of gamma irradiation for an acceptable sprout inhibition of onions is very much dependent on the pre-harvest growing conditions, state of dormancy of the onion bulb at the time of irradiation, the radiation doses employed and the post-irradiation storage environment, particularly the temperature and humidity, as well as the differences in cultivars.[170]

Umeda *et al.*[186] reported that irradiation of 'senshuki' onions within 20 to 60 days after the harvest at a dose of 0.07 kGy resulted in a complete sprout inhibition, but the variety 'sapporoki' did not respond in the same way. In an other work, Takano *et al.*[187] reported that in the variety 'sapporoki', complete suppression of sprouting was not possible when bulbs were irradiated to 0.15, 0.30, or 0.60 kGy at 27 or 104 days after harvest and stored at 5 °C, 10 °C or at ambient temperature. Sprout inhibition of onions is suggested to depend on the inner bud length.[187] In an other work on onion

varieties grown in Korea carried out by Park *et al.*[188] it has been reported that doses of 0.05, 0.07 and 0.1 kGy, respectively, given at 11, 32 and 66 days after harvest were sufficient to inhibit the subsequent sprouting of onions, while little sprout inhibition resulted when they were irradiated at 0.15 kGy, 96 days after harvest.

The results of careful studies by Thomas *et al.*[189] showed that regardless of the dose or the time of irradiation, sprouting did not occur in either non-irradiated or irradiated bulbs stored under tropical ambient temperature during the first 3 months. However, at the end of 5 months, a non-irradiated control sample showed 8–24% sprouting compared with 1–3% in irradiated lots. However, storage of onions at 19 °C resulted in considerable sprouting. The results of sprout inhibition studies carried out by Skou[190] on an onion variety grown in Denmark seem to be generally in agreement with the results of Thomas *et al.*[189]

Doses of either 0.05 or 0.1 kGy were found to be sufficient for sprout inhibition in four German-grown onion varieties stored at 10 °C (RH 80%) or 20 °C (RH 60%), respectively, if irradiation was carried out within 3 weeks after harvest.[181] Irradiation at 5 weeks after harvest inhibited sprouting at 20 °C but not at 10 °C. The effect of irradiation with 10 MeV electrons on the sprout inhibition of several onion varieties grown in Germany was also investigated in the literature.[191,192] The dose needed to obtain complete sprout inhibition was 0.05 kGy and it was independent of storage temperature for samples irradiated 4 weeks after harvesting. According to work carried out by Mahmoud *et al.*[193] on a variety grown in Hungary, sprouting can be inhibited by gamma irradiation at a dose of 0.05 kGy before the break of dormancy.

From studies performed on irradiated onions, it was concluded that it was necessary to use ionizing radiation with enough energy to penetrate the growth point of the bulbs for effective sprout inhibition. Ogata and Chachin[194] found that a higher dose rate gave better sprout inhibition than a lower dose rate.

A temporary increase in the respiration of onion bulbs following irradiation was observed by Rakitin and Krylov,[195] but this increase in respiration decreased subsequently during storage. Irradiation of onions in the dose range 0.02–0.06 kGy in the presence of air was found to induce increasing loss of ascorbic acid without seriously affecting the nutritional value.[196,197] No changes in levels of glucose, fructose or molic acid in four cultivars of onions irradiated with 10 MeV electrons at doses of 0.05 or 0.1 kGy and stored at 10 °C or 20 °C were observed.[181,192,198] Guo *et al.*[199] reported that the content and quality of soluble carbohydrates, lipids and protein in onions remained unchanged after irradiation for samples irradiated at the doses below 0.5 kGy and stored for 8 months. However, an acute decrease and damage to vitamin C content was reported by the same workers, immediately after irradiation at 0.1–0.5 kGy.

Garlic belongs to the onion family and the factors contributing to losses in stored garlic are essentially of a similar nature to those of onions. Doses in the range of 0.02–0.06 kGy applied shortly after harvest were found to provide 100% inhibition when bulbs are in the dormancy period. Near the end of the dormancy period, higher doses of 0.10–0.15 kGy were required to provide sprout inhibition.[200–202]

Mushrooms

Mushrooms continue to grow after harvest and thus, have a very short shelf-life. Quality deterioration takes place owing to accelerated physiological, morphological and microbial changes leading to browning and slimness as well as early breaking of the veil, expansion and darkening of cap and gills, and elongation of the stem.[203–205] A short shelf-life makes the mushroom unattractive to the consumer and reduces its economic value. Modified atmosphere packaging was reported[206] not to be suitable for mushroom owing to the fact that it can lead to reduced shelf-life,[207] weight loss,[208] generation of off-flavor,[209] increased bacterial load and an environment suitable for the growth of anaerobic pathogens.[210,211]

Commercial mushrooms are preferred at an immature stage with the cap unopened. Irradiation is used to retard the opening of the cap of mushroom. Irradiation alone and in combination with refrigeration has been reported to prolong shelf-life,[212] improve color and appearance,[213] inhibit veil and cap opening, decrease stem elongation and reduce moisture loss.[214,215] Reduction in microbial population, polyphenol oxidise activity, respiration and browning have also been reported for irradiated mushrooms.[216–218] Irradiated brown mushrooms have been observed to remain closed and to retain their light color during storage[219,220] if electrons of 1 MeV were used. However, later studies revealed that at similar dose levels, X-rays gave better results compared with electrons[221,222] if mushrooms were irradiated soon after harvest. Treatment with 1.0 kGy of X-rays was observed to prolong storage life up to 12 days at 10 °C, but the taste deteriorated to a great extent after 3–4 days storage.[223]

Bramlage and Lipton[224] reported that irradiation extended the market life of mushrooms by maintaining them in a fresh-like condition. Maxie *et al.*[225] observed a decrease in the extent of cap opening of California-grown brown mushrooms irradiated in the dose range 0.4–1.0 kGy when they were stored at 5 °C and 20 °C. Based on their detailed studies on mushroom irradiation, Campbell *et al.*[226] and Skou *et al.*[227] reported 1 kGy as the optimum dose for mushroom preservation, but higher doses tended to discolor the flesh too much. Michigan-grown white mushrooms packed in ventilated plastic tubs have been reported[228,229] to remain closed for 3 weeks at 0.5 °C (RH 98%), whether irradiated or not, but at 5 °C (RH 95%), non-irradiated mushrooms opened three times as fast as mushrooms irradiated at three different dose (0.25, 0.50 or 1.0 kGy) by gamma radiation. According

to Wahid and Kovacs[230] a dose of 2.5 kGy showed better results than 1.0 kGy in inhibiting the veil opening and stem elongation by mushroom during 8 days of storage at 14 °C (RH 80–90%).

At equivalent absorbed doses, X- or gamma rays treatment has been shown to be more effective in inhibiting cap opening than treatment with electrons of 1 and 3 MeV energies.[206] However, Skou *et al.*[227] reported no significant differences in the opening of two veils, cap expansion and stem growth when electrons of 10 MeV and gamma rays were used at similar dose levels. Prevention of bacteria and parasitic fungus growths was reported to be possible at a dose of 2 kGy during 8-days test storage at 10 or 20 °C.[227]

The effects of electron-beam irradiation on microbial counts, color, texture and enzyme activity of mushroom slices have also been studied in the literature[206] at dose levels of 0.5, 1.0, 3.1 and 5.2 kGy. Irradiation levels above 0.5 kGy reduced total plate counts, yeast and mold counts to below detectable levels and prevented microbial-induced browning. A 1 kGy dose was found to be ideal for preservation of the quality of sliced mushrooms.

13.7 Future of irradiation in fruits and vegetables

Reducing the wastage of crops after they are harvested is a major problem in most countries, especially in the developing and less developed countries. It is known that an important quantity of the world's food production, including fruits and vegetables, is lost owing to damage caused by bacteria, mold, insects and other pests. Irradiation offers a sustainable, environment friendly and less energy intensive treatment for the control of insect pests, spoilage-causing organisms and physiological process, especially in the case of perishable crops. As a preservation technique, food irradiation is moving to centre stage along with other technologies after decades of research, development and public debate. Irradiation of fruits and vegetables is beginning to play an important role in contributing to improved food safety and security and to increased trade as a proven sanitary, phytosanitary and preservation treatment. This role will be even more important in the near future. However, variability in effects creates, in most cases, difficulties in standardizing the irradiation treatment. Control of very many factors such as radiation dose, dose rate, degree of maturity, physiological status of fruits, temperature and atmosphere during and after treatment, pre- and post-harvest treatments and handling susceptibility of the microorganisms to the radiation used is needed for a successful and reliable treatment for a given commodity and cultivar. Therefore, there will be a need for further studies, especially in the cases of fresh fruits and vegetables, to adopt a treatment that is efficient and effective. On the other hand, the availability of a sufficiently developed infrastructure within a country is necessary in resolving a food trade or technical problem.

Several successful pilot-scale storage studies and market trials carried out in different countries over the world have shown the commercial potential and economic value of irradiation as a post-harvest treatment for specific commodities of tropical origin. The positive results of these studies encouraged some countries to establish commercial facilities for the treatment of bulb and tuber crops. The use of this technology is expected to increase further and play a substantial role in international food exports and imports in future provided that progress in the technical aspects and the trend towards increased regulatory approval of fruit and vegetable irradiation continue. To achieve these goals, further international co-trials and meetings are needed to consider the problems of drafting uniform guidelines and legislation pertaining to traffic in irradiated produce.

Another factor encouraging the application of irradiation to the preservation of fruits and vegetables is the ban on several fumigants used in the past for disinfestation. Continual reduction and banning of fumigants will lead to the expansion of the use of radiation to disinfest food. Following the ban many producers tried to use hot water dip treatment to disinfest mangoes and papayas, but this method was observed to cause physiological disorders in the fruits, particularly discoloration and browning of the pulps. The 1996 USDA/Animal Plant Inspection Service quarantine policy statement was considered to be a positive step towards greater use of irradiation treatment.

Extension of storage life makes irradiation particularly useful for tropical fruits, which are generally infested and require a prolonged shelf-life to reach consumer markets in good quality. It is believed that the major factors determining the future of food irradiation are the development of simple and reliable detection methods, the harmonization of legislation, the commitment of the food industry and consumer attitudes. Therefore, well defined standards for irradiated food at national and international levels, consumer education and consultation with consumers, and a standardized method of detecting irradiated foods and irradiation dosages will be the integral parts of future development in the area of food irradiation, and particularly in the area of fruit and vegetable irradiation. As pointed out in the literature, the future of food technology will be to combine various methods of preservation to obtain ultimate real gain from food irradiation.

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14

Thermal treatments of fresh fruit and vegetables

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14.1 Introduction

Heat treatments have been known to reduce decay and pests in fruits and vegetables since 1929 when Baker developed a vapour heat treatment against the Mediterranean fruit fly (Couey, 1989). With the development of fungicides and pesticides, the application of heat treatments became economically unattractive. Nowadays, as regulatory restrictions are put on the post-harvest use of chemicals, there is a renewed interest in alternative pest and disease control techniques. Although physical decontamination techniques are also subjected to many food regulations, the legal restrictions are often less severe than for chemical decontamination. Among physical decontamination techniques, heat treatment is the most important (Smelt *et al*., 2002). However, heat treatments are unsuitable for highly perishable products such as asparagus, nectarines or leafy vegetables as their shelf-life and marketability is reduced (Couey, 1989).

Fruits and vegetables can be heat treated in different ways. The different available technologies will be discussed in Section 14.2. Both conventional heat treatments, such as hot water, vapour heat and hot dry air treatments, as well as more advanced techniques like far infrared heating and heat treatment with electromagnetic radiation will be described. The selection of a specific technique for heat treatment depends on the properties of both the host (fruit or vegetable) and the organism that has to be inactivated. In Section 14.3 the effect of heat treatments on hosts and pathogens or pests will be highlighted including thermal inactivation modelling of the pathogen or pest. The success of a heat treatment depends on the specific heat transfer kinetics, which will be described in Section 14.4.

Practical applications will be described in Section 14.5. Future trends and sources of information will be the subject of Section 14.6 and 14.7, respectively.

14.2 Technologies

Heat may be applied to fruits and vegetables in several ways: by hot water, vapour heat, hot dry air, far infrared radiation or electromagnetic energy. The appropriate type of application depends on the heat tolerance of the commodity, its thermophysical properties and the size and shape of the product, the properties of the organism that has to be inactivated (lethal temperature and time) and the position on the host where the organism is located. Most insects will have to be eliminated in the core of the product, while fungi or bacteria causing decay are mainly situated at the surface or the first few cell layers under the peel of the product (Lurie, 1998).

For the conventional heating methods (hot water, vapour heat and hot dry air), the heat transfer efficiency is strongly related to the amount of water in the heating medium. Heat transfer to the surface of the product is strongly enhanced in water. As a consequence, shorter water treatments at reduced temperatures can have the same lethal effect as longer and more severe hot dry air treatments. However, the greater heat transfer efficiency of water does not entirely explain the disparity in insect and commodity tolerance to different heating media. Shellie and Mangan (2000) found that a less severe heat dose is required when fruits are heated in water because the decrease in O_2 and increase in CO_2 inside the fruit during immersion in hot water may impose an additional stress and may alter the tolerance of fruit fly larvae to heat. On the other hand, some commodities (e.g. grapefruit) are heat intolerant when heated with moist air and more tolerant when heated in air.

Supplementary treatments may be applied to reduce heat injury to the produce or on the other hand, to increase the heat susceptibility of the target organism. Preconditioning and application of periodic thermal treatments can be applied to reduce product injury (Jacobi *et al.*, 1995; Scheerlinck *et al.*, 2004). After the heat treatment, cooling in air (slow) or water (hydrocooling, fast) may be required to reduce the time that the product surface is exposed to high temperatures.

In this section, the details, the (dis)advantages and possible applications of each technology will be discussed.

14.2.1 Hot water

Hot water treatment is the best choice for delivering thermal energy to the fruit surface (Tang *et al.*, 2000). As a consequence, the technique was originally used for control of fungi, which are typically located at the surface

and in the first few cell layers under the peel of the commodity (Lurie, 1998). Later, applications were extended for insect disinfestation as an alternative to the more expensive vapour heat treatments. The cost of a commercial hot water immersion technology is about 10% that of a commercial vapour heat treatment system (Fallik, 2004). For fungal control, short term applications of 30 s to 10 min at relatively high temperatures (46–60 °C) are used (Barkai-Golan and Phillips, 1991). For insect control, temperatures are between 43 and 49 °C but the application times have to be prolonged to 30–120 min because the whole fruit and not only the surface has to be brought to a higher temperature. When the hot water is properly circulated through the load of product, a uniform temperature profile is quickly established (Couey, 1989). According to Barkai-Golan and Phillips (1991) the effective temperature range can be maintained without adding hot water if the commodity-to-water ratio is low and little heat is needed to warm the surface of the product. When the commodity-to-water ratio is high, heat must be added during the treatment. For insect control sophisticated equipment is available, but simple hot water tanks may be used for decay control.

When heat is applied by hot water, a less severe heat dose is required in comparison to hot dry air treatment because of an alteration of the atmosphere inside the fruit, which may impose an additional stress on fruit fly larvae (Shellie and Mangan, 2000). As a disadvantage, some commodities, for example some stone fruits and mangoes, may be intolerant to water heat treatments. The shelf-life of cherries was improved significantly by retarding brown rot fungi without alteration of fruit quality when treatment did not exceed 15 min at 45 °C, but treatment at 45 °C even for only 3 min caused severe damage to strawberries which made them more susceptible to diseases (Marquenie *et al*., 2002). It has been hypothesised by Hayes (1994) that inhibition of respiration during heating in water predisposes a fruit to injury, but Shellie and Mangan (2000) found no evidence of this in their research. For heat-sensitive commodities, heating temperatures and periods may have to be diminished; this can be established when the heat treatment is combined with other treatments. For example, to control brown rot fungi *Monilinia fructicola* and *Rhizopus stolonifer* on peaches and nectarines, ethanol was added to the hot water (Margosan *et al*., 1997). More information on combination treatments will be given in Section 14.6. Another solution may be to gradually increase the temperature (McGuire, 1991) or to apply cyclic heat treatments (Scheerlinck *et al*., 2004). To alleviate damage to mangoes it may be necessary to precondition in hot air (Jacobi *et al*., 1995). For 'Bing' sweet cherries, the success of hot water treatment for disinfestation of codling moth was greatly dependent on the moment of application (preferably just after harvest) and later on shipping conditions owing to quality loss (Feng *et al*., 2004).

A recent review of hot water treatments and hot water rinsing and brushing is given by Fallik (2004). Applications of hot water treatment for decay

control include apple, avocado, citrus fruits and plums. Treatment times range from 2–5 min.

In relation to insect disinfestations, hot water treatments are mainly tested and developed for tropical horticultural crops to control fruit flies, for example for guava (Gould and Sharp, 1992), mangoes (Shellie and Mangan, 2000; Hallman and Sharp, 1990; Sharp and Pichomartinez, 1990; Jacobi *et al*., 1995), stone fruit (Sharp, 1990) and lime (Gould and McGuire, 2000). Treatment times can be reduced if only insects found in the outer layers are to be killed. Gould and McGuire (2000) found that a treatment of 20 min at 49 °C was sufficient to kill mealybugs and all other tested arthropods found externally on limes, or under the calyx.

Next to decay and pest control, hot water treatments can be used to improve product quality either directly or through improved chilling resistance, ripening inhibition or sprouting inhibition (Fallik, 2004). Hot water dips of fresh broccoli, for example, could at the same time extend its shelf-life by delaying yellowing, removing *Lepidoptera* larvae and delaying the development of decay caused by fungal spotting and bacterial soft rot (Forney, 1995). Ideally, a temperature of 50 °C was applied for 2 min. However, a treatment at the same temperature for 3 min resulted in off-odours that reflected physiological injury caused by the hot water treatment (Forney and Jordan, 1998).

In Section 14.5 (Practical applications and costs) a special hot water treatment, namely hot water rinsing and brushing is described.

14.2.2 Vapour heat (water-saturated hot air)

In this section, heat treatment with water-saturated hot air will be discussed. Intermediate forms between vapour air and dry air can be applied, the effect depending on the water content of the moist hot air (Shellie and Mangan, 2000). The term vapour heat is applied when the relative humidity is greater than 90%. When applying vapour heat, heat transfer is accomplished by condensation of water vapour on the relatively cool product surfaces (Couey, 1989). The surface heat transfer rate can be as much as 10 000 times that obtained with dry air only and 50 times that of still water (Singh and Heldman, 2001). The first applications of vapour heat were in chambers without forced air and long gradual warming periods (approach times) were applied. In modern facilities, a faster and more homogeneous heating is accomplished by using forced water-saturated air which circulates through the pallets (Lurie, 1998). Also, a quick run-up treatment has been developed which consists of a short preheat period to a specified temperature followed by a gradual warming to 47 °C, similar to the approach time in the regular vapour heat treatment (Couey, 1989). When the interior of the fruit has reached the required temperature, there is a holding period for the length of time necessary to kill the insect. Afterwards, the product

is cooled down by air cooling or hydrocooling, the latter being the faster method (Lurie, 1998).

Vapour heat treatment was developed for insect control (Lurie, 1998) and is mainly applied in quarantine treatments for fruit flies in subtropical fruits. Experimental applications include decay control on vegetables and fruits, for example control of *Botrytis cinerea* in table grapes (Lydakis and Aked, 2003).

14.2.3 Hot dry air

Owing to the low heat transfer coefficient of dry air in comparison to that of water or vapour heat, longer heating times are required. When the air is dry, no condensation forms on the target commodity and the rate of heat transfer depends largely on the velocity of the air passing over the surface of the fruit (Barkai-Golan and Phillips, 1991). The heat transfer can be improved by air circulation. Air circulation can be accomplished by ventilation fans in the heating chamber or by applying forced hot air where the speed of air circulation is precisely controlled (Lurie, 1998).

As an advantage, the slower heating and the lower humidity used when applying hot dry air can cause less damage compared to hot water or vapour treatments. Ornelas and Yahia (2004) compared the quality of 'Hass' avocado fruit heated with dry (50% RH) or moist (95% RH) forced air at 38 °C for 6 h. Treatment with dry forced hot air reduced the incidence of chilling injury and decreased quality deterioration of the avocado fruits. The quality of grapefruit, mango, papaya and orange could also be better retained when applying forced hot air instead of hot water immersions (Shellie and Mangan, 2000; McGuire, 1991).

Heat input during treatment is more important in this application compared to hot water treatment, because of the lower heat-holding capacity of air compared to water (Barkai-Golan and Phillips, 1991). Once the core of the product has reached the lethal temperature, rapid cooling may be required to minimise the exposure time of the product surface to high temperature.

Applications of forced hot dry air are mainly quarantine treatments of subtropical fruits against insects. To eliminate the Caribbean fruit fly for example, the core of the treated fruits has to reach the lethal temperature of 44 °C. To reach this temperature in the core of carambolas, navel oranges and mangoes, the fruits have to be heated for 64.8 min, 108 min and 136 min, respectively when applying hot dry air of 48 °C (Sharp, 1992; Sharp and Hallman, 1992; Sharp and McGuire, 1996). For quarantine treatments against Mediterranean fruit fly, melon fly and oriental fruit fly on papayas, application at 48.5 °C of forced air for 3.5 h was necessary (Armstrong *et al*., 1995).

Little work has been done to develop air treatments specifically for decay control. Unlike insect control, only the surface of the product has to reach a lethal temperature, so application times can be kept shorter. Still, treatment times are considerably longer (one to several hours) compared to hot water or vapour heat treatments. Also, moist heat may be more effective than dry heat because moist spores have a higher physiological activity than dry spores (Barkai-Golan and Phillips, 1991). All these factors make hot water treatment more attractive for fungal control. However, further investigations of forced hot dry air could be of interest to finding a treatment which, at the same time, prevents fungal and insect diseases and beneficially affects commodity physiology (Barkai-Golan and Phillips, 1991; Lurie, 1998).

When applying hot dry air, commodities can start to dry out because of condensation. Solutions may be heating in stages, waxing or wrapping the product in plastic foil (Barkai-Golan and Phillips, 1991).

14.2.4 Far infrared radiation heating (FIR)

Far infrared radiation (FIR) heating technology may be an alternative to conventional heating methods because it can achieve rapid and contactless heating (Verboven *et al*., 2005). Infrared waves lie between the visible and microwave portions of the electromagnetic spectrum, the 'far infrared' waves being closer to the microwave region. Far infrared waves are thermal and can be used to cook (Shilton *et al*., 2002) or to heat food (e.g. in fast food restaurants), and to control food surface moisture (Datta and Ni, 2002). Hashimoto *et al*. (1992) found that *Escherichia coli* and *Staphylococcus aureus* suspended in a liquid medium below the lethal temperature were injured and killed by far-infrared irradiation. By estimating the temperature distribution within the suspension, it was suggested that the test bacteria are injured and inactivated in the very thin domain near the surface of the suspension.

The use and understanding of FIR heating in industrial food applications is still limited. Verboven *et al*. (2005) investigated the potential of FIR heating as an alternative to conventional heating for surface decontamination of strawberry. A Monte Carlo computational fluid dynamics (CFD) model was applied to simulate the three-dimensional temperature profile of strawberry. FIR heating achieved a more uniform surface heating than air convection heating but the heating rate was smaller, for the configuration and air velocity considered. Compared to hot water application (Scheerlinck *et al*., 2004), the FIR heating rates were more than five times smaller and the FIR surface temperature uniformity was inferior. Given these results, practical use of FIR for surface decontamination seems limited. However, periodic FIR heating and other configurations need to be investigated to be conclusive.

14.2.5 Radio frequency (RF) heating or microwave (MW) heating

Slow, non-uniform heating under conventional heating methods as described above may be solved by dielectric heating such as radio frequency heating (RF) and microwave heating (MW). This method involves a reduced run-up time and is suited to high temperature, short-time, continuous-feed treatments (Ferguson *et al*., 2000). Dielectric heating is generated by direct interaction between electromagnetic waves and foods instead of by slow heat conduction and convection as in conventional heating (Tang *et al*., 2000). RF and MW treatments involve the application of electromagnetic energy at 10 to 30 000 MHz (Wang and Tang, 2004). According to the Federal Communications Commissions (FCC) in the USA, industrial, scientific and medical usable frequencies are limited to 13.56, 27.12 and 40.68 MHz for RF, and 915 and 2450 MHz for MW. Because of the much longer wavelengths of RF compared to MW, RF energy penetrates dielectric materials more deeply than microwave energy (Wang *et al*., 2003). Also, RF treatment may lead to preferential heating of insects without adversely affecting product quality, owing to the larger dielectric loss factor of insects than that of dry host products (Wang *et al*., 2003). In addition, RF yields simple uniform field patterns as opposed to the complex non-uniform standing wave patterns in a microwave oven (Luechapattanaporn *et al*., 2004). Further, the focusing effect of small, spherical fruits may lead to excessive heating of the fruit centre (Datta and Anantheswaran, 2001). All these factors make RF heating more suitable than MW treatments for possible application.

RF treatments may be particularly interesting for insect control inside the horticultural product. Quarantine treatment protocols have been developed using RF treatments that can effectively control codling moth (Wang *et al*., 2001) and navel orangeworm (Wang *et al*., 2002b).

A great deal of effort has been made to apply radio-frequencies as a high-temperature short-time (HTST) method for the post-harvest pest control in nuts and dry products as alternatives to chemical fumigants (Tang *et al*., 2000; Wang *et al*., 2003; Mitcham *et al*., 2004; Wang and Tang, 2004). Dry nuts have a higher heat tolerance than fresh fruit. Fresh fruit suffers thermal damage (burn) at the points of contact with the container or with other fruit when heated with RF energy in air. This is the result of overheating caused by a concentration of electric fields around the contact areas that have the least resistance to RF energy. Immersing the fruit in a medium can solve the problem of contact surface overheating (Wang *et al*., 2003). The selected medium should have similar dielectric properties to the treated fruit (Wang *et al*., 2003). Ikediala *et al*. (2002) used 0.15% saline water as a medium to apply RF treatment against codling moth in cherries. A 99% mortality rate of codling moth larvae and eggs was obtained at 50 °C when treated for 7–10 min. Cherry quality was better or comparable with methyl bromide fumigated fruit. In larger fruits such as citrus and apples, non-uniform heating caused by varying RF fields can occur. Birla

et al. (2004) developed a fruit mover to evaluate possibilities for improving RF heating uniformity. With rotation and movement of fruit, the temperature uniformity in oranges and apples was significantly improved with less than 2.8 and 3.1 °C standard deviations, respectively. However, even when submerging and circulating apples in water, the range of variability in temperatures was problematic according to Hansen *et al*. (2004a). The authors concluded that commodities with higher thermal tolerances would be better candidates for RF post-harvest treatments.

Controversy exists about a so-called non-thermal effect of RF and MW treatments on microorganisms. Although some authors have reported on a specific effect (Fleming, 1944), most authors concluded that the effect of RF treatment was purely thermal (Ingram and Page, 1953; Brown and Morrison, 1954; Carroll and Lopez, 1969; Lechowich *et al*., 1969; Welt *et al*., 1994; Ponne *et al*., 1996). Part of the controversy may be due to large experimental variability in the complex systems of microorganisms in a dynamic environment (Ponne *et al*., 1996).

14.3 Effect on host and pathogen

14.3.1 Influence on pests and microorganisms and modelling thermal inactivation

There are a wide range of insect pests that are the target of post-harvest quarantine heat treatments but there is little variability in the maximum temperature (40–50 °C) at which they can survive. Heat has an influence on insect metabolism, respiration, nervous and endocrine systems, but there is still uncertainty about the real cause of insect mortality following a heat treatment (Neven, 2000). The temperature experienced by individual insects heated within fruit depends on the water or air temperature, the heat transfer coefficient, the thermophysical properties and the size of the fruit, and the location of the pest in the fruit, the latter being dependent on the life stage of the insect. For example, fruit fly eggs are typically laid no more than 5 mm from the fruit surface, while late instars can be located at the fruit centre (Waddell *et al*., 2000). Sensitivity to high temperatures will be influenced by the life stage of the target insect and thermal treatments have to be severe enough to kill the most tolerant life stage.

The use of models can assist in the identification of promising treatments while avoiding extensive in-fruit testing. Features that have to be taken into account for these models are the temperature–time history of fruit cores and the minimal treatment time at different temperatures to control the most tolerant life stage of the insect. Recent models include cumulative lethal effects on insect mortality in their models (Hansen *et al*., 2004b).

Thermal death kinetic models have been used to describe the influence of temperature on mortality, for example codling moth (Wang *et al*., 2002a) and Indianmeal moth (Johnson *et al*., 2003), navel orangeworm (Wang

et al., 2002b) and Mediterranean fruit fly (Gazit *et al*., 2004). The models are commonly based on the following equation (Equation 14.1):

$$\frac{\mathrm{d}(N/N_0)}{\mathrm{d}t} = -k(N/N_0)^n \qquad [14.1]$$

where k is the thermal death rate constant (min^{-1}), n is the kinetic order of the reaction, N and N_0 are the surviving and initial numbers of insects, and t is the exposure time (min). The temperature dependency of k can be described using an Arrhenius equation (Equation 14.2).

$$k(T) = k_{\mathrm{ref}} \exp\left(\frac{E_\mathrm{a}}{R}\left(\frac{1}{T_{\mathrm{ref}}} - \frac{1}{T}\right)\right) \qquad [14.2]$$

In this equation, E_a is the Arrhenius activation energy [$\mathrm{J\,mol}^{-1}$], R the universal gas constant [$\mathrm{J\,(mol\ K)}^{-1}$] and k_{ref} is the inactivation rate [min^{-1}] at the reference temperature T_{ref} [K].

As an example, thermal mortality curves of Mediterranean fruit fly eggs at four temperatures as described in Gazit *et al*. (2004) are given in Fig. 14.1.

In practice, individual insects in fruit will experience a 'ramped heating', which means that the actual temperature to which the insect is exposed will

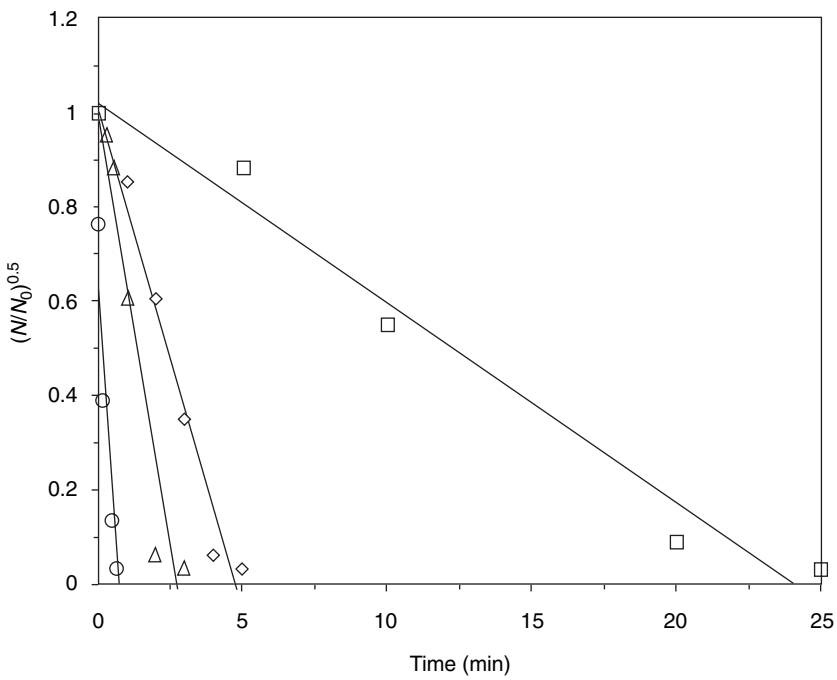

Fig. 14.1 Thermal mortality curves of Mediterranean fruit fly eggs at four temperatures using a 0.5 order kinetic model: □, 46 °C; ◇, 48 °C; △, 50 °C; ○, 52 °C (*Source*: Gazit *et al*., 2004).

slowly increase to lethal levels (Neven, 2000). A commonly used method to account for cumulated lethal effects is the degree–minute model (Shellie and Mangan, 1994) which suggests that the cumulated temperature beyond a threshold value multiplied by the duration of exposure is a critical factor that yields a certain level of insect mortality. Recently, Hansen *et al.* (2004b) used a cumulated lethal time model as proposed by Tang *et al.* (2000) to evaluate efficacy of heat treatments for codling moth in cherries. Here, the accumulative lethal effect for any given temperature–time combination is described in terms of equivalent total lethal time M_{ref} (in min) at a reference temperature, T_{ref} (°C) by Equation 14.3:

$$M_{\text{ref}} = \int_0^t 10^{(T(t)-T_{\text{ref}})/z}\,\mathrm{d}t \qquad [14.3]$$

where $T(t)$ is the recorded core temperature as a function of time t (in min) and z is the temperature difference required for a 10-fold change in the thermal death time curve (°C).

However, long exposure to elevated but non-lethal temperatures has also been shown to condition dipteran and lepidopteran species such that subsequent treatment at lethal temperatures is less effective (Waddell *et al.*, 2000). Waddell *et al.* (2000) examined the effect of thermal conditioning on the heat sensitivity of *Bactrocera tryoni* eggs. A kinetic model for conditioning was built. Figure 14.2 gives a representation of the extra treatment time at 46 °C which is required after two static temperature condition treatments.

Currently used heat treatments to reduce decay are fungistatic rather than fungicidal (Schirra *et al.*, 2000). Fungi can be inhibited by direct thermal inhibition, as well as by an enhanced resistance of the fruit. The variation in sensitivity to heat among fungi species and their life stages (mycelium, dormant spores, germinating spores, conidia) is considerable. Non-germinated spores are more tolerant to heating than germinated spores or mycelium. Other factors such as the moisture content of spores, age of the inoculum and inoculum concentration (Barkai-Golan and Phillips, 1991) affect the response of fungi to heat.

The classical approach to describe thermal inactivation of microorganisms is to use a first order decay reaction. Microbial inactivation by heat can be considered to obey first order kinetics because it is directly linked to the inactivation of some critical enzymes or enzyme systems, which is dynamically described – in analogy with chemical kinetics – by a first order decay reaction (Marquenie, 2002). The temperature dependence of the inactivation rate constant can then be described using the Arrhenius equation (McMeekin *et al.*, 2002). As an example, Marquenie *et al.* (2002) described inactivation of *Botrytis cinerea* and *Monilia fructigena* conidia by using a first order inactivation model (Equation 14.1 with $n = 1$) combined with the

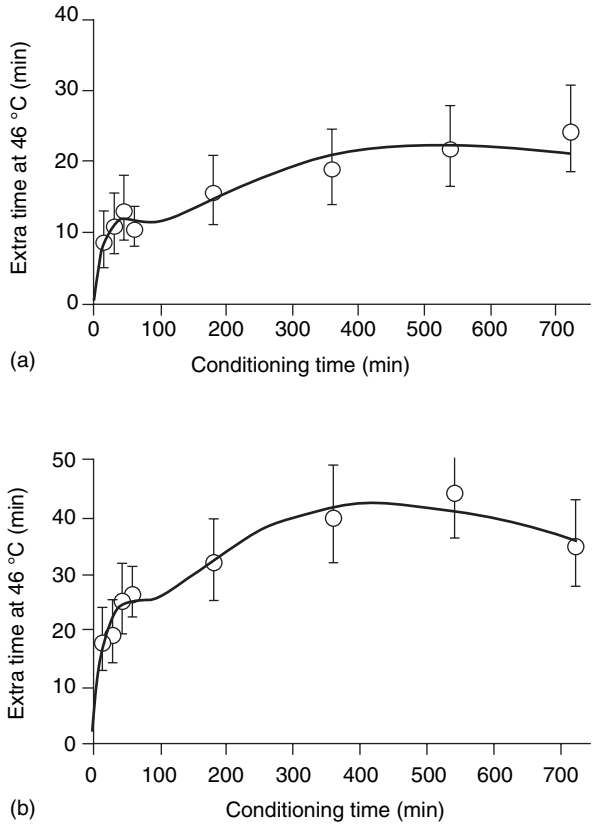

Fig. 14.2 Relationship between static temperature conditioning of *Bactrocera tryoni* eggs at 34 °C (a) and 38 °C (b) and the value of 'extra treatment time' required at the lethal temperature 46 °C to achieve 99% mortality, compared to eggs which had not been conditioned. Data values are plotted as open circles with 95% confidence limits as error bars. The solid lines are values calculated from a kinetic model for conditioning (*Source*: Waddell *et al*., 2000).

Arrhenius equation (Equation 14.2). Model predictions and observed numbers of conidia for *Botrytis cinerea* are shown in Fig. 14.3.

14.3.2 Influence on host quality

The primary obstacle to the widespread use of heat treatments is the sensitivity of many products to the temperatures required for effective treatment (Couey, 1989). Heat injury results in quality loss by external or internal damage, like for example browning and softening (Lurie, 1998). Sensitivity to heat is dependent on the exposure temperature as well as the

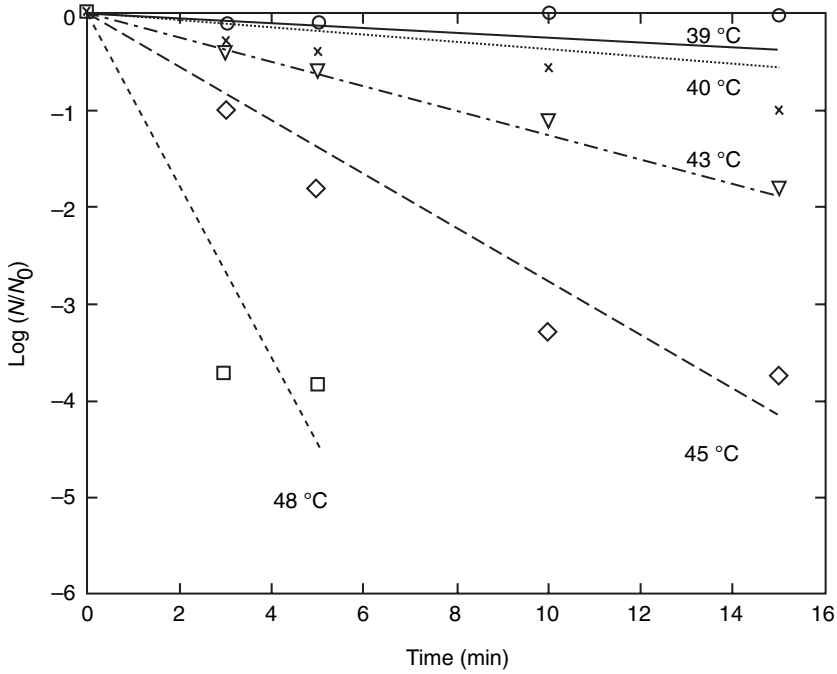

Fig. 14.3 Inactivation of conidia of *B. cinerea* as a function of temperature (°C) and treatment duration (min). Model predictions: —, 39 °C; ···, 40 °C; ·-·-, 43 °C; – –, 45 °C; ---, 48 °C) and observed values: ○, 39 °C; ×, 40 °C; ▽, 43 °C; ◊, 45 °C; □, 48 °C) (*Source*: Marquenie, 2002).

exposure time and is species- or even cultivar-specific. Analysis of the relationship between exposure temperature and time yields an interface between injured and non-injured fruits. This can be done for one fruit or for different types of fruits as was done by Couey (1989). Bananas, papayas, mangoes and litchis were grouped in a relatively heat-resistant fruit cluster, whereas peaches, raspberries and cantaloupes were heat sensitive.

When exposure temperatures or times are too high, fruits and vegetables can suffer from quality damage. However, applying moderately high temperatures of 35–40 °C can induce a tolerance to later exposure to normally lethal temperatures. The achieved thermotolerance is related to the production of heat shock proteins (HSP) (Vierling, 1991). The ability to build up thermotolerance can be employed during heat treatments. Gradually increasing the temperature or preconditioning at moderate temperatures can prevent damage to heat sensitive commodities. Not only thermotolerance but also tolerance to chilling injury can result from mild heat treatments. Several commodities, among which are tomato, avocado and cucumber, can keep longer at low temperatures after a heat treatment (Lurie, 1998). This response is partly related to the production of heat shock

proteins and partly to membrane alterations that reduce membrane leakage.

Apart from possibly causing damage, high temperatures may have a positive influence on quality. This can be directly by influencing ripening, preventing chilling injury or reducing browning, or indirectly by making the commodity more resistant to microbial diseases.

The effect on fruit ripening is a function of the exposure temperature and duration and how quickly the commodity is cooled following the heat treatment (Paull and Chen, 2000). According to Lurie (1998) heated fruit will be more advanced in some ripening characteristics than non-heated fruit while maintaining their quality longer during shelf-life at 20 °C. Exposing fruit to high temperatures attenuates some ripening processes while enhancing others. Ethylene production and response to exogenous ethylene are inhibited during heat treatment, but can be reversed when the fruits are removed from heat. A range of commodities soften more slowly when stored at high temperatures compared to storage at 20 °C. Apples that had been held at 38 °C for 3 or 4 days pre-storage were firmer than non-heated fruit, even after 6 months of storage at 0 °C and a subsequent shelf-life of 7 days at 20 °C (Conway *et al*., 1994). Flavour can be favoured because of a decrease in titratable acids alone or in combination with an increase in soluble solid content which results in a higher sugar to acid ratio. Also volatile production may be affected by a heat treatment. Respiration rates of the produce are generally enhanced during the first days of exposure to high temperatures, but at longer times at high temperatures the rate decreases (Lurie, 1998). When exposing the fruits to ambient temperature again, the respiration is lower than for non-heated fruits (Klein and Lurie, 1990).

Making fruits or vegetables more resistant to microbial diseases can be another indirect effect of heat treatments. First, wax layers may melt and consequently fill cracks, microwounds and stomata. The occlusion of possible gaps against wound pathogens as well as the encapsulation and inactivation of early-germinated spores have been considered as additional factors in fruit protection against decay (Schirra *et al*., 2000). Porat *et al*. (2000) demonstrated that hot water baths for citrus fruits smoothed the wax layer and thus covered and sealed stomata and cracks which are possible invasion sites for bacteria. Second, it has been stated that heat could lead to the induction of natural resistance in the fruit (Klein *et al*., 1997), although Schirra *et al*. (2000) reported that a hot water dip by itself did not elicit lignification or phytoalexin production in citrus fruit unless the fruit was pathogen-challenged or wounded. Alternatively, Porat *et al*. (2000) suggested that hot water brushing by itself induced the production of the pathogen-related proteins chitinase and β-1,3 glucanase in 'Star Ruby' grapefruit.

Readers are referred to Lurie (1998) and Paull and Chen (2000) for extensive reviews on the effects of heating on commodity quality.

14.4 Heat transfer kinetics

Knowledge of heat transfer kinetics is important to find the most suitable thermal treatment for a specific application. The source of thermal energy as well as the properties of the treated product affect the efficiency of heat transfer from the medium to the target position in the product. Both product quality retention and inactivation efficiency will largely depend on the heat transfer kinetics.

Conventional heating consists of convective heat transfer from the heating medium (water or air) to the fruit surface and conductive heat transfer from the surface to the core. Convective heat transfer from the medium to the product surface ($r = r_0$) is described by the following equation:

$$-\lambda \left.\frac{\partial T}{\partial r}\right|_{r=r_0} = h[T(r_0, t) - T_\infty] \qquad [14.4]$$

where λ is the thermal conductivity of fruit ($W\,m^{-1}\,°C^{-1}$), h is the surface heat transfer coefficient ($W\,m^{-2}\,°C^{-1}$), r is the radial coordinate (m), r_0 is the fruit radius, T is the fruit temperature (°C) and T_∞ is the temperature of the heating medium (°C). The left-hand side of the equation represents the heat flow into the product; the term on the right-hand side is the heat flow from the heating medium, which is proportional to the surface heat transfer coefficient h. The value of h is dependent on the product and the heat transfer medium and may vary from $5\,W\,m^{-2}\,°C^{-1}$ in still air and $200\,W\,m^{-2}\,°C^{-1}$ in moving air to 50–$10\,000\,W\,m^{-2}\,°C^{-1}$ in circulating water and $100\,000\,W\,m^{-2}\,°C^{-1}$ for condensing water vapour (Singh and Heldman, 2001).

From the product surface, the heat will be further transferred to the core by conduction. Conductive heat transport in a product can be described by the Fourier equation (Incropera and De Witt, 1990):

$$\rho c_p \frac{\partial T}{\partial t} = \lambda \nabla^2 T \qquad [14.5]$$

with ρ the product density ($kg\,m^{-3}$) and c_p the heat capacity ($J\,kg^{-1}\,°C^{-1}$).

Temperature profiles in products can be predicted from Equation 14.5 subjected to boundary conditions (Equation 14.4) and a known initial temperature distribution. To demonstrate the influence of the applied medium the simulated core temperatures of an apple with a diameter of 8 cm heated by different heating media at 52 °C are given in Fig. 14.4 according to Tang *et al.* (2000). As we can see from the figure, the core temperature slowly increases from the initial temperature to the temperature of the heating medium. Apple cores reached a temperature of 50 °C after 42 min when heated in circulating water at 52 °C. To reach the same core temperature, apples had to be heated for 83 min in circulating air at $4\,m\,s^{-1}$ and even 143 min in circulating air at $1\,m\,s^{-1}$ (Tang *et al.*, 2000).

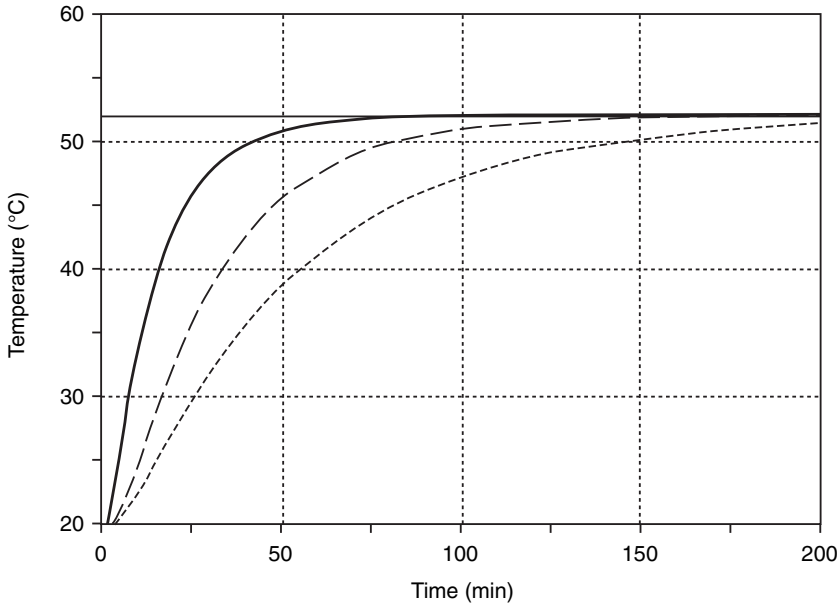

Fig. 14.4 Simulated core temperatures of an apple (d = 8 cm diameter) when heated by water or air (at different air velocities u): —, water heating; – –, air heating at $u = 4\,\text{ms}^{-1}$; ---, air heating at $u = 1\,\text{ms}^{-1}$ (*Source*: Tang *et al*., 2000).

On the other hand, heating profiles are dependent of the product characteristics such as size and thermal diffusivity ($\alpha = \lambda\ \rho^{-1}\ C^{-1}$). Shellie and Mangan (2000) subjected four commodities (mango, papaya, grapefruit and orange) to three different heating media (water and forced air with and without a water vapour-pressure deficit) and compared the heating rates (see Fig. 14.5). Papaya and mango fruit heated more rapidly than oranges and grapefruit, even though they weighed more than the citrus fruit and had a higher density than the other fruits. Mangoes and papaya fruit had an average density of 1.03 and 0.91 g ml^{-1}, respectively, and heated about 20 min faster than oranges (0.88 g ml^{-1}) and grapefruit (0.80 g ml^{-1}).

The research of Shellie and Mangan (2000) also suggests that differences in the surface heat transfer coefficient have a greater relative influence on the rate of heating at the fruit centre than differences in fruit thermal diffusivity.

A model-based approach to design periodic thermal treatments for surface decontamination of strawberries, and fruit in general, was developed by Scheerlinck *et al*. (2004). The model accounts for the actual shape of the strawberry, surface decontamination kinetics and temperature-related product quality objectives. It was shown that by applying a periodical thermal treatment the temperature increase inside the fruit could be reduced while still achieving a sufficiently high surface temperature.

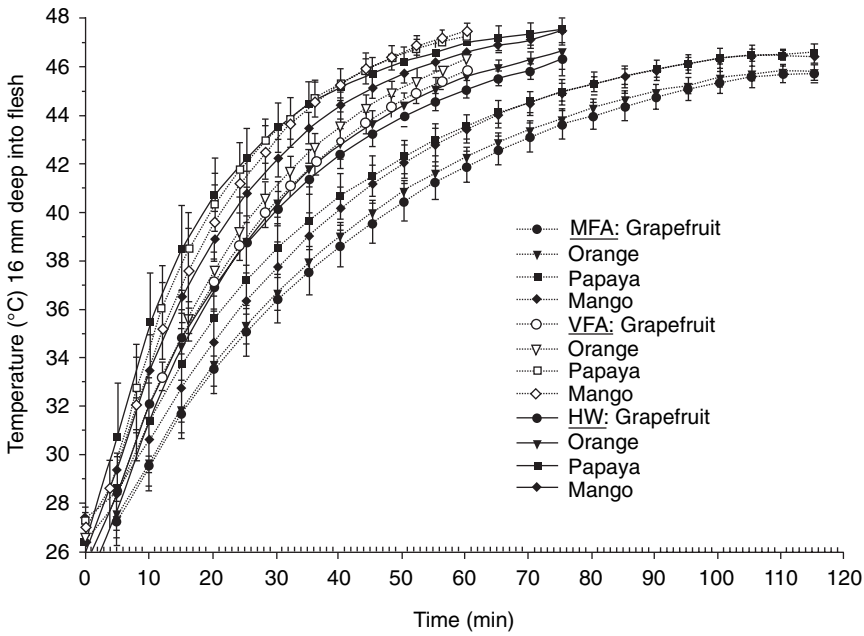

Fig. 14.5 Average temperature 16 mm deep into grapefruit (circle), orange (triangle), papaya (square) or mango (diamond) during exposure to 48 °C in forced vapour-pressure-deficit air (MFA) (closed symbol, dotted line), forced vapour-saturated air (VFA) (open symbol, dotted line) or water (HW) (closed symbol, solid line) until the average temperature of the slowest heating type of fruit reached 46 °C. Standard errors based upon mean of four replications with three of each fruit type per replication (*Source*: Shellie and Mangan, 2000).

14.5 Practical applications and costs

According to Hallman (2000), researchers are often not aware of the differences between the experimental versus commercial setting and the effect these contrasts could have on treatment efficacy. He states that perhaps the main reason that thermal quarantine treatments have not failed more often is that they are quite robust. Treatments are designed to provide a very high level of control, and the levels of infestation usually found in fresh agricultural commodities traded internationally are extremely low resulting in low probabilities of finding live insects after an insufficient quarantine treatment. The major requirements for heating technology systems are being effective against pathogens or pests whilst minimising the thermal impact on the product quality, and at the same time being economically viable (Ferguson *et al*., 2000).

Heat has been approved as a quarantine treatment by the US Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) against pests (mainly fruit flies) for several perishable commodi-

ties (USEPA, 1996). The capital costs for heat treatment are only slightly higher than that for methyl bromide. Operating costs for heat treatments, on the other hand, are eight times higher than those for methyl bromide, attributable primarily to longer treatment times and high energy costs. Although the costs are higher, the relative proportion of this cost is small when compared to the value of the commodity (USEPA, 1996). Hot water treatments are considerably cheaper than heat treatments with vapour or forced dry air. The cost of a commercial hot water immersion technology is about 10% of that of a commercial vapour heat treatment system (Fallik, 2004). The main components of a hot water immersion unit are the treatment tank, a heat exchanger unit, a water circulation system and a temperature controller. A uniform consistent temperature profile exists throughout the treatment tank at or slightly above the set point temperature. The unit is easily assembled, simple to operate and affordable (Tsang *et al*., 1995). However, for quarantine treatments the use of hot water is restricted to mangoes because of the high rate of damage in other commodities (USEPA, 1996). In Florida, a hot water treatment at 55 °C for 3 min is used as a quarantine treatment for mangoes. Forced hot air treatment is used on mangoes from Mexico and on papayas from Chile, Brazil and Hawaii. Vapour heat is used on mangoes from Mexico and on papayas and pineapples from Hawaii (Ben-Yehoshua and Porat, 2005).

In recent years, hot water treatments have become increasingly commercially accepted for decay control by the introduction of a hot water rinsing and brushing method (HWRB) (Fallik, 2004). The new technique for rinsing and disinfecting fruit and vegetables with hot water and brushes was patented (Israeli patent 116965) and introduced commercially in 1996 (Fallik, 2004). First, fruits are rinsed from above with non-heated tap water from several nozzles while being brushed for about 10 s in order to remove the heavy dirt, pesticides and fungal spores. Fruits continue to roll over brushes directly into the hot water rinse stage at temperatures between 48 and 63 °C for 10–25 s, depending on produce type and cultivar. The hot water is pressurised in order to recycle. Fruits are then dried with forced-air fans for less than 2 min inside a 3–4 m long tunnel. A schematic representation of a hot water rinsing and brushing machine as part of a sorting line is given in Fig. 14.6.

In comparison to hot water immersion, the applied temperature is relatively high (up to 65 °C instead of 55 °C) and the exposure time is drastically reduced (to a few seconds instead of few minutes). The advantages of hot water rinsing and brushing over hot water immersion are the increased energy directed to the contaminants, a reduced volume of water use and wastewater generation, and reduced water uptake by the produce. The technique was designed to be part of a commercial packing house sorting line (Porat *et al*., 2000) and has been commercially adopted to clean and disinfect sweet peppers (Fallik *et al*., 1999), mangoes (Prusky *et al*., 1999), corn and melons (Porat *et al*., 2000) and tomato, kumquat, organic citrus fruit

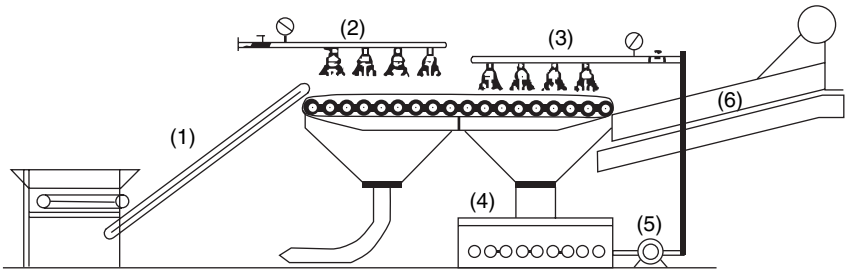

Fig. 14.6 Hot water rinsing and brushing machine: (1) conveyor; (2) tap water rinsing and brushing unit; (3) hot water rinsing and brushing unit. Water is recycled; (4) hot water container; (5) water pump to pressurise and recycle the hot water; (6) forced-air dryer (*Source*: Fallik, 2004).

and litchis. Soon the technology will be introduced for sweet potato. In Israel there are about 250 units with a capacity of 500 kg h^{-1} to 30 tonnes h^{-1} (Fallik, personal communication). Also in Spain and several countries in South and Latin America the technique is already commercially used (Ben-Yehoshua and Porat, 2005). For an overview of applications of hot water immersion and hot water rinsing and brushing treatments, the readers are referred to Fallik (2004).

Another commercially applied technique is heating of fungicidal solution especially for citrus disinfection, as it saves costs, cuts dosages and markedly reduces toxic residues (Ben-Yehoshua and Porat, 2005). Application of the fungicides imazalil and thiabendazole as hot (55 °C) solutions increases their fungicidal efficacy and allows their dosage to be reduced from 1000 ppm to 250 ppm (Schirra *et al*., 1998).

14.6 Future trends

Although heat treatments have potential for insect or fungal control, they are usually not as efficient as fungicides, they have no residual effects and they can be injurious to the host commodity (Barkai-Golan and Phillips, 1991). To overcome these problems, combinations with other treatments such as application of chemical compounds, biological agents, physical treatments or controlled atmospheres can be applied. Combination treatments can directly retain product quality, for example waxing or plastic wrapping to prevent condensation (when applying vapour heat-or hot dry air) or water loss (after hot water treatment) (Barkai-Golan and Phillips, 1991). On the other hand, combining treatments may allow the exposure temperatures and times to be less severe and thus can also indirectly result in better quality preservation. This is particularly interesting when it concerns heat-susceptible commodities. Marquenie *et al*. (2002) found that combining hot water treatment with pulsed light or ultraviolet treatment could reduce

fungal development in strawberries and cherries at temperatures that did not cause external damage to the heat-sensitive strawberries. An integrated approach that includes biological control, hot water treatment and modified atmosphere packaging was shown to be a good strategy for controlling post-harvest diseases of peaches during long-term storage, which could be easily implemented in commercial scale (Karabulut and Baykal, 2004).

The use of antagonists of fungal pathogens, which alone are good phytosanitary treatments but have no residual effect, may also offer a residual protection to heat treatments. Leverentz *et al*. (2000) found that the residual protection from bacterial and yeast antagonists against the pathogen *Penicillium expansum* on 'Gala' apples added to the control provided by the heat treatment.

Heated fungicides may be more effective than non-heated ones. The mechanism of control with heated fungicide mixes may be related in part to the direct effect of heat or to increased chemical activity, but control may also be improved by increased penetration and deposition of fungicide on the product when the treatment solution is heated (Wells and Harvey, 1970). Levels of chemicals usually employed at ambient temperatures can be reduced without compromising fruit quality and treatment efficacy. Hot drench with fungicides appears to be an attractive practical solution and is already being implemented in many packing houses, for example for citrus (Schirra *et al*., 2000; Ben-Yehoshua and Porat, 2005).

In Section 14.2 it has been stated that part of the beneficial effect of hot water application can be due to the change of the internal atmosphere of the fruit or vegetable. To obtain the same effect in hot dry air, the heat treatment can be applied in a controlled atmosphere. Neven and Mitcham (1995) found that combining a controlled atmosphere and temperature treatment (CATTS) looks promising for quarantine treatments of cherries, and even better for apples and pears. Shellie and Mangan (2000) showed that the altered atmosphere that developed in the fruits of mango, papaya, grapefruit and orange during controlled atmosphere–temperature treatment enhanced the disinfestation efficacy of heat. Hot water treatment could be used as a disinfectant for tomatoes prior to storage in modified atmosphere packages in order to reduce microbial growth, cracking and decay (Suparlan, 2003).

Apart from combinations with conventional heat treatments, new techniques based on thermal radiation are being investigated. Applications of electromagnetic radiation have great potential for quarantine disinfestation as high-temperature-short-time treatment, whereas far infrared radiation could be used for surface decontamination.

14.7 Sources of further information and advice

Research groups involved in post-harvest heat treatments are listed below. This list is intended to be neither comprehensive nor exclusive.

Department of Biological Systems Engineering, Washington State University, 213 L.J. Smith Hall, Pullman, WA 99164-6120, USA
http://www.bsyse.wsu.edu/tang/

This group studies fundamental principles and strategies to develop postharvest quarantine and phsytosanitary treatments based on microwave and radiofrequency energy to control insect pest in fruits and nuts for domestic and international trade. The laboratory is equipped with specialised pilot-scale RF and MW sterilisers for packaged foods, as well dielectric property systems for measurements over a wide range of frequency and temperature, and computer simulation packages to study electric field distributions.

Postharvest Tropical Commodities Research Unit, US Pacific Basin Agricultural Research Center, P. O. Box 4459, Hilo, Hawaii 96720
Tel: 808.959.4336. Fax: 808.959.5470.
http://pbarc.ars.usda.gov/

US Department of Agriculture, EPA (Environmental Protection Agency)
www.epa.gov

Among other research concerning environmental protection, the EPA performs research on alternatives to methyl bromide application among which are heat treatments. The results of the methyl bromide alternative case studies can be found at:
http://www.mbao.org/heatcom2.html

United States Department of Agriculture, Agricultural Research Service, Crop Quality and Fruit Insect Research Unit, 2301 S. International Boulevard, Weslaco, TX 78596, USA
http://weslaco.ars.usda.gov/

ARO (Agricultural research organisation), Postharvest Science of Fresh Produce, Volcani Centre, P.O. Box 6, Bet Dagan 50250, Israel
http://www.agri.gov.il/

Mann Laboratory, Department of Vegetable Crops, University of California, One Shields Ave, Davis, CA 95616-8631, USA
http://rics.ucdavis.edu/postharvest2/

In the frame of post-harvest integrated pest management research, benefits of post-harvest temperature management or temperature treatments for control of decay and insects have been examined.
HortResearch, Private Bag 921609, Auckland, New Zealand
http://www.hortresearch.co.nz/

Flanders Centre/Laboratory of Postharvest Technology, Willem de Croylaan 42, 3001 Heverlee, Belgium
www.vcbt.be

Books and book chapters on the subject:

MANGAN R L and HALLMAN G J (1998), 'Temperature treatments for quarantine security: New approaches for fresh commodities', in *Temperature Sensitivity in Insects and Application in Integrated Pest Management*, Hallman G J and Denlinger D L (eds), Boulder, Colorado, Westview Press, 201–34.

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SHARP J L and HALLMAN G J (1994), *Quarantine Treatments for Pests of Food Plants*, Westview Press, Boulder, Colorado, USA.

Supplier of hot water rinsing and brushing machines:
Juran Metal Works Ltd, 23 Shmotkin Street, Rishon Le Zion, 75363 Israel
http://www.juran.co.il/

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15

Antimicrobial films and coatings for fresh fruit and vegetables

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15.1 Introduction

Edible films are generally defined as continuous matrices that can be prepared from edible materials such as proteins, polysaccharides and lipids. They can be used as film wraps or pouches for food, or formed as film coatings on food or between food components (Cagri *et al*., 2004). When consumed with the food, an edible film or coating becomes an ingredient of the food.

Waxes such as carnauba wax have been used in fresh fruit and vegetable coatings to reduce moisture loss and add gloss since the 1930s in the United States (Cagri *et al*., 2004). Edible films/coatings are also currently used in a variety of other applications, including collagen casings for sausages, chocolate coatings for dry nuts and fruits and shellac coatings for chocolates and other confectioneries (Donhowe and Fennema, 1994; Cagri *et al*., 2004).

Interest in the application of edible coatings to fruits and vegetables has increased because they can be used to maintain fresh quality by controlling oxygen and carbon dioxide exchange between the product and the ambient atmosphere and by providing microbial stability for the product by incorporating antimicrobial agents (Wong *et al*., 1994; Cuq *et al*., 1995). Both functional approaches reduce the vulnerability of the product to microbial decay.

This chapter provides general information about edible films and coatings and their use with fruits and vegetables to reduce microbial growth. It reviews potential antimicrobial agents for antimicrobial films/coatings and summarizes methods for testing antimicrobial activity of films/coatings. Results of scientific investigations of edible films and coatings that

incorporate antimicrobials have been summarized. The focus of this chapter is on edible coatings formed on fruits and vegetables. However, many of the investigations involve study of antimicrobials in edible films that have been formed independent of a fruit or vegetable surface. Recommendations for future research topics are also included in this chapter.

15.1.1 Characteristics and functions of edible films and coatings

Edible films/coatings can control migration of gas, moisture, oil and fat, and solutes, as well as retain volatile flavor compounds. They can also improve structural integrity and mechanical handling properties and carry food additives so that they help maintain the quality of foods during marketing and even after packaging is opened (Mellenthin *et al.*, 1982; Kester and Fennema, 1986; Nisperos-Carriedo *et al.*, 1990; Donhowe and Fennema, 1994; Krochta and Mulder-Johnston, 1997).

The additives which can be incorporated into edible films and coatings can be selected to improve general coating performance such as strength, flexibility and adherence, to enhance product color, flavor and texture, and to control microbial growth (Cuppett, 1994). As an example, whey protein films/coatings can incorporate effective amounts of edible antimicrobial agents such as potassium sorbate, ethylenediaminetetra-acetic acid (EDTA), nisin and lysozyme (Han, 2000). The diffusion rates and the antimicrobial activities of the incorporated antimicrobial agents and the stability of film/coating forming solutions can be controlled by altering the pH and the volume ratio of the whey protein and the plasticizer (e.g. glycerol), because the charge density and the cavity size of the three dimensional protein network of the whey protein film is affected (Han, 2000).

Sensory appeal is an important functionality of an edible film/coating (Chen, 1995). Sensorial characteristics such as color, gloss, transparency, roughness or sticking can be modified. Edible films/coatings can protect or separate small pieces or food portions for individual consumption (Debeaufort *et al.*, 1998).

15.1.2 Use of edible coatings with fruit and vegetables

Interest in fitness has led to an increased demand for fresh fruits and vegetables (Labuza and Breene, 1988). High quality and microbial safety of fruits and vegetables have become increasingly important with the increased consumption of fruits and vegetables. In October 1998, the US Food and Drug Administration Center for Food Safety and Applied Nutrition (FDA CFSAN) and the US Department of Agriculture (USDA) jointly issued the *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*. This guide is intended to help growers, harvesters, packers and shippers address microbial safety hazards (Pabrua and Williams, 2004).

To understand the development and function of edible coatings for fresh fruit and vegetable products, it is necessary to understand fresh fruit and vegetable post-harvest physiology (Baldwin and Baker, 2002). Oxygen (O_2) levels inside coated fruits and vegetables should not become so low that they experience anaerobic reactions, which can result in off-flavors, abnormal ripening and spoilage (Kader, 1986). Respiration rates, storage temperature and type and thickness of applied coatings affect the production of ethylene. Ethylene stimulates ripening and senescence, thereby shortening shelf-life (Baldwin, 1994). Reduction of the O_2 concentration to less than 10% controls the respiration rate and slows down senescence. An O_2 level below 8% decreases ethylene production. However, an adequate O_2 concentration must be available to maintain aerobic respiration. A minimum of 1–3% O_2 is required to avoid a shift from aerobic to anaerobic respiration. Carbon dioxide (CO_2) levels above 5% prevent or delay many responses to ethylene by fruit tissue, including ripening. Possible heat accumulation from respiration inside films/coatings of fruits needs to be avoided because it can raise the tissue temperature, the rate of transpiration and thus shorten the shelf-life (Robertson, 1993).

The use of edible coatings with selective gas permeability to modify internal atmospheres of fruits and vegetables has been studied extensively during the past 20 years (Cuq *et al*., 1995; Cisneros-Zevallos and Krochta, 2003). Coatings of wax, cellulose derivatives, starch, gums, pectin and proteins were suggested for fruits such as grapes, apricots, bananas, guavas, mangoes and pineapples to lessen absorption of oxygen by the fruit and thereby slow respiration (Kester and Fennema, 1986; Hagenmaier and Baker, 1994; Debeaufort *et al*., 1998). Selective mass transfer properties are required for edible coatings to allow fruits and vegetables to respire slowly while limiting dehydration during storage (Ghaouth *et al*., 1991).

Growth of microorganisms on the surfaces of fruits and vegetables can result in production of uncharacteristic appearance, odors, and invasion of the interior of fruits and vegetables, accelerating further decay (Labuza and Breene, 1988). Spoilage of fruits and vegetables results in large economic loss (Wills *et al*., 1989). It has been estimated that 25–80% of harvested fresh fruits and vegetables are lost due to spoilage (Baldwin, 1994). There is also potential for the growth of human pathogens that can cause human disease. These microorganisms usually come from mishandling and cross-contamination (Labuza and Breene, 1988).

Surface treatments by spraying or dusting with antimicrobial agents or by dipping fruits and vegetables in antimicrobial solutions are widely practiced to improve microbial stability (Labuza and Breene, 1988). However, the antimicrobial agent rapidly diffuses away from the food surface into the food interior, with a resulting loss of the minimum inhibitory concentration required to inhibit microbial growth at the food surface. Another approach is the use of edible coatings that can provide a semipermeable barrier to gases and water vapor and incorporate antimicrobial agents that diffuse to

the coating-food interface and/or coating-air interface and inhibit target microorganisms (Baldwin, 1994; Vermeiren *et al*., 2002). Thus, the antimicrobial agent is maintained at the food surface where it is desired. Fruits and vegetables provide potential applications for antimicrobial coatings (Brody *et al*., 2001). Microorganisms on the surface or damaged area of fruits and vegetables can rapidly multiply through failure in temperature control and re-storage after opening. Microbial stability, related to post-processing contamination or multiplication, can be controlled by antimicrobial agents in coatings (Cagri *et al*., 2004).

Antimicrobial coatings have advantages over direct applications of the antimicrobial agents because the coating can be designed to slow antimicrobial diffusion from the surface. By slowing antimicrobial diffusion into food, the preservative activity at the surface of food is maintained. Thus, smaller amount of antimicrobials would be needed in an edible coating and only low levels of antimicrobials come into contact with the food to achieve a target shelf-life, compared to dipping, dusting or spraying antimicrobial agents onto the surface of the food.

When antimicrobial coatings are designed, care must be taken to maintain adequate O_2 levels inside fruits and vegetables. The O_2 should be maintained at a certain level to avoid anaerobic respiration and avoid the germination of bacterial pathogens such as *Clostridium botulinum* from their spores if the antimicrobial agents in the coating cannot prevent it.

15.2 Antimicrobial coatings for fresh fruit and vegetables

15.2.1 Pathogenic and spoilage microorganisms of concern to fruits and vegetables

Consumption of ready-to-eat (RTE) raw fruits and vegetables has increased since 1990 (Brackett, 1997; Ray, 2004) and is likely to become more popular because of their convenience in preparation (Brackett, 1997). Foodborne disease can be caused by the consumption of contaminated products. The incidence of foodborne disease from fruit and vegetable sources has increased (Tauxe, 1997; Tauxe *et al*., 1997).

Human pathogens that have growth potential on vegetables and fruits include *Salmonella*, *Escherichia coli* O157:H7, *Shigella*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter jejuni* (Ray, 2004). Fresh produce items associated with some foodborne diseases in recent years in the USA are listed in Table 15.1. Fruits and vegetables can be contaminated with *Campylobacter* spp. directly from fecal material from animals and infected humans or indirectly from sewage and contaminated water (Ray, 2004). Low temperature storage of fruits and vegetables may shift the conditions for the growth of psychrotrophic microorganisms and foodborne pathogens capable of growth at 5 °C. These microorganisms

Table 15.1 Survival of bacteria on fruits and vegetables

Produce	Product	Microorganism (microbial load)	Significance	Reference
Fruits	Watermelon	*Salmonella*	Foodborne diseases	Ray (2004)
	Citrus	*Vibrio cholerae*	Foodborne diseases	Pawsey (2002)
Vegetables	Sprouts	*Salmonella*, *E. coli* O157:H7	Foodborne diseases	Ray (2004)
	Lettuce	*E. coli* O157:H7, *Shigella* spp.	Foodborne diseases	Ray (2004)
	Cabbage	*L. monocytogenes*	Foodborne diseases	Ray (2004)
	Carrots	*E. coli*	Foodborne diseases	Ray (2004)
	Tomatoes	*Salmonella*	Foodborne diseases	Ray (2004)
	Scallions	*Shigella* spp.	Foodborne diseases	Ray (2004)
	Bean sprouts	Coliforms (~7 log cfu g^{-1})		Jinneman *et al.* (1995)
	Frozen corn	Coliforms (MPN < 20 g^{-1})		Barnard *et al.* (1982)
		E. coli (MPN < 3 g^{-1})		
	Frozen peas	Coliforms (MPN < 20 g^{-1})		Barnard *et al.* (1982)
		E. coli (MPN < 3 g^{-1})		

Note: MPN = most probable number.

include *Clostridium botulinum* type E, *Y. enterocolitica*, *E. coli*, *L. monocytogenes* and *Aeromonas hydrophilia* (Palumbo, 1986).

Microbial spoilage is another concern. It greatly impairs flavor, aroma and appearance of the product and is responsible for substantial economic loss (Nisperos-Carriedo *et al*., 1990). Types of spoilage vary with the kind of fruit or vegetable and even with the variety. Fruits and vegetables are spoiled by both fungi and bacteria (Goepfert, 1980). Table 15.2 lists examples of some common types of microbial spoilage of fruits and vegetables and the molds normally associated with them (Brackett, 1997). Fungal pathogens are the major cause of post-harvest spoilage of fruits and vegetables (Wong *et al*., 1994). The most common pathogens causing rot in harvested vegetables are fungi such as *Alternaria*, *Botrytis*, *Diplodia*, *Monilinia*, *Penicillium*, *Phomopsis*, *Rhizopus* and *Sclerotinia* and the bacteria *Erwinia* and *Pseudomonas*. The most commonly occurring types of microbial spoilage of fruits and vegetables are *Alternaria* rot, Anthracnose, Bacterial soft rot, Black rot, Black mold rot, Blight, Blue mold rot, Brown rot, *Cladosporium* rot, Crown rot, Downy mildew, *Fusarium* rot, Finger rot, Gray mold rot, Green mold rot, Leather rot, Lenticel rot, Phytophora rot, Pineapple black rot, Pink mold rot, *Rhizopus* soft rot, Soil rot, Sour rot (Oospora rot, Watery soft rot), Stem-end rot and Tuber rot (Frazier and Westhoff, 1988; Jay, 1996; Pitt and Hocking, 1997).

Microbial contamination is caused by (1) plant pathogens acting on the stems, leaves, flowers or roots of the plant, (2) saprophytic microorganisms, which may be secondary invaders after action of a plant pathogen as in the case of various rots (Frazier and Westhoff, 1988), and (3) microorganisms from a processing plant or human contact. The major sources of the microbial contamination from processing and humans are the lack of field sanitation and sanitation in handling fruits and vegetables, use of poor quality water for washing, poor quality air in the plant, transportation in dirty trucks and insects (Frazier and Westhoff, 1988; Brackett, 1997; Tauxe, 1997). Microbial populations increase in fruits and vegetables damaged by processing operations (Brackett, 1997). Fruits usually have 10^3–$10^4\,g^{-1}$ molds (Webb and Mundt, 1978) and $\geq 10^6\,g^{-1}$ bacteria (Frazier and Westhoff, 1988). Vegetables have approximately 10^3–$10^4\,g^{-1}$ molds (Webb and Mundt, 1978) and 10^3–$10^7\,g^{-1}$ bacteria (Splittstoesser, 1970).

The identification of spoilage and pathogenic microorganisms of fruits or vegetables makes possible the application of available or potential antimicrobial films/coatings for the prevention of spoilage and foodborne illness.

15.2.2 Edible coating materials

Polysaccharides, proteins and lipids are the major substances used to form a continuous matrix for edible coating formation. The choice of the substance depends on the specific application, i.e. type of food product and

Table 15.2 Microbial spoilage of fruits and vegetables

Produce	Product	Genus or species of microorganisms	Spoilage	Reference
Fruits	Apples	*Penicillium, Cryptosporiopsis malicorticus, Phylctaena vagabunda*	Blue rot, Lenticel rot	Pitt and Hocking (1997), Jay (1996)
	Apricots	*Alternaria, Botrytis, Aspergillus niger, Monilia fructicola*	*Alternaria* rot, Gray mold rot, Black mold rot, Brown rot	Pitt and Hocking (1997), Frazier and Westhoff (1988)
	Avocados	*Colletotrichum, Rhizopus*	Anthracnose, *Rhizopus* soft rot	Frazier and Westhoff (1988)
	Bananas	*Colletotrichum musae, Fusarium roseum, Verticillium theobromae, Ceratocystis paradoxa*	Anthracnose, Crown rot	Pitt and Hocking (1997), Jay (1996)
	Blackberries	*Penicillium, Botrytis, Rhizopus, Mucor*	Blue mold rot, Gray mold rot, *Rhizopus* soft rot	Pitt and Hocking (1997), Frazier and Westhoff (1988)
	Cherries	*Cladosporium herbarum, Monilia fructicola*	*Cladosporium* rot, Brown rot	Pitt and Hocking (1997), Jay (1996)
	Citrus fruits	*Alternaria, Geotrichum candidum, Penicillium digitatum, P. italicum, P. ulaiense, Botytis cinerea, Aspergillus* spp. *Rhizopus* spp., *Diplodia natalensis, Phomopsis citri, Phytophthora citrophthora*	*Alternaria* rot, Sour rot, Green mold rot	Pitt and Hocking (1997), Jay (1996), Suppakul *et al.* (2003)
	Grapes	*Botrytis cinerea, Kloeckera apiculata*	Gray mold rot	Pitt and Hocking (1997), Jay (1996)
	Kiwi	*Botrytis cinerea*	Stem-end rot	Pitt and Hocking (1997)
	Mangoes	*Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Dothiorella*	Anthracnose, Stem-end rot	Pitt and Hocking (1997)
	Papayas	*Colletotrichum, Mycosphaerella caricae, Fusarium*	Anthracnose, Stem-end rot, Black rot, *Fusarium* rot	Pitt and Hocking (1997)
	Peaches	*Cladosporium herbarum, Monilinia fructicola, Trichothecium*	*Cladosporium* rot, Brown rot, Pink rot	Pitt and Hocking (1997), Jay (1996)

	Pears	*Penicillium, Cryptosporiopsis malicorticus, Phylctaena vagabunda*	Blue rot, Lenticel rot	Pitt and Hocking (1997), Jay (1996)
	Pineapples	*Ceratocystis paradoxa, Penicillium purpurogenum, Fusarium moniliforme*	Black rot, Leather rot	Pitt and Hocking (1997)
	Strawberries	*Botrytis, Phytophthera cactorum, Mucor, Rhizoctonia solani*	Gray mold rot, Leather rot, *Rhyzopus* soft rot, Black rot	Pitt and Hocking (1997), Frazier and Westhoff (1988)
	Water melon	*Colletotrichum lagenarium*	Anthracnose	Pitt and Hocking (1997)
Vegetables	Asparagus	*Fusarium*	*Fusarium* rot	Jay (1996)
	Beans	*Colletotrichum lindemuthianum, Rhizoctonia solani*	Anthracnose, Soil rot	Pitt and Hocking (1997)
	Cabbage	*Botrytis, Alternaria, Phytophthora, Fusarium*	Gray mold rot	Pitt and Hocking (1997), Jay (1996)
	Carrots	*Alternaris*	Black rot	Jay (1996)
	Celery	*Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani, Sclerotinia*	Watery soft rot	Pitt and Hocking (1997), Jay (1996)
	Eggplant	*Phomopsis*	Blight	Jay (1996)
	Green beans	*Rhizopus, Pythium*	*Rhizopus* soft rot	Jay (1996)
	Lettuce	*Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani, Alternaria, Bremia, Phytophthora*	Downy mildew	Pitt and Hocking (1997), Jay (1996)
	Onions	*Aspergillus, Ceratocystis fimriata, Colletotrichum*	Black rot, Anthracnose	Jay (1996)
	Potatoes	*Fusarium*	Bacterial rot, Tuber rot	Pitt and Hocking (1997), Jay (1996)
	Spinach	*Erwinia, Pseudomonas, Clostridium, Bacillus, Botrytis*	Bacterial soft rot, Gray mold rot	Frazier and Westhoff (1988)
	Sweet potatoes	*Alternaria, Ceratocystis fimbriata, Rhizopus*	*Alternaria* rot, Black rot, *Rhizopus* soft rot	Pitt and Hocking (1997), Frazier and Westhoff (1988)
	Tomatoes	*Alternaria, Rhizopus, Botrytis, Cladosporium, Fusarium, Trichothecium roseum, Sclerotinia, Phytophthora, Pythium, Diaporthe*	*Alternaria* rot, *Rhizopus* soft rot, Gray mold rot, Green mold rot	Pitt and Hocking (1997), Frazier and Westhoff (1988)

main deterioration mechanisms (Cuq *et al*., 1995). Depending on the application, edible coatings need to fulfill a variety of requirements including good sensory qualities, high barrier and appropriate mechanical properties, physicochemical and microbial stability, application with simple technology and low cost of raw materials and process.

Coatings with substantial gas and moisture barrier properties are required for many applications (Cuq *et al*., 1995). Edible coatings with selective gas permeability can be applied to reduce degradation of some fresh fruits and vegetables. The exchange of O_2 and CO_2 between fruit or vegetables and the environment is a fundamental physiological phenomenon of post-harvest products (Burton, 1974; Cameron and Reid, 1982). The type of coating material applied alters the relative effects on the skin permeability to O_2 and CO_2 (Cuq *et al*., 1995). The control of gas exchanges results in improved control of the ripening of fruits (Debeaufort *et al*., 1998). For example, waxing increased the carbon dioxide content and decreased the oxygen content in the internal atmosphere of films/coatings for orange (Cuq *et al*., 1995). Water barrier efficiency of coatings is also desirable to retard the surface dehydration of fruits and vegetables (Debeaufort *et al*., 1998).

Edible coatings that have little or no taste are desirable to prevent detection during consumption (Contreras-Medellin and Labuza, 1981). If edible coatings have a particular taste or flavor, their sensorial characteristics need to be compatible with those of the food application (Biquet and Labuza, 1988). Some polysaccharide, protein and composite film/coating materials for fruits and vegetables are listed in Table 15.3.

Polysaccharides

Coatings for fruits have been developed from polysaccharides that attempt to create a modified atmosphere inside the fruit that will delay ripening and senescence in a manner similar to the more costly controlled atmosphere storage (Baldwin, 1994). The CO_2 and O_2 permeabilities of the polysaccharide-based coatings result in retardation of ripening in many fruits (Baldwin, 1994). Polysaccharide coatings exhibit effectiveness in control of gas exchange rather than retardation of water loss, owing to their good oxygen but poor moisture barrier properties (Baldwin, 1994). Careful control of relative humidity is important in maintaining control of gas exchange by edible coatings. High relative humidity (RH) conditions cause swelling of the polysaccharide matrix, resulting in increased permeability owing to increased gas solubility and diffusivity in the film/coating (Kumins, 1965). The incorporation of hydrophobic compounds such as fatty acids in a polysaccharide film to produce a composite film decreases moisture transfer and thus reduces increase in gas permeability at high RH (Coma *et al*., 2001).

Cellulose, starch and chitosan have been used to form edible films/coatings on foods to provide an oxygen or lipid barrier and to improve appearance, texture and handling (Krochta and Mulder-Johnston, 1997).

Table 15.3 Polysaccharide, protein and composite film/coating materials for fruits and vegetables and their functions

Produce	Product	Film and coating materials	Functions	References
Fruits	Apple	Carboxymethyl cellulose, chitin/chitosan, dextrin, zein, Composite I[a], mixture of SPI[b] and carboxymethyl cellulose, Nature Seal®	Both: oxygen (O_2) and carbon dioxide (CO_2) barrier. Dextrin: reduced browning. Zein: improved gloss and firmness and reduced weight loss. Composite I: semi-permeable modified atmosphere. Mixture of SPI and carboxymethyl cellulose: decrease in water vapor permeability and water loss. Nature Seal®: reduced browning	Murray and Luft (1973), Chu (1985), Drake *et al.* (1988), Davies *et al.* (1989), Baldwin *et al.* (1996), Xie and Hettiarachchy (1997), Baldwin and Baker (2002)
	Banana	Carboxymethyl cellulose, Composite I[a]	O_2 and CO_2 barrier Composite I: semi-permeable modified atmosphere	Banks (1984)
	Cherry	Semperfresh™	Moisture loss reduction	Drake *et al.* (1988)
	Lime	Composite I[a]	Semi-permeable modified atmosphere	Motlagh and Quantick (1988)
	Mango	Composite I[a], TAL Pro-long, Semperfresh™	Semi-permeable modified atmosphere Semperfresh™: improved firmness and color and reduction in weight loss	Dhalla and Hanson (1988), Carrillo-Lopez *et al.* (2000)
	Orange	Carboxymethyl cellulose	O_2 and CO_2 barrier	Nisperos-Carriedo *et al.* (1990)
	Peach	Beeswax coconut oil emulsion, chitin/chitosan	O_2 and CO_2 barrier	Erbil and Muftugil (1986), Davies *et al.* (1989)
	Pear	Carboxymethyl cellulose, chitin/chitosan, Composite I[a]	O_2 and CO_2 barrier Composite I: semi-permeable modified atmosphere	Elson *et al.* (1985), Meheriuk and Lau (1988), Davies *et al.* (1989)
	Prune	Starch, hydroxylpropyl starch derivative	Both: shelf life extension Hydroxylpropyl starch derivative: low O_2 permeability and semi-permeability of CO_2	Jokay *et al.* (1967)

Table 15.3 *Continued*

Produce	Product	Film and coating materials	Functions	References
	Strawberry	Chitin/chitosan, Composite II[c]	Chitin/chitosan: delayed spoilage. Composite II: changes in internal fruit atmosphere (O_2 decrease and CO_2 increase)	El Ghaouth *et al.* (1991a), Diab *et al.* (2001)
Vegetables	Bell pepper	Cellulose, chitin/chitosan	Cellulose: O_2 and CO_2 barrier. Chitin/chitosan: reduced respiration, color loss, wilting, fungal infection and the rate of ripening	El Ghaouth *et al.* (1991b), Lerdthanangkul and Krochta (1996)
	Carrot	Casein, casein-monoglyceride, xanthan gum	Casein, casein-monoglyceride: moisture retention. Xanthan gum: improved color	Avena-Bustillos and Krochta (1993), Mei *et al.* (2002)
	Celery	Carboxymethyl cellulose	Moisture barrier	Mason (1969)
	Cucumber	Chitin/chitosan	Degradation control, reduction in microbial degradation	El Ghaouth *et al.* (1991b)
	Green bell pepper	Sodium caseinate	O_2 and CO_2 barrier	Lerdthanangkul and Krochta (1996)
	Tomato	Zein, carboxymethyl cellulose, chitin/chitosan, Semperfresh™	Zein: moisture and oxygen barrier. Caroboxymethyl cellulose: O_2 and CO_2 barrier. Chitin/chitosan: retardation of ripening. Semperfresh™: delay changes in firmness, titratable acidity, pH, soluble solids, sugars, ascorbic acid and lycopene	Nisperos and Baldwin (1988), El-Ghaouth *et al.* (1992), Park *et al.* (1994 a,b), Tasdelen and Bayindirli (1998)
	Zucchini	Casein	Moisture barrier	Avena-Bustillos and Krochta (1993)

[a] Carboxymethyl cellulose/sucrose fatty acid esters/mono- and diglycerides; [b] soy protein isolate; [c] pullulan/sorbitol/sucrose fatty acid ester.

Cellulose

The usefulness of cellulose is extended by chemical modification to methyl cellulose (MC), hydroxylpropyl cellulose (HPC), hydroxylpropylmethyl cellulose (HPMC) and carboxymethyl cellulose (CMC) (Krochta and Mulder-Johnston, 1997). HPC is the only edible and biodegradable cellulose-derived polymer that is thermoplastic and, therefore, capable of injection molding and extrusion (Krochta and Mulder-Johnston, 1997).

Cellulose-based edible films/coatings have been used for fruit and vegetable coatings (Brody *et al*., 2001). Sucrose ester–carboxymethyl cellulose formulations retarded color development and retained acids and firmness compared to controls when tested on apples (Baldwin, 1994).

Starch

Starch films have a low permeability for O_2 and semi-permeability for CO_2 (Donhowe and Fennema, 1994). A solution of hydroxylpropyl starch derivative, which was high in amylose starch (70% w/v) with 12% glycerol as plasticizer, extended the shelf-life of pitted prunes (Jokay *et al*., 1967).

Chitin

Chitin is the second most abundant polysaccharide after cellulose and is widely distributed in nature (Hirano and Nagao, 1989). Research on edible film/coating development has focused on chitosan [(1,4)-linked 2-amino-2-deoxy-β-D-glucan], a deacetylated form of chitin (Hirano and Nagao, 1989). Chitosan is produced commercially by de-acetylating chitin obtained from shellfish waste. It is biodegradable but has not yet been approved as a food ingredient in the USA.

Chitosan films that are clear, tough and flexible and good oxygen barriers can be formed by casting from acidic (pH 2.5–4.9) aqueous solution (Sandford, 1989; Kaplan *et al*., 1993; Begin and Van Calsteren 1999).

Zhang and Quantick (1997) and El Ghaouth *et al*. (1992) suggested use of chitin and chitosan to make a transparent film for application as an edible film/coating on fruits and vegetables. Chitosan-based coatings can protect foods from fungal decay and modify the atmospheres of fresh fruits (Knorr, 1991; Krochta and Mulder-Johnston, 1997).

Many workers have noted that chitosan is inherently inhibitory to certain strains of bacteria and fungi (Popper and Knorr, 1990; Knorr, 1991). Chelation of essential metals such as zinc and the agglomeration capacity of the polycation for the anionic microbes are the proposed antimicrobial mechanisms of chitosan (Brody *et al*., 2001). Coma *et al*. (2002) reported an anti-*L. monocytogenes* effect of chitosan coating from 1% (w/w) chitosan film-forming solution.

Chitosan films/coatings retarded ripening and prolonged shelf-life of tomatoes, cucumber, strawberries and bell pepper fruit without affecting

their ripening characteristics (Hirano and Nagao, 1989; El Ghaouth *et al.* 1991; Cuq *et al.*, 1995).

Other polysaccharides

Pectin, guar gum, locust bean gum, tara gum, alginate, carageenan and pullulan films/coatings have been applied to a variety of foods because of their good oxygen barrier properties (Kester and Fennema, 1986; Aoyama *et al.*, 1993; Conca and Yang, 1993; Krochta and Mulder-Johnston, 1997). Guar gum, locust bean gum, tara gum and sodium alginate gel were used to extend freshness of fruits and vegetables (Lidster, 1981; Kumake, 1984; Aoyama *et al.*, 1993). Lidster (1981) suggested the use of xanthan gum as a post-harvest dip to prevent water loss from cherries.

Lipids

Many lipid compounds such as animal and vegetable fats, including natural waxes and derivatives, acetoglycerides and surface-active agents, have been used to make edible films/coatings (Kester and Fennema, 1986). They have very good moisture barrier properties, but stability (particularly oxidation), texture and sensory quality (opacity, waxy taste) are issues (Brody *et al.*, 2001).

Lipid compounds are often used to form moisture barrier films/coatings. For example, wax coating on fruits and vegetables reduced weight loss induced by desiccation during storage by 40–75% (Kaplan, 1986). The rate of transmission of water through a lipid film increases as the length of the lipid hydrocarbon chain decreases and the degree of unsaturation or branching of acyl chains increases (Kamper and Fennema, 1984, 1985). Oxygen permeability decreases as unsaturation (or branching) increases and the length of carbon chain decreases (Brody *et al.*, 2001).

The most well-known basic material for the coating of fresh fruits and vegetables is wax (Wong *et al.*, 1994). The most successful wax coatings have been made of beeswax, carnauba, candelilla, paraffin and rice bran wax (Kester and Fennema, 1986).

Wax coatings can be designed to (1) form an efficient barrier to moisture loss, (2) have a selective permeability to gases, (3) control migration of water-soluble solutes to retain the natural color pigments and nutrients, and (4) incorporate additives such as coloring, flavor or preservatives that impart specific functions and properties such as antimicrobial properties (Hagenmaier and Shaw, 1990).

Wax is commercially applied to many fruits and vegetables to reduce dehydration and to increase consumer appeal by decreasing shriveling and shrinkage. Fruits and vegetables that are coated include oranges, lemons, grapefruits, apples, tomatoes, rutabagas, cherries, cucumbers, apricots, bananas, dates, grapes, guavas, mangoes, peaches, nectarines, pears,

persimmons, pineapples, peppers, cantaloupes, honeydew melons, asparagus, beans, beets, carrots, celery, eggplants, kohlrabi, okra, parsnips, peppers, potatoes, radishes, squashes, sweet potatoes, sweetcorn, pumpkins and turnips (Hardenberg, 1967; Hall, 1981; Baldwin, 1994; Cuq *et al*., 1995).

Commercial wax coatings for citrus fruit are often composed of shellac and other ingredients (Baldwin, 1994). Green apples and pears were coated with paraffin, which resulted in delayed yellow color development, softening and onset of mealiness. All these observations were explained by increased CO_2 and reduced O_2 levels in the internal atmosphere of the apples and pears (Baldwin, 1994).

A finely divided crystalline-paraffin wax emulsion, paraffin wax, a sisal-paraffin, sugarcane and polyethylene wax emulsions increased shelf-life and decreased bruising of bananas (Baldwin, 1994). Carnauba wax was tested for its effect on internal atmospheres of mangoes and quality factors during storage at 10 or 15 °C with 90–99% RH followed by simulated marketing conditions of 20 °C with 56% RH. The coating created modified atmospheres, reduced decay and improved appearance by imparting a subtle shine. The carnauba wax coating significantly reduced water loss compared to uncoated mangoes (Baldwin *et al*., 1999). A candelilla coating extended shelf-life and slowed ripening of bananas (Siade and Pedraza, 1977).

Aqueous wax emulsions, consisting of vegetable (sisal, sugarcane and carnauba) waxes and mineral petroleum (paraffin) with and without shellac and emulsifiers increased shelf-life of mangoes, pineapples, bananas, papayas, guavas and avocados (Dalal *et al*., 1971). Room temperature storage life of guavas was extended 80% by coating with a 3% carnauba–paraffin wax emulsion (Srivastava *et al*., 1962).

Applications of fungicides in wax-based emulsions or water suspensions have been studied primarily on citrus fruits (Cohen, 1981; Cuppett, 1994). It has been reported that waxing of citrus fruit can adversely affect the fruit flavor (Cohen *et al*., 1990; Hagenmaier and Baker, 1993). This might be due to production of volatiles associated with anaerobic conditions, such as ethanol, methanol and acetaldehyde. Harvested tangerine fruits, especially those that are waxed, are particularly susceptible to off-flavor development (Cohen *et al*., 1990). Some commercial wax coating companies and their products were listed by Baldwin (1994).

Proteins

Proteins are also being studied for use in coatings for fruits and vegetables. Edible protein films are quite moisture sensitive, but their hydrophilicity makes them excellent barriers to non-polar substances such as oxygen and volatile flavor compounds (Miller and Krochta, 1997). An increase in crystallinity, density, orientation, molecular weight or cross-linking results in a

decrease in polymer permeability. Complicated protein structures make the control of these factors quite challenging (Miller and Krochta, 1997).

Proteins that have been studied for film formation include whey protein, collagen, gelatin, corn zein, wheat gluten, soy protein isolate, casein, maize and β-lactoglobulin. Edible protein films/coatings generally require plasticizers to improve durability or emulsifiers to increase the hydrophobic particle distribution in composite emulsion-based edible films (Debeaufort and Voilley, 1995). The large water vapor permeability of protein films/coatings can be lowered by including wax or other lipid materials in the formulation (Gontard *et al*., 1992; Avena-Bustillos and Krochta, 1993; McHugh and Krochta, 1994).

Perez-Gago and Krochta (1999) showed that aqueous solutions of native whey protein can form transparent, flexible and water soluble films with good barrier and mechanical properties. Heat denaturation of the whey protein solutions at specified conditions results in films with improved barrier and mechanical properties that are insoluble in water (Perez-Gago and Krochta, 1999, 2001). The modified properties of these denatured whey protein films are based on the addition of hydrophobic and disulfide bonds between adjacent protein molecules.

Collagen is a fibrous, structural protein in animal tissue that can be converted into edible and biodegradable films (Krochta and Mulder-Johnston, 1997). Collagen film is an excellent oxygen barrier at 0% RH, but oxygen permeability (OP) increases rapidly with increasing RH. Collagen is the most commercially successful edible protein film. Collagen casings have largely replaced natural gut casings for sausages (Krochta and Mulder-Johnston, 1997).

Gelatin is obtained by hydrolytic cleavage of collagen chains. Gelatin coatings reduce oxygen, moisture and oil migration and can carry an antioxidant or antimicrobial (Krochta and Mulder-Johnston, 1997).

Zein-based coatings were developed for whole apple as an alternative to shellac for a high-gloss fruit coating. The zein coated apples had similar firmness and weight loss values to those of commercial shellac- and carnauba-coated apples (Baldwin and Baker, 2002). Park *et al*. (1994a) applied zein coatings on the surfaces of tomatoes and reported that the coatings delayed color change, softening and weight loss.

Egg albumen and soy protein coatings significantly reduced moisture loss from coated raisins (Bolin, 1976). A soy protein coating was also used on dried fruits and vegetables as a moisture and oxygen barrier (Cole, 1969).

Other materials

Fruits and vegetables have been coated with an aqueous emulsion of lecithin–methyl anthranilate, a hydrolyzed lecithin coating, acrylate and methacrylate polymers, polymers of vinyl acetate and organic latex and copolymers (vinyl acetate, acrylate, ethyl acrylate and propyl acrylate) (Baldwin, 1994). All these films were claimed to have low water vapor

transmission and adequate permeability to O_2 and CO_2. Except methyl anthranilate, these polymers are acceptable for secondary direct food additives or indirect food additives (FDA, 1991). Microbial stability could be extended by adding fungicides (Baldwin, 1994).

Krochta *et al*. (1996) provided methods of preserving the natural color of fresh vegetables and fruits by applying an edible hygroscopic coating of either a hygroscopic salt or a lower alkyl polyhydric alcohol and storing the coated vegetable or fruit in a gas permeable plastic container capable of maintaining an internal humidity of between 90 and 100%. Preferred hygroscopic salts are $CaCl_2$, $MgCl_2$, NaCl and KCl. Preferred lower alkyl polyhydric alcohols are glycerol, polyglycerol, propylene glycol, sorbitol, mannitol 10 and polyethylene glycol 6000 (Krochta *et al*., 1996).

Composite films containing both lipid and hydrocolloid components have also been developed. A composite coating of alginic acid, casein and acetylated monoglyceride that cross-links with the addition of calcium into a three dimensional network was developed by the USDA Agricultural Research Service. This coating increased water vapor resistance, decreased respiration and decreased ethylene evolution of cut apples (Wong *et al*., 1994). Other examples of composite materials are listed in Table 15.3.

Mixtures of sucrose fatty acid esters have been commercially available since the 1980s for coating fruits and vegetables, under the trade names of TAL Pro-long (Courtaulds Group, London) and Semperfresh™ (United Agriproducts, Greeley, Colorado) (Park, 1999). Both TAL Pro-long and Semperfresh™ consist of sucrose esters of fatty acids, carboxymethyl cellulose sodium salt and mono- and diglycerides of fatty acids. TAL Pro-long has proven effective in extending the shelf-life of bananas, limes, apples and mangoes (Nisperos-Carriedo *et al*., 1990). Mango fruits also exhibited retarded ripening and therefore increased shelf-life when coated with 0.75–1.0% TAL Pro-long and stored at 25 °C (Dhalla and Hanson, 1988).

Semperfresh™ edible fruit coating was found to be significantly effective at storage temperatures of 12 and 23 °C in delaying changes in tomatoes in firmness, titratable acidity, pH, and contents of soluble solids, sugars, ascorbic acid and lycopene of tomatoes (Tasdelen and Bayindirli, 1998). Semperfresh™ was applied in combination with a shellac-wax to citrus fruits. This resulted in fruit with higher turgidity, less decay and enhanced good flavor, wherease ethanol levels were increased (Baldwin, 1994). Cherries coated with Semperfresh™ showed reduced moisture loss. However, the stem discoloration, which greatly influences consumer perception of cherry quality, was not reduced by Semperfresh™ (Drake *et al*., 1988). Semperfresh™ was also used to prolong the storage life of mangoes. Titratable acidity, firmness and green color were higher in coated fruits and weight loss and pH were lower compared with the non-coated fruit. Semperfresh™ retarded ascorbic acid decrease in mangoes (Carrillo-Lopez *et al*., 2000).

Another commercial coating, Nature Seal® (EcoScience Corp., Orlando, FL), was tested on cut apples. Browning of the cut surface was reduced by the coating (Xie and Hettiarachchy, 1997).

15.2.3 Antimicrobial agents suitable for fruits and vegetables

Common antimicrobial agents for food products are organic acids (e.g. sorbic, propionic and benzoic) and their salts, sulfites, nitrites, antibiotics, bacteriocins (e.g. nisin, pediocin), enzymes (e.g. lysozyme) and alcohols, metals and fungicides (e.g. benomyl, imazalil) (Suppakul *et al*., 2003). Several other compounds have been proposed or tested including carbon dioxide, sulfur dioxide, grapefruit seed extract, peroxide, ozone, chlorine oxide, eugenol, cinnamaldehyde, allyl isothiocyanate, and EDTA (Brody *et al*., 2001). Future work in antimicrobial films/coatings may focus on the application of natural or biologically derived antimicrobial materials that are bound or incorporated into films (Hoover and Steenson, 1993).

Organic acids and their salts

Benzoic acid, lactic acid and sorbic acid are potential antimicrobial agents for incorporation in edible films/coatings for fruits and vegetables (Cagri *et al*., 2004). Potassium sorbate or sorbic acid, which have a wide range of bacteriostatic and mycostatic properties, can be used by dipping to reduce the total number of viable bacteria at both refrigeration and elevated temperatures (Suppakul *et al*., 2003). Human pathogens including *L. monocytogenes*, *E. coli* and *Salmonella* spp. in fruits and vegetables were inhibited by organic acids and their salts (Cagri *et al*., 2004).

Films that were constructed from cellulose derivative and fatty acids to control the release of sorbic acid and potassium sorbate were considered very practical for application to fruits and vegetables (Hotchkiss, 1995).

Antimycotics

Edible films/coatings can contain antimycotics (fungicides) for use as antimicrobial films/coatings (Brody *et al*., 2001). Application of fungicides in wax-based emulsions or water suspensions has been studied primarily on citrus fruit, where the fungicide would not be consumed. Benomyl, thiabendazole and imazalil are applied to citrus fruits to suppress their post-harvest deterioration caused by *Penicillium* spp., *Diplodia natalensis*, *Phomopsis citri* and *Phytophthora citrophthora* (Cohen, 1981; Cuppett, 1994). Fungicides used with a wax emulsion controlled post-harvest diseases of peaches and nectarines caused by *Monilinia fructicola* and *Rhizopus stolonifer* (Wells, 1971). Imazalil-bound ionomer controlled the contamination of peppers (Halek and Garg, 1989). Captan (*N*-[(trichloromethyl)thio]-

4-cyclohexene 1,2-dicarboximide), dithane M45 (coordination of zinc and manganese ethylenebisdithiocarbamate), sodium *o*-phenyl phenate and thiram [tetramethylthiuramdisulfide: bis(dimethylthiocarbamoyl disulfide)] showed potential as fungicide treatments to control *Colletotrichum*, *Phytophthora*, *Rhizopus*, *Botrytis*, *Penicillium* and other pathogens on tomatoes when applied in a wax solution (Domenico *et al*., 1972).

Nisin

Nisin interacts with the sulfur-containing compounds in the bacterial membrane, disrupting their semi-permeable function and causing lysing of the cells (Thomas *et al*., 2000). Nisin has been tested as an antimicrobial agent to be incorporated into polymeric film materials (Siragusa *et al*., 1999; Natrajan and Sheldon, 2000). Nisin has been accepted by regulatory authorities in some countries, such as Japan, for food use (Brody *et al*., 2001).

MC/HPMC films containing nisin were effective in inhibiting *S. aureus* and *L. monocytogenes* (Cooksey, 2000). Eswaranandam *et al*. (2004) studied the effectiveness of partial replacement of the plasticizer glycerol with citric, lactic, malic and tartaric acids on the antimicrobial activities of nisin (205 IU g^{-1} protein)-incorporated soy protein film against *L. monocytogenes*, *E. coli* O157:H7 and *S. gaminara*. They found that malic acid (2.6%)-incorporated soy protein film without nisin had the fewest survivors of *L. monocytogenes*, *E. coli* O157:H7 and *S. gaminara*.

Lysozyme

Lysozyme may be applied to antimicrobial films/coatings. Lysozyme is a 15 kDa single-chain protein (Shah, 2000). Lysozyme inactivates bacteria via hydrolysis of glucosidic linkages in the peptidoglycan of cell walls. Specifically, lysozyme hydrolyses β-1,4 linkages between *N*-acetylmuramic acid and 2-acetyl-amino-2-deoxy-D-glucose residues in bacterial cell walls, resulting in cell lysis (Shah, 2000). Lysozyme is usually active against a number of Gram-positive bacteria such as *L. monocytogenes* (Losso *et al*., 2000). Lysozyme has been widely used to control lactate fermentation by *Clostridium tyrobutyricum* in semi-hard and hard, brine-salted cheeses (Walzem *et al*., 2002).

Appendini and Hotchkiss (1997) investigated the efficiency of lysozyme immobilized on different polymers. Cellulose triacetate (CTA) containing lysozyme yielded the highest antimicrobial activity. The viability of *Micrococcus lysodeikticus* was reduced in the presence of immobilized lysozyme on CTA film. Antimicrobial activity of lysozyme in soy protein isolate films and corn zein films was also demonstrated by Padgett *et al*. (1998).

Plant extracts

Compounds of plant origin including extracts of grapefruit seed, cinnamon, allspice, clove, thyme, rosemary, onion, garlic, radish, mustard, horseradish

and oregano showed antimicrobial activity (Brody *et al*., 2001; Suppakul *et al*., 2003). These compounds can be added into foods to extend microbial shelf-life without labeling as antimicrobial agents or preservatives (Suppakul *et al*., 2003). Chung *et al*. (1998), Hong *et al*. (2000) and Ha *et al*. (2001) recently reported antimicrobial properties of food packaging films incorporating some plant extracts, including grapefruit seeds and *Coptis chinensis* (Huang Lian). The effect of the incorporation of these extracts on sensory properties has not been reported. However, it is recommended that the influence of incorporation of any antimicrobial compounds into edible films and coatings on sensory properties be studied.

Silver ion antimicrobials

Silver ion antimicrobials including silver-zeolite salts and silver (oxide) ions have been accepted for food contact uses by regulatory authorities in Japan (Brody *et al*., 2001). These silver ion antimicrobials may have potential for use in antimicrobial edible films and coatings in a limited concentration. In the USA, the standard for silver content in drinking water is set at <50 ppb on the basis of a silver-containing medicine that causes angina symptoms (Brody *et al*., 2001). Silver is permitted in certain foodstuffs as a colorant even though the number of the foodstuffs is very small (Vermeiren *et al*., 2002).

Commercial antimicrobial materials containing silver-zeolite or silver (oxide) ions have not been approved for use with food-related applications by FDA. However, some of the materials have been marketed in some European countries and Japan (Brody *et al*., 2001; Vermeiren *et al*., 2002). The silver ion antimicrobial-containing materials have been incorporated in plastic resin such as acrylonitrile butadiene styrene, polypropylene and low density polyethylene (Brody *et al*., 2001).

Silver zeolites are crystalline aluminosilicate materials continuously releasing a small amount (~10 ppb) of silver ions resulting in long-term antimicrobial activity (Matsuura *et al*., 1997). Silver ions act against microorganisms by displacing other essential metal ions such as Ca^{2+} or Zn^{+} (Vermeiren *et al*., 2002). The binding of silver to microbial DNA can inhibit transport processes such as phosphate and succinate uptake and can interact with cellular oxidation processes as well as the respiratory chain (Vermeiren *et al*., 2002).

Schierholz *et al*. (1998) pointed out that silver is probably the most useful among heavy metals as it combines a high antimicrobial activity with a significantly low human toxicity. However, silver zeolite and silver ions do not inhibit microorganisms efficiently in nutrient-rich culture media (Brody *et al*., 2001). Films and coatings containing the silver zeolite or silver ions may be designed to prevent post-microbial contamination occurring on the surface of packaging materials, which do not provide a nutrient-rich environment for microorganisms.

15.2.4 Evaluation methods for antimicrobial films and coatings

Several methods have been used by researchers to evaluate the antimicrobial properties of films/coatings. Data obtained from these methods have been published in many scientific journals and, thus, the methods are considered reliable and reproducible. The evaluation methods can be divided into three main categories, according to nature of the test. One of the categories measures the antimicrobial activity of the film/coating formation solution. A second category measures the antimicrobial activity of films. The third category measures the effect of films/coatings on the microbial growth on foods. Recently applied methods to evaluate the effect of edible antimicrobial films and coatings on the inhibition of microorganisms are shown in Table 15.4.

Inhibition zone test (antimicrobial effectiveness of film/coating formation solution)

A lawn of a target microorganism on agar plates (8.5 cm diameter) is formed by overlaying a 0.5–0.8% (w/v) agar seeded with the target microorganism. Microbial density of the lawn ranges are 10^4–10^6 cfu/plate. A film-forming solution is dropped on the lawn of a target microorganism. Different dilutions of the film-forming solution can be tested. Dishes are refrigerated at 4 °C for 3 h to allow diffusion of the antimicrobial agent and then incubated at 30 °C for 24–48 h (Sebti *et al.*, 2002).

Disc diameter test (antibacterial effectiveness of film discs)

A lawn of a target microorganism is prepared on the agar plate as described in the inhibition zone test. Circular film discs (0.5–2.0 cm diameter) containing antimicrobial agents are placed on the lawn. After appropriate incubation, the clear zone of the growth inhibition in the bacterial lawn is visually examined and the size of the clear zone around the film disc is measured at the nearest 1 mm (Coma *et al.*, 2002; Sebti *et al.*, 2002; Eswaranandam *et al.*, 2004). Results as a relative percentage of inhibition (RPI) are calculated using the following relation: RPI (%) = (diameter of inhibition zone of film samples/diameter of inhibition zone of film sample without fatty acid) × 100 (Coma *et al.*, 2001; Cutter *et al.*, 2001; Sebti *et al.*, 2002).

Surface spreading test (antibacterial effectiveness of film discs)

The piece of film is cut into a desired size (0.5–2.0 cm diameter) and is placed on top of the solidified agar medium. A microbial inoculum is then spread all over the plate (10^4–10^6 cfu/plate) and the plate is incubated (Halek and Garg, 1989). This test simulates the situation of post-contamination by microorganisms on the surface of edible films/coatings.

Direct inoculation method (antibacterial effectiveness of film discs)

A certain volume (e.g. 15 µl) of a microbial suspension (10^5–10^9 cfu ml^{-1}) is inoculated on film discs. The films are incubated for a selected time (e.g.

Table 15.4 Effects of edible antimicrobial films and coatings on the inhibition of microorganisms

Methods for testing inhibition	Base materials for films/ coatings	Target microorganisms	Antimicrobial agents
Disc diameter test	Soy protein isolate, corn zein	*Lactobacillus plantarum, E. coli*	Lysozyme, nisin, EDTA
Disc diameter test	Whey protein isolate (WPI)	*L. monocytogenes, E. coli* O157:H7*, Salmonella enterica* subsp. *enterica* serovar Typhimurium DT104	*p*-aminobenzoic acid (PABA), sorbic acid
Disc diameter test	Hydroxy propyl methyl cellulose (HPMC)	*Micrococcus luteus, Listeria innocua, Staphylococcus aureus*	Nisin
Inhibition zone assay, disc diameter test	Hydroxy propyl methyl cellulose (HPMC)	*Micrococcus luteus, L. monocytogenes, S. aureus*	Nisin
Disc diameter test, plate counting method	Chitosan	*L. innocua* 430, *L. monocytogenes*	Chitosan
Disc diameter test, plate counting method	Soy protein isolate	*L. monocytogenes*, *E. coli* O157:H7, *Salmonella gaminara*	Citric acid, lactic acid, malic acid, tartaric acid, nisin
Direct inoculation method	Soy protein isolate, spray-dried wheat gluten, egg albumen protein, WPI	*L. monocytogenes*	Nisin

Media/food used for tests	Effects	Reference
Lactobicilli MRS agar, trypticase soy agar	Inhibition of bacterial growth	Padgett *et al.* (1998)
Trypticase soy agar + 0.6% yeast extract (TSAYE)	Inhibition of bacterial growth	Cagri *et al.* (2001)
Nutritive agar, tryptose agar	All bacteria were inhibited by the films. The more the stearic acid concentration increased, the less was the residual inhibitory activity of films. Electrostatic interactions between stearic acid and nisin were especially presumed to be responsible for the lower antimicrobial activity of HPMC films	Coma *et al.* (2001)
Nutritive agar, tryptose agar	All bacteria were inhibited by the films. Antimicrobial activity on *L. monocytogenes* and *S. aureus* were reduced when stearic acid was incorporated. A decrease of film pH induced an increasing antimicrobial activity in the film	Sebti *et al.* (2002)
Tryptose broth and agar, Emmental cheese	Bactericidal activity with 1% (w/v) chitosan in the film forming solution	Coma *et al.* (2002)
Brain, heart infusion agar, nutrient agar	Inhibition of bacterial growth. Malic acid (2.6%)-incorporated film had the highest inhibition of *L. monocytogenes*, *E. coli* O157:H7, *S. gaminara* (5.5, 6.8, 3.0 log cfu ml^{-1}, respectively).	Eswaranandam *et al.* (2004)
Films	Inhibition increased as the nisin concentration increased. ~2 log reduction (inoculum size: ~5 log cfu ml^{-1}) by 160 IU nisin in whey protein isolate films	Ko *et al.* (2001)

Table 15.4 *Continued*

Methods for testing inhibition	Base materials for films/ coatings	Target microorganisms	Antimicrobial agents
Direct inoculation method, plate counting method	Soy protein isolate	*L. monocytogenes*	Lauric acid, nisin
Plate counting method	Methyl cellulose, stearic acid or palmitic acid	*Zygosaccharomyces rouxii*, *Zygosaccharomyces mellis*	Benzoic acid
Plate counting method	Gelatin	*Brochothrix thermosphacta*, *E. coli* O157:H7, *Lactobacillus sakei, Leuconostoc mesenteroides, L. monocytogenes, Salmonella* Typhimurium	Lysozyme, nisin, EDTA
Plate counting method	Semperfresh™	Endogenous molds and yeasts	Modified atmosphere
Plate counting method	WPI	*L. monocytogenes, E. coli* O157:H7, *S. enterica* subsp. *enterica* serovar Typhimurium DT104	*p*-aminobenzoic acid (PABA), sorbic acid
Plate counting method	Calcium caseinate and WPI	Endogenous microorganisms of total aerobes, total *Enterobacteriacea*, lactic acid bacteria, *Brochothrix thermosphacta,* presumptive *Pseudomonsas* spp.	Spice powders
Plate counting method	WPI	*L. monocytogenes,* mesophilic aerobic bacteria (MAB), lactic acid bacteria (LAB) and yeast/mold	*p*-aminobenzoic acid (PABA), sorbic acid

Media/food used for tests	Effects	Reference
Turkey bologna	Films containing both lauric acid and nisin completely inhibited cells from a 10^6 cfu after 8 h of exposure to 1% peptone medium at 22 °C. Films with lauric acid, nisin or both reduced the cell number of turkey bologna by 1 log after 21 days at 4 °C.	Dawson *et al.* (2002)
Taiwanese-style fruit preserves	Complete inhibition with 72–82 μg/(g preserve) benzoic acid	Chen *et al.* (1999)
Ham, bologna	Coating treatment resulted in bactericidal effects up to 4 log cfu cm^{-2} on *B. thermosphacta*, *Lactobacillus sakei*, *Leuconostoc mesenteroides*, *L. monocytogenes*, *S.* Typhimurium and inhibited the growth of thesemicroorganisms during the 4 weeks of storage at 8 °C. No inhibition effect was observed on *E. coli* O157:H7 on bologna	Gill and Richard (2000)
Cherries	Semperfresh™ coating increased fungal spoilage slightly.	Yaman and Bayindirh (2001)
Bologna, summer sausage	WPI-based films with PABA and sorbic acid decreased the number of *L. monocytogenes*, *E. coli* and *S.* Typhimurium by 3.4 to 4.1, 3.1 to 3.6, and 3.1 to 4.1 logs, respectively on bologna and summer sausage after 21 days at 4 °C.	Cagri *et al.* (2002)
Ground beef containing ascorbic acid at 0.5% (w/w)	A 1 log reduction in total aerobes. No significant inhibition effects on *Enterobacteriacea*, lactic acid bacteria, *Brochothrix thermosphacta,* presumptive *Pseudomonsas* spp.	Ouattara *et al.* (2002b)
Hot dog	The number of *L. monocytogenes* remained relatively unchanged. Populations of MAB, LAB and yeast/mold on WPI-1.0%-PABA casings were 1 to 3 logs lower compared to collagen and natural casings.	Cagri *et al.* (2003)

5 days). Visual observation, plate counting tests and epifluorescence tests (Coma *et al*., 2002) can be conducted to examine antimicrobial activity of the films. For example, if plate counting tests are used, after incubation, the film discs are placed into stomacher bags. The disc is diluted with a buffer or 0.1% peptone water in the bag and then stomached. The homogenate from stomaching is plated onto agar plates after appropriate dilutions and then the plates are incubated. The number of colonies is counted after incubation (Ko *et al*., 2001; Dawson *et al*., 2002; Eswaranandam *et al*., 2004).

Plate counting method (effects of films/coatings on the microbial quality of foods)
Food samples are coated or contacted with films. Each treated sample is inoculated with a desired number of microbial inoculum (e.g. 10^4 cfu). Inoculation can be done before or after coating or film contact by spotting and spreading. Inoculated samples are incubated for a certain time at a certain temperature (e.g. 4 °C, 10 °C, 25 °C) and sampled after every selected period of time. Samples are blended with a buffer solution or 0.1% peptone water by a stomacher. From the resulting homogenate, serial dilutions are prepared and the appropriate dilutions are spread onto agar plates. The plates are incubated as desired. Survival of microbial cells is measured by their colony forming ability on the plates. Reduction of growth rate and reduced maximum growth population indicate improved microbial safety, and the extended lag period shows the prolonged shelf-life with microbial quality assurance (Chen *et al*., 1999; Yaman and Bayindirh, 2001; Ouattara *et al*., 2002a).

Headspace gas composition assay (effects of films/coatings on the microbial quality of foods)
The rate of increase in CO_2 in the headspace of sealed glass jars may be used as a measure of mold growth. Food samples are coated or contacted with films either before or after inoculation with a target number of fungal spores (e.g. 10^4 spores). The samples are placed in glass jars. The jars are sealed and incubated at 25 °C. The headspace gas composition is measured during storage using gas chromatography (Weng and Hotchkiss, 1991, 1992).

15.2.5 Results of studies using evaluation methods

Inhibition zone test
A HPMC film-forming solution containing nisin (5×10^4 IU ml^{-1}) and stearic acid (15% w/w HPMC) inhibited *Micrococcus luteus* IP 270 (Sebti *et al*., 2002). Data indicated that a decrease of film pH induced an increasing film antimicrobial activity against *M. luteus* IP 270.

Disc diameter test

Soy protein isolate films were prepared by a heat-press method and corn zein films were made by the heat-press method and a casting method. Those films incorporated either lysozyme or nisin in combination with EDTA and were evaluated for inhibition against *Lactobacillus plantarum* and *E. coli* (Padgett *et al*., 1998). The concentrations of lysozyme, nisin and EDTA incorporated into the films were 2.5–141 mg g^{-1} film, 0.01–40 mg g^{-1} film, and 15–60 mM, respectively. The lysozyme and nisin maintained their antimicrobial activities against *L. plantarum* and *E. coli* throughout both the heat-press and cast film-forming processes. The addition of EDTA increased the inhibitory effect of films against *E. coli*. The cast films showed larger inhibitory zones, compared to the heat-press films when the same levels of lysozyme or nisin were used.

Clear zones of inhibition of spoilage bacteria, *Brochothrix thermosphacta*, were observed around discs of whey protein film containing lysozyme (Han, 2000). He reported 25 mg g^{-1} as the critical inhibitory concentration.

Cagri *et al*. (2001) developed low pH (5.2) whey protein isolate (WPI)-based edible films containing *p*-aminobenzoic acid (PABA) or sorbic acid (SA). The WPI films containing 0.5–1.5% PABA or SA inhibited *L. monocytogenes*, *E. coli* O157:H7 and *S.* Typhimurium DT104.

Sebti *et al*. (2002) incorporated nisin into a HPMC-based film and found that the film had antimicrobial activity against *L. monocytogenes* and *S. aureus*. The antimicrobial activity was reduced when the amount of stearic acid incorporated together with nisin increased.

Zones of inhibition of *L. monocytogenes* by soy protein films increased in the presence of nisin (205 IU g^{-1} protein) and 0.9%, 1.8% and 2.6% citric acid (1.6, 2.4 and 4.0 mm), lactic acid (<0.5, 1.5 and 2.0 mm), malic acid (1.5, 3.0 and 5.5 mm) or tartaric acid (2.0, 3.5 and 4.8 mm) in the films, compared with a control (soy protein films without organic acid) at pH 4.55, 3.85 and 3.35 (<0.5, 1.0 and 2.0 mm) (Eswaranandam *et al*., 2004).

Surface spreading test

Antimicrobial activity of edible films and coatings, analyzed by a surface spreading test method, has not been reported. A fungicide (benomyl) was chemically coupled to an ionomeric packaging film containing pendent carboxyl groups (Halek and Garg, 1989). The film was cut into 1 inch × 1 inch (2.5 cm × 2.5 cm) and was placed on top of a solidified agar medium. Spores of *Aspergillus flavus* and *Penicillium notatum* were spread on the whole surface of the medium. Growth was checked daily for 21 days. Both microorganisms were successfully inhibited by the film, which was shown as zones of inhibition on the agar medium after incubation (Halek and Garg, 1989).

Direct inoculation test

Effects of soy protein isolate (SPI) films incorporating nisin (120 IU/film disk; 0.02 g) on inhibition of *L. monocytogenes* were studied by Ko *et al.* (2001). About a 2 log reduction (from 5.2 to 3.0) of *L. monocytogenes* by the films occurred by 60 min. There was no further reduction in bacterial counts from 60–120 min incubation at ambient temperature. SPI films incorporating 4% nisin and 8% lauric acid completely inhibited detectable cells of *L. monocytogenes* from 10^6 cells after 8 h exposure (Dawson *et al.*, 2002).

Plate counting method

Chitosan films, made from dilute acetic acid solutions, inhibited the growth of *Rhodotorula rubra* and *Penicillium notatum* when the film was applied directly to the colony forming organism (Chen *et al.*, 1996). Since chitosan is soluble only in slightly acidic solutions, chitosan films would be prepared with the film forming solution containing an organic acid and the salt, which can result in improved antimicrobial properties (Suppakul *et al.*, 2003).

Cagri *et al.* (2002) reported that whey protein isolate films (pH 5.2) incorporating 0.5–1.0% *p*-aminobenzoic acid (PABA) and/or sorbic acid (SA) reduced the numbers of *L. monocytogenes*, *E. coli* and *S.* Typhimurium in bologna and summer sausage slices by 3.4–4.1, 3.1–3.6 and 3.1–4.1 logs, respectively, after 21 days at 4 °C.

Dawson *et al.* (2002) reported that soy protein isolate films incorporating 4% nisin and 8% lauric acid reduced the number of *L. monocytogenes* in turkey bologna from 10^6 to 10^5 after 21 days. The films with lauric acid alone reduced the cells by 1 log in turkey bologna after 21 days at 4 °C.

The effects of partial replacement of glycerol (a plasticizer) with citric, lactic, malic and tartaric acids on the antimicrobial activity of soy protein films incorporating nisin against human pathogens on agar plates were studied (Eswaranandam *et al.*, 2004). Malic acid (2.6%)-incorporated soy protein films decreased *L. monocytogenes*, *E. coli* O157:H7 and *S. gaminara* log number cfu ml^{-1} from 8.3, 8.9 and 9.0 to 5.5, 6.8 and 3.0, respectively. Citric acid (2.6%)-incorporated films reduced *L. monocytogenes*, *E. coli* O157:H7 and *S. gaminara* by 0, 0.5 and 0.8 log cfu ml^{-1}, respectively, whereas lactic acid (2.6%)-incorporated films produced reductions of 1.2, 1.6 and 5.8 log cfu ml^{-1}, and tartaric acid (2.6%)-incorporated films gave reductions of 0, 0.1 and 2.5 log cfu ml^{-1}, respectively.

Headspace gas composition assay

Antimicrobial activity in edible films and coatings, tested by headspace gas composition assay, has not been reported. Antimicrobial activity in a synthetic film (LDPE film) incorporating imazalil was analyzed by this assay by Weng and Hotchkiss (1991, 1992). The antimicrobial activity of the LDPE film was tested on *Penicillium* sp. and *Aspergillus toxicarius*. An LDPE film incorporating imazalil (2000 mg kg^{-1} film) delayed the growth of

A. toxicarius. With 1000 mg kg^{-1} imazalil, the growth of *Penicillium* sp. was markedly delayed. LDPE film containing 1000 mg kg^{-1} imazalil inhibited both molds growing on Cheddar cheese (Weng and Hotchkiss, 1992).

The results of antimicrobial edible film and coating studies published between 1998 and 2004 are summarized in Table 15.4, together with the evaluation methods used.

15.2.6 Results from other antimicrobial film and coating studies

Thiabendazole was used in a carnauba wax formulation for papaya fruit to control post-harvest diseases of anthracnose (*Colletotrichum glocosporiodes*), Stem-end rot (*Ascochyta caricaepapayae* Tarr and *Botryodiplodia theobromae* Pat.), Peduncle rot (*Fusarium* spp. and *Cladosporium* spp.) and *Rhizopus* rot for up to 14 days at 10 °C followed by ripening at room temperature. Slightly improved control of the diseases was achieved when thiabendazole in wax was applied immediately after a short hot water dip (54 °C, 1.5 min) (Couey and Farias, 1979).

The effects of a chitosan coating containing the fungicide, iprodione (Rovral®) on *Botrytis cineria* inoculated strawberries were studied (Ghaouth *et al*., 1991). The application of fungicide within the coating significantly reduced the microbial growth at 4 and 13 °C when compared to the untreated control. The coating also prevented outgrowth of mold for a longer period of time than did the use of the aqueous dip (Ghaouth *et al*., 1991).

Edible coatings composed of methyl cellulose, glycerol and fatty acid (stearic acid or palmitic acid) were tested as the preservative carriers for inhibition of surface microbial growth (Chen *et al*., 1999). Taiwanese-style fruit preserves obtained from plum were covered by edible coatings containing benzoic acid. The edible coatings showed antimicrobial activity against *Zygosaccharomyces rouxii* and *Zygosaccharomyces mellis*, without significantly different sensory qualities from those of uncoated samples (Chen *et al*., 1999).

TAL Pro-long coating, consisting of sucrose esters of fatty acids, (carboxymethyl) cellulose sodium salt and mono- and diglycerides of fatty acids, increased resistance to some fungal rots in apples, pears and plums. The microorganisms causing the rots include *Sclerotina* spp. and *Rhizopus nigricans* (Baldwin, 1994).

15.3 Recommendations

Edible films/coatings are very promising systems for the improvement of food preservation and quality (Debeaufort *et al*., 1998). Several issues associated with edible films/coatings must be addressed in developing commercial products.

15.3.1 Selection of film/coating materials and the thickness of films/coatings

Modification of internal atmospheres by the use of edible coatings can increase disorders associated with high carbon dioxide or low oxygen concentration (Ben-Yehoshua, 1969). Even though some edible coatings have been successfully applied to fresh produce, other applications have adversely affected quality (Park, 1999). Park *et al*. (1994a, b) reported that tomatoes coated with a 2.6-mm zein film produced alcohol and off-flavors internally, which were attributable to an internal gas composition too low in O_2 and too high in CO_2. Anaerobic fermentation of banana was accelerated and incidence of decay in cucumbers increased (Risse *et al*., 1987). Smith *et al*. (1987) summarized the effects of the modification of internal atmosphere by the use of coatings on the physiological disorders of core flush, flesh breakdown, accumulation of ethanol and generation of off-flavors.

Selection of edible coating materials and the thickness of coating need to be made based on the characteristics of the fruit or vegetable to be coated. Gas permeation properties and internal gas compositions for fruit and vegetable coated with the edible films/coatings should be properly measured and predicted so that films/coatings can be designed to have appropriate permeability of gases for fruits and vegetables. Cisneros-Zevallos and Krochta (2003) investigated the dependence of coating thickness on viscosity of coating solution applied to Fuji apples by a dipping method. The results indicate the possibility of controlling coating thickness and internal gas composition based on coating solution properties.

15.3.2 Effect of relative humidity

Edible polymers make good O_2, aroma and lipid barrier films at low-to-intermediate RH, but their barrier properties decrease as the RH increases (Krochta and Mulder-Johnston, 1997). The optimum RH for the storage of fresh fruits and vegetables varies from product to product. Generally, levels of 85–95% represent a compromise between preventing excessive weight loss while providing some control of microbial spoilage (Robertson, 1993). Thus, edible polymer materials and coating thicknesses that provide desirable barrier properties at high RH must be selected. In addition, the RH must be controlled within a reasonable range. Otherwise, if the RH drops to too low a value, the barrier property of an edible coating may become too great, leading to anaerobic respiration in the fruit or vegetable.

15.3.3 Adhesion of coating

Knowledge of interactions between the coating solution and the surface of food with different commodities will be useful in providing a more pre-

dictable coating performance (Cisneros-Zevallos and Krochta, 2003). In order for edible coatings to be successfully applied to the desired food, interactions among the coating molecules (cohesion) and interactions between the coating molecules and the food surface molecules (adhesion) should be carefully considered (Miller and Krochta, 1997). Lack of attention to these facts has probably resulted in inconsistent and unsatisfactory results in many studies (Krochta and Mulder-Johnston, 1997). Solution temperature, solvent evaporation rate, solvent characteristics and the concentration of the film-forming polymer molecules in the solution are the important processing parameters influencing cohesion and adhesion (Miller and Krochta, 1997).

The degree of cohesiveness of the coating matrix is a critical parameter affecting the functional properties of edible edible coatings (Banker, 1966). It is difficult to obtain adequate adhesion of the coating to the food product, for instance when a hydrophobic coating-forming solution is used to protect a hydrophilic food product. In such cases, surface-active agents can be coated on the food or added to the film-forming solution, or a material capable of adhering to both components can be applied as an intermediate precoating (Cuq *et al*., 1995).

Binding a lipid material onto a cut surface covered with juice presents a considerable technical problem. One solution is to use some means of setting the coating material by forming a tight matrix. For example, an emulsion mixture of casein and acetylated monoglyceride will form a coagulum by adjusting the pH to the isoelectric point of 4.6. The lipid molecules are presumably trapped within the matrix of the casein coagulum (Wong *et al*., 1994).

15.3.4 Influence of antimicrobial incorporation on film/coating properties

Both aroma and oxygen permeabilities in edible films/coatings can be affected by the incorporation of antimicrobials (Miller and Krochta, 1997). Besides the barrier efficiency, edible films/coatings have to be organoleptically compatible with foods. Antimicrobial agents should not alter color and flavor significantly.

Cagri *et al*. (2004) mentioned that nisin may interact differently with proteins of different films. When enzyme is used as an antimicrobial agent for a protein-based film/coating, enzyme interaction with the protein of the film/coating must be considered. More fundamental research is necessary to understand the transfer mechanisms of solutes such as enzymes through edible films/coatings (Debeaufort *et al*., 1998).

15.3.5 Labeling of coated foods

The food industry's main concern about introducing active components to edible films/coatings is that consumers may consider the components

unacceptable (Vermeiren *et al*., 1999). In addition, food processors considering the use of protein-based films must be aware that a small portion of the population has intolerance and/or allergic reactions to certain proteins. Several proteins that have been tested as coating/film materials have the potential to produce an allergic reaction, including film-forming proteins from wheat, nuts, peas, beans and milk, as well as protein-based antimicrobials. Use of protein-based films as coatings on foods must be declared appropriately to the consumer, no matter how small the amount used (Krochta and Mulder-Johnston, 1997). Coated fruits and vegetables should be labeled identifying the source of coating (e.g. vegetable-, animal-, resin-, petroleum- or beeswax-based coating) (Baldwin and Baker, 2002).

Testing of natural preservatives to replace synthetic preservatives is a worldwide trend with the potential to affect many consumer food products. Future research on combinations of naturally derived antimicrobial agents could enhance the merits of antimicrobial films/coatings in terms of food safety, shelf-life extension and environmental friendliness (Nicholson, 1998; Coma *et al*., 2001).

15.3.6 Combination of edible films/coatings with other hurdles

The combined use of an edible film/coating with other treatments may be considered to enhance the stability of minimally processed products (Wong *et al*., 1994). Uses of coatings in combination with hot water dips, fumigation and extended cold storage have been investigated (Baldwin, 1994). Combination use with gamma irradiation has been proposed (Ouattara *et al*., 2002a,b). Vachon *et al*. (2003) reported that γ-irradiation treatment combined with an edible coating significantly delayed mold growth. In the future, edible films/coatings combined with other hurdle technology may reduce the need for energy intensive operations and costly controlled atmosphere storage (Baldwin, 1994).

15.4 Sources of additional information

- Types, methods of preparation, properties and applications of edible polymers: Kester and Fennema (1986), Krochta and Mulder-Johnston (1997).
- Comparison of the oxygen permeability of edible polymer films and conventional synthetic polymer films: Miller and Krochta (1997)
- Antimicrobial food packaging: Han (2000)
- Edible coatings as carriers of food additives, fungicides and natural antagonists: Cuppett (1994)
- Antimicrobial agents for potential use in food packaging materials: Suppakul *et al*. (2003)

- Considerations in developing antimicrobial films/coatings: Hotchkiss (1995), Miller and Krochta (1997), Brody *et al*. (2001), Suppakul *et al*. (2003)
- Commercial wax coating companies, products and applications: Baldwin (1994)
- Commercial antimicrobial materials and their trade names and manufactures: Vermeiren *et al*. (2002), Suppakul *et al*. (2003).

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16

Modified atmosphere packaging (MAP) and the safety and quality of fresh fruit and vegetables

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16.1 Introduction

Recently, there has been a rapid growth in the market for fresh prepared fruit and vegetable (i.e. produce) items. The main driving force for this market growth is the increasing consumer demand for fresh, healthy, convenient and additive-free prepared produce items. However, fresh prepared produce items are highly perishable and prone to the major spoilage mechanisms of enzymic discoloration, moisture loss and microbial growth. Good manufacturing and handling practices along with the appropriate use of modified atmosphere packaging (MAP) are relatively effective at inhibiting these spoilage mechanisms, thereby extending shelf-life. Shelf-life extension also results in the commercial benefits of less wastage in manufacturing and retail display, long distribution channels, improved product image and the ability to sell convenient, value-added, fresh prepared produce items to the consumer with reasonable remaining chilled storage life.

This chapter explains the factors that affect fresh produce shelf-life and outlines how extended shelf-life can be achieved by using MAP. In addition, the effects of MAP and novel MAP gases [high oxygen (O_2), argon (Ar) and nitrous oxide (N_2O)] on fresh produce quality, microbial growth and safety are highlighted. Finally, future trends and research directions are predicted and sources of further information and advice are listed.

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16.2 Background information

Unlike other chilled perishable foods that are modified atmosphere (MA) packed, fresh produce continues to respire after harvesting and any subsequent packaging must take into account this respiratory activity. The depletion of O_2 and enrichment of carbon dioxide (CO_2) are natural consequences of the progress of respiration when fresh produce is stored in hermetically sealed packs. Such modification of the atmosphere results in a respiratory rate decrease with a consequent extension of shelf-life (Kader *et al.*, 1989). MAs can passively evolve within hermetically air-sealed packs as a consequence of produce respiration. If a produce item's respiratory characteristics are properly matched to film permeability values, then a beneficial equilibrium MA (EMA) can be passively established. However, in the MAP of fresh produce, there is a limited ability to regulate passively established MAs within hermetically air-sealed packs. There are many circumstances when it is desirable rapidly to establish the atmosphere within produce packs. By replacing the pack atmosphere with a desired mixture of O_2, CO_2 and nitrogen (N_2), a beneficial EMA may be established more rapidly than a passively generated EMA. For example, flushing packs with N_2 or a mixture of 5–10% O_2, 5–10% CO_2 and 80–90% N_2 is commercial practice for inhibiting undesirable browning and pinking on prepared leafy green salad vegetables (Day, 1998).

The key to successful retail MAP of fresh prepared produce is currently to use packaging film of the correct permeability so as to establish optimal EMAs of typically 3–10% O_2 and 3–10% CO_2. The EMAs attained are influenced by produce respiration rate (which itself is affected by temperature, produce type, variety, size, maturity and severity of preparation); packaging film permeability; pack volume, surface area and fill weight; and degree of illumination. Consequently, establishment of an optimum EMA for individual produce items is very complex. Furthermore, in many commercial situations, produce is sealed in packaging film of insufficient permeability resulting in development of undesirable anaerobic conditions (e.g. <2% O_2 and >20% CO_2). Microperforated films, which have very high gas transmission rates, are now commercially used for maintaining aerobic EMAs (e.g. 5–15% O_2 and 5–15% CO_2) for highly respiring prepared produce items such as broccoli and cauliflower florets, baton carrots, bean sprouts, mushrooms and spinach. However, microperforated films are relatively expensive, permit moisture and odour losses, and may allow for the ingress of microorganisms into sealed packs during wet handling situations (Day, 1998).

16.3 Factors that affect fresh produce shelf-life

The primary goal of MAP for fresh produce is the extension of shelf-life. It should be stressed that this extension of produce shelf-life may allow for

the growth of pathogenic bacteria to higher levels as compared with air-stored samples. Since fruit and vegetables continue to respire after harvest, there are many other factors that affect the post-harvest shelf-life extension of fresh produce and the success of MAP (Day, 2001a; Farber *et al*., 2003).

Respiration rates of fruit or vegetables are inversely proportional to achievable shelf-life and higher respiration rates are associated with shorter shelf-life (Day, 1993; Lee *et al*., 1995). Generally speaking, those produce items with increased wounding, as in the case of fresh-cut produce, will have a shorter shelf-life owing to their increased respiration rates. Respiration, which can be measured by the production rate of CO_2 or by the consumption rate of O_2, also results in the production of heat and water vapour (Zagory, 1995). Consequently, a goal of MAP is to decrease produce respiration rate, which can be successfully achieved with decreased O_2 levels (e.g. 2–5%) and good chilled storage (i.e. 0–5 °C). However, O_2 concentrations below 1–2% can lead to anaerobic respiration and the production of off-odours and flavours (e.g. ethanol, aldehydes and ketones), as well as creating conditions for the growth of anaerobic pathogenic bacteria such as *Clostridium botulinum*. As described later, high O_2 (70–100%) combined with CO_2 for MAP has been tested and shown to have beneficial effects on product quality (Amanatidou *et al*., 1999; Day, 2001a); however, more research is required to support and explain this novel MAP concept (Wszelaki and Mitcham, 2000; Kader and Ben-Yehoshua, 2000).

Senescence, the natural form of produce deterioration, is endogenously controlled and results in the breakdown of plant membranes. It is marked by chlorophyll loss, a decrease in protein content and tissue softening. Senescence is driven by an increase in respiration, as well as by an increase in ethylene production in some climacteric produce items, for example tomatoes, apples and pears. The delay of senescence is the main goal in the preservation of fresh produce by MAP, since senescence accounts for a large proportion of post-harvest losses (Lee *et al*., 1995). Therefore, it is a reasonable assumption that reducing ethylene production may effectively delay senescence (Farber *et al*., 2003).

The plant hormone, ethylene, plays an important role in produce shelf-life and can cause a marked increase in respiration rates as well as enhancing ripening and senescence (Day, 1993; Nguyen-the and Carlin, 1994). In some produce items, accelerated ageing and the initiation of ripening can occur following exposure to ethylene concentrations as low as $0.1\,\mathrm{mol\,l^{-1}}$ (Lee *et al*., 1995). As senescence begins, spoilage caused by the growth of indigenous microorganisms can be augmented (Farber *et al*., 2003). Also, different biological structures of assorted produce varieties contribute to the sensitivity response to ethylene, as well as to the response to O_2 and CO_2 levels. Furthermore, different stages of maturity, cultivar and post-harvest storage conditions also influence the sensitivity of produce items to ethylene (Lee *et al*., 1995).

Post-harvest control measures taken to minimize the production of ethylene include storage in a controlled or modified atmosphere at optimal chilled temperatures (just above the chilling or freezing injury threshold) and oxidizing any ethylene by various chemical and physical means. MAP can maintain the quality of respiring produce items by greatly reducing the damaging effects of exposure to ethylene. In MAP, CO_2 can inhibit ethylene action as well as autocatalytic production of ethylene by climacteric products such as apples, pears and tomatoes. However, CO_2 levels above 15–20% can cause undesirable physiological damage to whole leaf plants and therefore it is important to take into account the specific CO_2 tolerance level of individual produce items before designing a MA package for a particular commodity (Day, 1993).

As previously mentioned, the control of produce respiration and ethylene production by MAP can help maintain produce quality and extend shelf-life. However, the control of produce respiration and ethylene production is also highly dependent on optimal chilled temperature control throughout the entire supply chain, from 'farm to fork'. Furthermore, the microbiological safety of MA packed produce items is also dependent on controlled chilled temperature storage and the individual characteristics of each produce item. For example, most MA packed fresh-cut (i.e. prepared) produce items that are stored at optimal chilled temperatures, tend to spoil overtly before becoming microbiologically unsafe. However, incorrect storage at elevated temperatures will greatly enhance the food safety risks of MA packed fresh-cut produce items, by allowing for the growth of pathogenic bacteria. Hence, storage at optimal chilled temperatures cannot be overemphasised. Recent advances in the chilled storage industry show promise for improved temperature control of produce items during transport as well as during retail and domestic storage (Farber *et al*., 2003).

16.4 Effects of MAP on fresh produce microbial growth and safety

16.4.1 Spoilage microorganisms

Fresh produce can support the growth of a plethora of spoilage microorganisms. The most commonly encountered microorganisms on fresh produce are *Pseudomonas* spp., lactic acid bacteria such as *Leuconostoc mesenteroides* and *Lactobacillus* spp., *Erwinia herbicola, Flavobacterium, Xanthomonas, Enterobacter agglomerans,* yeasts and moulds (Nguyen-the and Carlin, 1994; Zagory, 1999). Although these microorganisms can be responsible for the spoilage of fresh produce, the type and magnitude of microbial growth can vary greatly for different produce items and storage conditions. Storage temperature has a huge influence on determining the type and magnitude of microbial growth found on chilled produce items. For example, good chilled storage conditions (e.g. 0–5 °C) lead to the

preferential growth of psychrotrophic (cold-loving) microorganisms and a subsequent decrease in the growth of mesophilic (warm-loving) microorganisms (Farber *et al*., 2003).

Good chilled storage (0–5 °C) not only decreases the growth rate of spoilage and pathogenic microorganisms on fresh produce but also increases the inhibitory effects of MAP by increasing the solubility of antimicrobial CO_2 into fruit and vegetable tissues. For example, the depleted levels of O_2 and elevated levels of CO_2 used in MAP generally inhibit the growth of aerobic spoilage bacteria such as *Pseudomonas* spp., but can favour the growth of lactic acid bacteria. This can hasten the spoilage of produce that is sensitive to lactic acid bacteria, such as lettuce, chicory leaves and carrots (Nguyen-the and Carlin, 1994). The effects of MAP on yeasts are negligible since they are capable of both aerobic and anaerobic respiratory growth. However, depleted levels of O_2 (2–5%) and elevated levels of CO_2 (>10%) cause growth inhibition of moulds since they are aerobic microorganisms (Molin, 2000).

A possible concern when using MAP for respiring fresh produce arises from the potential for pathogenic bacteria, which may be resistant to depleted levels of O_2 (2–5%) and elevated levels of CO_2 (>10%), to outgrow spoilage microorganisms that may be susceptible to the same MAP conditions (Bennik *et al*., 1998). This dynamic interaction between the growth of pathogenic bacteria and spoilage microorganisms has been extensively reviewed for meat and milk products; however, further research is still required for the MAP of fresh produce (Nguyen-the and Carlin, 1994; Francis and O'Beirne, 1998).

16.4.2 Pathogenic bacteria

Fresh prepared produce that is MA packed can be vulnerable from a food safety standpoint since depleted levels of O_2 (2–5%) and elevated levels of CO_2 (>10%) may inhibit the growth of microorganisms that usually warn consumers of spoilage, while the growth of pathogenic bacteria may be encouraged. In addition, slow growing pathogenic bacteria may have the opportunity further to increase in numbers owing to the extended shelf-life of MA packed fresh produce. Of most concern in relation to MA packed fresh produce are the psychrotrophic pathogenic bacteria such as *Listeria monocytogenes* and *Aeromonas hydrophila.* Also, non-proteolytic *Clostridium botulinum, Salmonella* spp., *Escherichia coli* O157 : H7 and *Shigella* spp. are capable of growth and can be potential health risks when present on MA packed fresh produce (NACMCF, 1999).

Clostridium botulinum

C. botulinum spores are commonly found in agricultural soils and consequently on the surfaces of fresh produce. Proteolytic *C. botulinum* has difficulty growing and producing toxin at temperatures below 12 °C, pH <4.6,

a water activity <0.95 and NaCl concentrations >10% (Lund and Peck, 2000). Non-proteolytic *C. botulinum* can grow at a minimum of 3.3 °C, pH >5.0, water activity >0.97 and NaCl concentrations >4%. Therefore, there is some concern about the use of MAP with respect to non-proteolytic *C. botulinum* (Zagory, 1995). As described previously, the level of O_2 in MA packed fresh produce packs can be depleted rapidly, particularly if the produce is temperature abused and the produce respiration increases. This scenario would lead to the development of an anaerobic environment that is ideal for the growth and toxin production of *C. botulinum* (Francis *et al*., 1999). However, in a study that looked at this potential in lettuce, cabbage, broccoli, carrots and green beans that had been packed under vacuum or in air, Larson *et al.* (1997) found that these produce items were almost always grossly spoiled before any significant toxin production was detected. Many other research studies have also shown similar results and a study by Larson and Johnson (1999) demonstrated the ability of produce spoilage microorganisms to protect against the overgrowth of pathogenic bacteria.

Fresh mushrooms and tomatoes have been shown to contain spores of *Clostridium* spp. and therefore the possibility of botulism associated with these MA packed produce items must not be ignored (Zagory, 1995). However, the acidic nature of tomatoes (pH <4.6) does not provide suitable growth conditions for *C. botulinum*. This supposition was supported by the results of Hotchkiss *et al.* (1992) who demonstrated that MA packed tomatoes (1.0–2.9% O_2), stored at 13 °C and 23 °C, only became toxic after becoming severely spoiled. The initial level of O_2 used for high respiring produce items such as mushrooms can be very important since it will deplete more rapidly, resulting in an anaerobic environment that is conducive to toxin production (Sugiyama and Yang, 1975). The industry practice of using microperforated MAP films discourages the growth of *C. botulinum*, although mushroom shelf-life is shortened.

The absence of outbreaks of botulism linked to MA packed fresh produce items indicates that *C. botulinum* is probably competitively inhibited by the natural microbial flora and storage conditions of these products. However, more research is required to examine the potential for growth of *C. botulinum* in a wide variety of MA packed fresh produce items, stored at mildly abusive temperatures (e.g. 7–12 °C). In addition, other hurdles to *C. botulinum* growth, besides good chilled temperature storage, need to be investigated so as to prevent potential toxin production (Farber *et al*., 2003).

Listeria monocytogenes

L. monocytogenes is a psychrotrophic and pathogenic bacterium that can remain largely unaffected by MAP, while the normal microflora are inhibited (NACMCF, 1999; Amanatidou *et al*., 1999). Consequently, *L. monocytogenes* can grow to potentially harmful levels, at low chilled temperatures, during the extended shelf-life of a MA packed fresh produce item (Francis and O'Beirne, 1997, 1998). For example, Berrang *et al.* (1989a) showed that

the growth of *L. monocytogenes* inoculated onto broccoli, asparagus and cauliflower was not affected by MA conditions of 3% CO_2/18% O_2/79% N_2 for 10 days at 10 °C. Also, Beuchat and Brackett (1990) clearly demonstrated that the levels of *L. monocytogenes* increased significantly on lettuce stored at chilled temperatures in a MA of 3% O_2/97% N_2. In addition, Francis and O'Beirne (1997) reported that MAP, under predominantly N_2, stimulated the growth of *L. monocytogenes* on fresh lettuce at 8 °C. Furthermore, elevated CO_2 levels (10–20%) have been reported to stimulate the growth of *L. monocytogenes* in a surface model system (Amanatidou *et al*., 1999).

Jacxsens *et al*. (1999) investigated the growth of *L. monocytogenes* and *Aeromonas* spp. on fresh-cut vegetables, packaged under either a MA (i.e. 2–3% O_2/2–3% CO_2/94–96% N_2) or air, and clearly found that the sensory quality of the produce items deteriorated to unacceptable levels before *L. monocytogenes* and *Aeromonas* spp. levels increased significantly. They concluded that the growth of psychrotrophic pathogenic bacteria was more influenced by the type of fresh-cut vegetable and the temperature of storage, rather than the MA conditions, and this conclusion was also supported strongly by the research results of Nguyen-the and Carlin (1994) and others (Farber *et al*., 2003).

Francis and O'Beirne (1998) and many other researcher groups have recommended that more investigations need to be carried out to examine thoroughly the influence of different MAP conditions, competing background microflora and storage temperatures on the survival and growth of *L. monocytogenes* on MA packed fresh-cut produce items.

Aeromonas hydrophila

A. hydrophila is a psychrotrophic and pathogenic bacterium that can be found on a wide variety of foods, as well as in most aquatic environments, and can cause gastroenteritis and occasionally septicaemia (Kirov, 1997). A microbiological survey of 97 fresh prepared salads found *A. hydrophila* to be present in 21.6% of them (Fricker and Tompsett, 1989). Similar to *L. monocytogenes, A. hydrophila* can grow at chilled temperatures and growth does not seem to be affected by depleted O_2 levels (e.g. 2–5%) and elevated CO_2 levels up to 50% (Francis *et al*., 1999). As mentioned previously, CO_2 levels >50% are detrimental to fresh produce quality, even though these elevated levels have been found to inhibit the growth of *A. hydrophila* and *L. monocytogenes* (Bennik *et al*., 1995). Furthermore, Berrang *et al.* (1989b) determined that the shelf-life of broccoli, asparagus and cauliflower was extended by MAP (i.e. 11–18% O_2/2–10% CO_2/balance N_2) but the growth of naturally occurring or inoculated *A. hydrophila* was not inhibited, at storage temperatures of 4 °C and 15 °C.

Other pathogenic microorganisms

Other pathogenic microorganisms such as *Salmonella* spp., *Shigella* spp, *E. coli* 0157 : H7 and various enteric viruses, such as hepatitis A, can survive

and grow on fresh produce and they have been implicated in a few food poisoning outbreaks. Hence, there is concern about their growth behaviour under MAP conditions (Zagory, 1995; Francis *et al*., 1999; Amanatidou *et al*., 1999; NACMCF, 1999). However, reassuringly, Farber *et al.* (2003) have extensively reviewed the relevant literature and concluded that MA packed produce items have an excellent food safety record. They stated that to their knowledge, only two MA packed produce items, i.e. coleslaw mix and ready-to-eat salad vegetables, have been directly implicated in food-borne illness outbreaks of botulism and *Salmonella* Newport, respectively. They also stated that there has been a noticeable increase in the consumption of fresh fruit and vegetables during the last two decades, and more consumers are now choosing the more convenient fresh-cut produce items. Since there has been a parallel rise in the number of produce-linked food-borne outbreaks, it is important that vigilance is maintained with respect to the safety of MA packed fresh-cut produce.

16.5 Effects of novel MAP gases on fresh produce quality and safety

16.5.1 High O_2 MAP

Information gathered by the author during 1993–1994 revealed that a few UK prepared produce companies had been experimenting with high O_2 (e.g. 70–100%) MAP and had achieved some surprisingly beneficial results. High O_2 MAP of prepared produce was not exploited commercially during that period, probably because of the inconsistent results obtained, a lack of understanding of the basic biological mechanisms involved and concerns about possible safety implications. Intrigued by the concept of high O_2 MAP, the Campden & Chorleywood Food Research Association (CCFRA), UK, carried out limited experimental trials on prepared iceberg lettuce and tropical fruits, in early 1995. The results of these trials confirmed that high O_2 MAP could overcome the many disadvantages of traditional low O_2 MAP. High O_2 MAP was found to be effective in inhibiting enzymic discolorations, preventing anaerobic fermentation reactions and inhibiting microbial growth. In addition, the high O_2 MAP of prepared produce items within inexpensive hermetically sealed plastic films was found to be very effective in preventing undesirable moisture and odour losses and ingress of microorganisms during wet handling situations (Day, 1998).

The experimental finding that high O_2 MAP is capable of inhibiting aerobic and anaerobic microbial growth can be explained by the growth profiles of aerobes and anaerobes. It has been hypothesised that active oxygen radical species damage vital cellular macromolecules and thereby inhibit microbial growth when oxidative stresses overwhelm cellular protection systems (Gonzalez Roncero and Day, 1998; Amanatidou, 2001). Also

intuitively, high O_2 MAP inhibits undesirable anaerobic fermentation reactions (Day, 1998).

Polyphenol oxidase (PPO) is the enzyme primarily responsible for initiating discoloration on the cut surfaces of prepared produce. PPO catalyses the oxidation of natural phenolic substances to colourless quinones which subsequently polymerise to coloured melanin-type compounds (McEvily *et al*., 1992). It is hypothesised that high O_2 (and/or high argon) levels may cause substrate inhibition of PPO or alternatively, high levels of colourless quinones subsequently formed may cause feedback product inhibition of PPO.

16.5.2 Argon and nitrous oxide MAP

Argon (Ar) and nitrous oxide (N_2O) are classified as miscellaneous additives and are permitted packaging gases for food use in the European Union (EU). Air Liquide S.A. (Paris, France) has stimulated commercial interest in the potential MAP applications of using Ar and, to a lesser extent, N_2O. Air Liquide's broad range of patents claim that in comparison with N_2, Ar can more effectively inhibit enzymic activities, microbial growth and degradative chemical reactions in selected perishable foods (Brody and Thaler, 1996; Spencer, 1999). More specifically, an Air Liquide patent for fresh produce applications claims that Ar and N_2O are capable of extending shelf-life by inhibiting fungal growth, reducing ethylene emissions and slowing down sensory quality deterioration (Fath and Soudain, 1992). Of particular relevance is the claim that Ar can reduce the respiration rates of fresh produce and hence have a direct effect on extension of shelf-life (Spencer, 1999).

Although Ar is chemically inert, Air Liquide's research has indicated that it may have biochemical effects, probably owing to its similar atomic size to molecular O_2 and its higher solubility in water and density compared with N_2 and O_2. Hence, Ar is probably more effective at displacing O_2 from cellular sites and enzymic O_2 receptors, with the consequence that oxidative deterioration reactions are likely to be inhibited. Notwithstanding, more independent research is needed to understand better the potential beneficial effects of Ar and N_2O (Day, 1998).

16.5.3 Novel MAP research

Two industrially funded research clubs were set up at CCFRA to investigate in detail the interesting effects of novel MAP on fresh prepared produce. A High O_2 MAP Club ran from April 1995 to September 1997 and as a follow-up, a Novel Gases MAP Club ran from January 1998 to December 1999. These clubs were supported by a total of nine prepared produce suppliers, five gas companies, four packaging film suppliers, three retailers,

two suppliers of non-sulphite dips, two manufacturers of MAP machinery and two gas instrument companies. In addition, further investigations were carried out during a three-year EU FAIR funded project, which started in September 1996. The overall objective of this project was to develop safe commercial applications of novel MAP for extending the quality shelf-life of a wide range of fresh prepared produce items. Other aims included investigations of the effects of novel MAP on non-sulphite dipped prepared produce, labile nutritional components, and microbial and biochemical spoilage mechanisms. The major focus of this research was on high O_2 MAP, followed by Ar MAP, and to a minor extent, N_2O MAP (Day, 1998).

In summary, the following major results and achievements were made during the course of CCFRA's Club and EU funded novel MAP research:

- High O_2 compatible MAP machines were used safely and successfully during the course of the project's experimental trial work. A non-confidential guidelines document on the safe use of high O_2 MAP was published (BCGA, 1998).
- Enzymic discolorations of prepared non-sulphite dipped potatoes and apples were generally more effectively inhibited by anaerobic (<2% O_2) MAP combinations of N_2, Ar and CO_2, compared with high O_2 MAP. However, high O_2 MAP was found to have certain odour and textural benefits for prepared potatoes and apples. Also, high O_2 MA packed non-sulphite dipped prepared potatoes and bananas were found to have a longer achievable shelf-life, in comparison with equivalent low O_2 (8%) MA packed control samples.
- For most prepared produce items, under defined storage and packaging conditions, high O_2 MAP was found to have beneficial effects on sensory quality in comparison with industry-standard air packing and low O_2 MAP. High O_2 MAP was found to be effective for extending the achievable shelf-lives of prepared iceberg lettuce, sliced mushrooms, broccoli florets, cos lettuce, baby-leaf spinach, raddichio lettuce, lollo rossa lettuce, flat-leaf parsley, cubed swede, coriander, raspberries, strawberries, grapes and oranges (Day, 2001a).
- Ar-containing and N_2O-containing MAP treatments were found to have negligible, variable or only minor beneficial effects on the sensory quality of several prepared produce items, in comparison with equivalent N_2-containing MAP treatments.
- High O_2 MAs were found to inhibit the growth of several generic groups of bacteria, yeasts and moulds, as well as a range of specific food pathogenic bacteria and spoilage microorganisms, namely *A. hydrophila*, *Salmonella enteritidis*, *Pseudomonas putida*, *Rhizopus stolonifer*, *Botrytis cinerea*, *Penicillium roqueforti*, *Penicillium digitatum* and *Aspergillus niger* (e.g. Fig. 16.1 and Fig. 16.2). High O_2 MAs alone were not found to inhibit or stimulate the growth of *Pseudomonas fragi*, *Bacillus cereus*, *Lactobacillus sake*, *Yersinia enterocolitica* and *L. monocytogenes*, but the

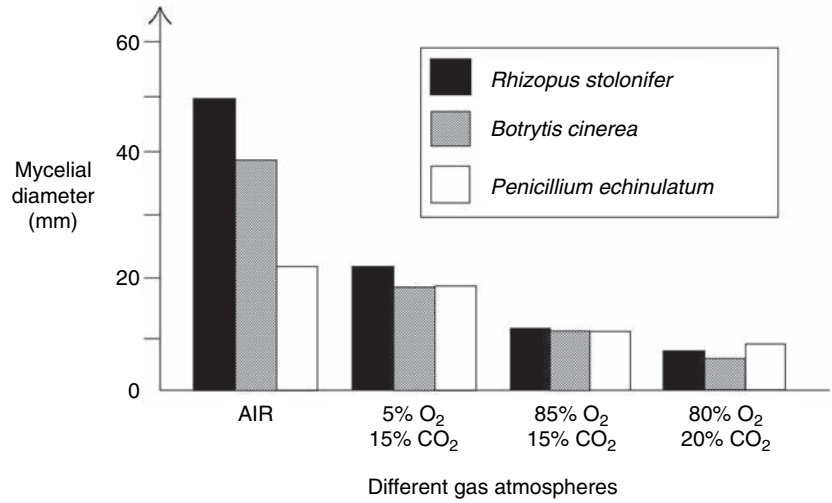

Fig. 16.1 Inhibition of fungal growth by different MAs.

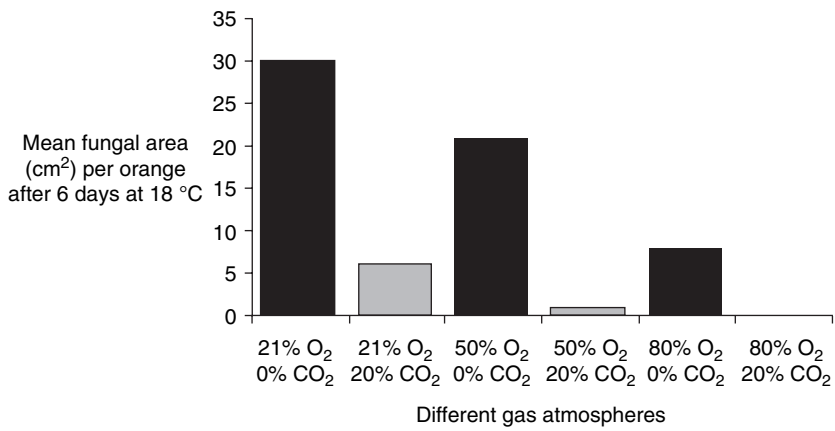

Fig. 16.2 Inhibition of fungal growth on *Penicillium digitatum* infected oranges under different MAs.

addition of 10–30% CO_2 inhibited the growth of all these bacteria. Ar-containing and N_2O-containing MAs were found to have negligible antimicrobial effects on a range of microorganisms, when compared with equivalent N_2-containing MAs.

- Respiration rates of selected prepared produce items were not found to be significantly affected by high O_2 or high Ar MAs, but were substantially reduced by the addition of 10% CO_2.

- High O_2 and high Ar MAP did not prevent the enzymic browning of non-sulphite dipped apple slices, but no further browning took place after pack opening.
- Ar-containing MAs were found to inhibit the activity of mushroom PPO, when compared with equivalent N_2-containing MAs. In contrast, no significant inhibition of mushroom PPO activity was found under 80% O_2/20% N_2 when compared with 20% O_2/80% N_2. However, the incorporation of 20% CO_2 into high O_2 MAs may inhibit mushroom PPO as well as the activity of other prepared produce PPOs (Sapers, 1993).
- High O_2 MAP increased membrane damage of apple slices, whereas high Ar MAP decreased membrane damage. However, apple slices stored under O_2-free MAs suffered the most membrane damage, which adversely affected tissue integrity, cell leakage and texture. By comparison, high O_2 and high Ar MAP were not found adversely to affect the cell permeability, tissue exudate or pH of prepared carrots.
- High O_2 and high Ar MAP were found to have beneficial effects on ascorbic acid retention, indicators of lipid oxidation and inhibition of enzymic browning on prepared lettuce.
- High O_2 MAs increased the peroxidase activity of *B. cinerea*, but the addition of 10% CO_2 substantially reduced this activity.
- In comparison with air packing and low O_2 MAP, high O_2 MAP was not found to decrease preferentially single antioxidant (ascorbic acid, β-carotene and lutein) levels in prepared lettuce but did induce the loss of certain phenolic compounds, even though desirable total antioxidant capacity (TRAP) values after chilled storage were increased.
- Extracts from high O_2 MA packed prepared lettuce and onions did not have any cytotoxic effects on human colon cells.
- Ingestion by volunteers of fresh lettuce resulted in an increase in human plasma TRAP values, obtained from blood samples taken at timed intervals, through the absorption of phenolic compounds and single antioxidant molecules. This increase in human plasma TRAP values was significantly higher than after ingestion of lettuce that had been chilled (5 °C) and stored for three days (Serafini, 2001).
- Ingestion by volunteers of chilled stored lettuce packed under air and high O_2 MAs resulted in measurable increases in human plasma TRAP values, whereas virtually no increases in TRAP values were measured after ingestion of equivalent lettuce packed under low O_2 MAs (Serafini, 2001).
- A guidelines document was compiled which outlines good manufacturing and handling practices for fresh prepared produce using high O_2 MAP and non-sulphite dipping treatments (Day, 2001a).

Partly as a result of the interest stimulated by CCFRA's Club and EU funded novel MAP research, several research studies and reviews have

recently appeared in the scientific literature (e.g. Gözükara, 2000; Kader and Ben-Yehoshua, 2000; Wszelaki and Mitcham, 2000; Amanatidou, 2001; Perez and Sanz, 2001; Jacxsens *et al*., 2002; Van der Steen *et al*., 2002; Hoogerwerf *et al*., 2002; Allende *et al*., 2004). These studies have shown some interesting effects of high O_2 MAP and indicated the direction of future research. Novel MAP (particularly, high O_2) has the potential to maintain the quality and assure the microbial safety of fresh prepared produce. The commercial implementation and success of this new technology may encourage greater consumption of conveniently packed fresh prepared produce and help towards improving the health and well-being of consumers. The publication of practical guidance on high O_2 MAP and non-sulphite dipping has facilitated limited commercial exploitation of this new technology to date (Day, 2001a), but further refinement of the technology will be necessary before high O_2 MAP becomes a mainstream preservation method for fresh prepared produce.

For example, Arun Foods Limited (Littlehampton, West Sussex, UK) has produced a range of salads and stir-frys for the commercial retail market using high O_2 MAP technology (Day, 2002). These high O_2 MA packed products were presented in a tray and lidding film format and were assigned a chilled shelf-life of 7–8 days in comparison with only 3–4 days in control air packs (Dr Steve Yeo, Arun Foods Limited, personal communication, June 2002). Also, a soft fruit supplier in Belgium has used high O_2 MAP to extend the chilled shelf-life of its product range (Dr Frank Devlieghere, Universiteit Gent, Belgium, personal communication, June, 2002). In addition, the author is aware of other companies who have or are trailing high O_2 MAP for fresh prepared produce and chilled ready meal applications (Day, 2002).

Specifically with regard to the high O_2 MAP of fresh prepared produce, the following future research directions are suggested (Day, 2001a):

- Further investigate the potential applications of an innovative dual-action O_2 emitter/CO_2 scavenger active packaging sachet that has been developed by Standa Industrie (Caen, France) and marketed by Emco Packaging Systems (Worth, Kent, UK). Initial trials carried out by CCFRA and LinPac Plastics Limited (Pontefract, Yorkshire, UK) in association with several soft fruit suppliers in the UK have clearly demonstrated the shelf-life extending potential of this active packaging device (McGrath, 2000). This O_2 emitter/CO_2 scavenger sachet enables high O_2 levels to be maintained within high O_2 MA packs of respiring fresh prepared produce whilst simultaneously controlling CO_2 below levels that may cause physiological damage to produce. Also, the inclusion of this sachet within high O_2 MA packs of fresh prepared produce that have a high intrinsic respiration rate and/or produce volume/gas volume ratio will prevent excessive depletion of in-pack O_2 levels and build-up of in-pack CO_2 levels. Furthermore, this sachet could also be

utilised in low O_2 MA packs of fresh prepared produce to prevent the development of undesirable anaerobic conditions during chilled storage.
- Thoroughly investigate the potential synergy of high O_2 MAP and other active packaging devices (e.g. moisture absorbers, ethylene scavengers and antimicrobial films) and suitable edible coatings and films (Day, 1993; Baldwin *et al*., 1995; Nussinovitch and Lurie, 1995; Rooney, 1999). Selection criteria for promising active packaging devices and edible coatings and films should be based on their technical efficacy, cost, regulatory status and consumer acceptability (Day, 2000).
- Carry out further underpinning research investigations on the effects of high O_2 MAP on the various spoilage and pathogenic microorganisms associated with fresh prepared produce items. Also, further research is merited on the effects of high O_2 MAP on the beneficial nutritional components present in fresh produce and on the complex biochemical reactions and physiological processes that occur during storage.
- Establish optimal high O_2 MAP applications for extending quality shelf-life and assuring the microbial safety of further fresh prepared produce items and combination food products that consist of respiring produce and non-respiring food items (e.g. ready meals, pizzas, kebabs, etc.). Initial trials carried out by CCFRA have demonstrated that high O_2 MAP is capable of extending the achievable shelf-life of several chilled ready meals, in comparison with CO_2/N_2 MAP and industry-standard air packing (Day, 2001b).

16.6 Future trends and research directions

With regard to the more general aspects of fresh whole and prepared produce, the following knowledge gaps, future trends and suggested research directions are highlighted, in order to assist researchers in the future (Day, 2001a; Farber *et al*., 2003):

- Study the interactions of the background microflora with foodborne pathogenic bacteria in various MAs used for produce, as well as the effects of different gaseous environments on the survival and growth of pathogenic bacteria on whole and fresh-cut produce.
- Examine the potential growth of *C. botulinum* in a wide variety of MA packed produce stored at mildly abusive temperatures (i.e. 7–12 °C). In addition, other hurdles besides temperature need to be examined to prevent botulinum toxin production.
- Examine the influence of different atmospheres, background microflora and storage temperatures on the survival and growth of *L. monocytogenes* and *E. coli* 0157:H7 on MA packed whole and fresh-cut produce.
- Provide packaging film permeability data on commercial laminations and coextrusions at realistic chilled temperatures (0–10 °C) and relative

humidities (85–95%). At the present time, virtually all gas permeability data (Table 16.1) is quoted for single films at unrealistic storage temperatures and relative humidities (e.g. 23 °C and 0% RH).
- Provide extensive respiration rate data on a wide variety of fresh prepared produce items at different chilled temperatures and under various gaseous storage conditions. At the present time, most respiration rate data available are for whole produce items stored in air (Day, 2001a).
- Provide data on the physiological tolerance of fresh prepared produce items to low (and possibly high) O_2 levels and elevated CO_2 levels. Currently, extensive data are available on the tolerance of whole produce items to low O_2 and high CO_2 levels (Kader *et al.*, 1989) but there is a dearth of information on the tolerance of fresh prepared produce items to varying gaseous levels.
- Provide information on the residual effects of MAP on individual fresh prepared produce items after subsequent pack opening and storage in air.
- Thoroughly investigate an integrated approach to minimal processing techniques, which cover the entire chain from 'farm to fork' so as to maintain the quality and assure the microbial safety of fresh prepared produce (Ahvenainen, 1996).
- Carry out further investigations on new and innovative natural preservatives, such as those produced by lactic acid bacteria and those derived from herbs and spices (Kets, 1999).
- Devise improved washing and decontamination procedures for fresh prepared produce that are based on safe non-chlorine alternatives.
- Develop peeling and cutting machinery that can process fresh produce more gently and hence extend the quality shelf-life of fresh prepared produce.
- Devote more resources into refrigeration equipment, design and logistics so that optimal storage temperatures for fresh prepared produce can be maintained throughout the entire chill chain.

16.7 Conclusions and sources of further information and advice

- Combinations of O_2 CO_2, and N_2 are most often used in MAP. Among them, CO_2 is the only gas with a direct antimicrobial growth effect. Although other gases such as Ar, N_2O, ozone, sulphur dioxide, ethylene oxide, chlorine and propylene oxide have been investigated, they have not been applied commercially to any great extent owing to safety, regulatory and cost considerations.
- The generally recommended optimal level of O_2 in MAP is 2–5%, from a food safety and quality standpoint, although the O_2 level can easily fall <1–2% in hermetically sealed MA packs of fresh produce.

Table 16.1 Oxygen and water vapour transmission rates of selected packaging materials for fresh produce

Packaging film[a] (25 μm)	Oxygen transmission rate (cm^3m^{-2} $day^{-1}atm^{-1}$ 23 °C:0% RH[b]	Relative permeability at 23 °C:0% RH	Water vapour transmission rate (g m^{-2} day^{-1}) 38 °C: 90% RH[b]	Relative water vapour transmission rate at 38 °C: 90% RH
Aluminium (Al)	<0.1[c]	Barrier	<0.1[c]	Barrier, <10
Ethylene vinyl alcohol (EVOH)	0.2–1.6[d]	<50	24–120[d]	Variable
Polyvinylidene chloride (PVdC)	0.8–9.2		0.3–3.2	Barrier, <10
Modified nylon (MXDE)	2.4[d]		25	Semi-barrier, 10–30
Polyester (PET)	50–100	Semi-barrier 50–200	20–30	Semi-barrier, 10–30
Polyamide (nylon) (PA6)	80[d]		200	Very high, 200–300
Modified polyester (PETG)	100		60	Medium, 30–100
Metallised orientated polypropylene (MOPP)	100–200[c]		1.5–3.0[c]	Barrier, <10
Polyvinyl chloride (plasticised) (PVC)	2000–5000[e]	Medium 200–5000	200[e]	Very high, 200–300
Orientated polypropylene (OPP)	2000–2500		7	Barrier, <10
High density polyethylene (HDPE)	2100		6–8	Barrier, <10
Polystyrene (PS)	2500–5000		110–160	High, 100–200
Orientated polystyrene (OPS)	2500–5000		170	High, 100–200
Polypropylene (PP)	3000–3700		10–12	Semi-barrier, 10–30
Polycarbonate (PC)	4300		180	Very high, 100–200
Low density polyethylene (LDPE)	7100	High 5000–10000	16–24	Semi-barrier, 10–30
Polyvinyl chloride (highly plasticised) (PVC)	5000–10000[e]		200[e]	Very high, 200–300
Ethylene vinyl acetate (EVA)	12000	Very high 10000–15000	110–160	Very high, 100–200
Microperforated (MP)	>15000[f]	Extremly high >15000	Variable[f]	Extremely high, >300
Microporous (MPOR)	>15000[f]		Variable[f]	Extremely high, >300

[a] Most packaging films for fresh produce are not single films but laminates and co-extrusions.
[b] Conditions of O_2 and water vapour transmission rate measurements are not at realistic chill conditions.
[c] Dependent on pinholes.
[d] Dependent on moisture.
[e] Dependent on moisture and level of plasticiser.
[f] Dependent on film and degree of microperforation or microporosity.

- A concern when using MAP for fresh produce arises from the potential of pathogenic bacteria, which may be resistant to moderate levels of CO_2, to outgrow spoilage microorganisms that may be inhibited by the same MA conditions. The use of packaging film with the correct permeability and good chilled temperature storage will ensure that spoilage will occur before botulinum toxin production is an issue.
- Endogenous microflora are partly responsible for the spoilage of fresh produce and can vary greatly for each produce item. Their growth is also influenced by storage temperatures and conditions. The elimination or significant inhibition of spoilage microorganisms should not be practiced as their interactions with pathogenic bacteria may play an integral role in ensuring the safety of MA packed fresh produce.
- Currently, there is concern about psychrotrophic pathogenic bacteria such as *L. monocytogenes* and *A. hydrophilia,* as well as non-proteolytic *C. botulinum,* although a number of other microorganisms, especially *Salmonella* spp., *E. coli* O157:H7 and *Shigella* spp., can be potential health risks when present on MA packed fresh produce.
- Only two MA packed produce items, i.e. coleslaw mix and ready-to-eat salad vegetables, have been implicated in food poisoning outbreaks of botulism and *Salmonella* Newport, respectively (Farber *et al*., 2003).
- High O_2 MAP has been found to be effective in inhibiting enzymic discoloration, preventing anaerobic fermentation reactions and inhibiting microbial growth on fresh prepared produce. In addition, the high O_2 MAP of prepared produce items within inexpensive hermetically sealed plastic films has been found to be very effective in preventing undesirable moisture and odour losses and ingress of microorganisms during wet handling situations (Day, 2001a).
- Ar and N_2O are capable of extending shelf-life by inhibiting fungal growth, reducing ethylene emissions, reducing respiration rates of fresh produce and slowing down sensory quality deterioration (Spencer, 1999).
- Additional research is merited to investigate further the influence of MAP on the safety and quality of fresh produce. The reference sources below contain a wealth of further information, advice and research ideas.

16.8 References

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17

Natural antimicrobials for preserving fresh fruit and vegetables

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17.1 Introduction

Fresh vegetables and fruits are known to supply several types of health promoting compounds that have been associated with protection from chronic diseases such as cancer, diabetes, hypertension as well as other medical conditions (Block and Thomson, 1995). Consumer awareness about the benefits of raw plant foods has greatly increased the demand for a variety of fruits and vegetables, as well as extended the periods of the year in which this wider choice of produce is available. This demand is satisfied by an extension in the storage and market life of home-grown crops along with the proper application of current technologies in transportation across continents. Refrigeration, controlled and modified atmospheres, and chemical treatments allow richer nations to have most of the more common types of fresh fruits and vegetables on a year-round basis. However, some of the currently used methods for preserving commodities are now being questioned. Consumers and vegetable industries are demanding commodities free of pesticide residues, mycotoxins, harmful microorganisms and any other contaminant that compromises produce quality.

In particular, the use of synthetic fungicides to control post-harvest diseases has many limitations and disadvantages, such as progressively restrictive legislation, social rejection and development of resistance in disease-causing microorganisms. Furthermore, the market for post-harvest fungicides is relatively small and it has become difficult to sustain the costs of new registration or to support previous ones. In addition, the rapid rise in demand for organically produced fruits and vegetables is increasing the demand for natural pesticides (Suslow, 2000). These issues have elicited

widespread interest in the development of alternatives to synthetic fungicides for controlling post-harvest diseases of fresh fruits and vegetables. Currently, no natural antimicrobial compound is used in industrial practice, although natural compounds such as phenyl pyrroles derived from pyrrolnitrin, a metabolite produced by *Pseudomonas* spp. (Nevill *et al*., 1988), and the strobilurins derived from a substance produced by the fungus *Strobilurus tenacellus* (Ammermann *et al*., 1992; Godwin *et al*., 1992) have served as models for the synthesis of fungicides with low toxicity.

This review summarizes part of the vast amount of research carried out on natural compounds of plant, microbial and animal origin that seem most promising for future development in the control of post-harvest diseases. Other reviews of interest are those reported in Barkai-Golan (2001), Roller (2003) and Tripathi and Dubey (2004). Refer to the original studies for more detailed information.

17.2 Compounds of plant origin

Plants produce a number of compounds with antimicrobial activity widely used in the food, cosmetic and drug industries. Plant-derived insecticides, including pyrethrins, have been discovered and widely used in agriculture. However, relatively little effort has been devoted toward the development of plant-derived compounds as substitutes for synthetic fungicides.

17.2.1 Plant extracts

Research on the *in vitro* effects of plant extracts on post-harvest pathogens are numerous; in contrast, relatively few reports of *in vivo* trials are available. To the best of our knowledge, one of the first documented applications of a plant extract against plant pathogens, including post-harvest pathogens, has been that of Ark and Thompson (1959) with the use of *Allium* extracts. They obtained complete control of brown rot of peaches caused by *Monilinia fructicola* by treatment with 5%, 10% and 20% of a deodorized aqueous extract of commercial powdered garlic. Extracts of *Allium* species inhibit the *in vitro* growth of *Aspergillus parasiticus*, *A. niger*, *A. flavus* and *A. fumigatus* (Sharma *et al*., 1981; Yin and Tsao, 1999), and many other spoilage fungi of grains, legumes and processed food. More recently, water and ethanol extracts of garlic cloves applied at 1%–5% were effective in controlling *Penicillium digitatum* and *P. italicum* (disease reduction up to 92%); however, this was not as effective as the fungicide treatment, which gave 100% control (Obagwu and Korsten, 2003). The antimicrobial activity of garlic (*Allium sativum* L.), other *Allium* species (onion [*A. cepa* L.] and leek [*A. porrum* L.]) is due to allicin (2-propenyl-2-propenethiol sulphinate) contained in the tissues of these species as a precursor (alliin), which is activated by the enzyme alliinase when bulb tissues are disrupted. Allicin

readily permeates through phospholipid membranes and is thought to act by reacting with critical thiol groups in the cell, affecting several physiological processes, including respiration and RNA synthesis (Miron *et al*., 2000). Another compound in garlic, ajoene, has been reported as antifungal (Yoshida *et al*., 1987). Other compounds such as phenolics (Cao *et al*., 1996; Yin and Tsao, 1999) and antifungal proteins such as allivin (Wang and Ng, 2001) are also thought to be responsible for the inhibition of fungi. Generally, the activity of *Allium* extracts diminishes during storage and is lost by heating (Sharma *et al*., 1981; Yin and Tsao, 1999); crude juices or aqueous extracts are more active than ethyl acetate, ether, chloroform or ethanol extracts (Sharma *et al*., 1979; Abdou *et al*., 1992; Obagwu and Korsten, 2003).

Extracts from 345 plants were evaluated *in vitro* by Wilson *et al*. (1997) for their antifungal activity against *Botrytis cinerea*. They used a rapid assay to determine the antifungal activity in both plant extracts and essential oils. Among the tested extracts, 13 applied at 10% dilution of the crude extract showed high levels of antifungal activity, the most active being *Allium* sp. and *Capsicum* sp. extracts. Petroleum ether extract from *Origanum syriacum* L., (wild marjoram) growing wild in Lebanon, was active against *B. cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp. and *Fusarium oxysporum* f. sp. *melonis* (Abou Jawdah *et al*., 2002). Of the 19 aqueous extracts of leaves and stems from plants indigenous to Mexico, eight were active *in vitro* against sporulation of *Rhizopus stolonifer* and three of them (from *Annona cherimola* M., *Bromelia hemisphaerica* L. and *Carica papaya* L.) were also active *in vivo* on 'ciruela' fruits (*Spondias purpurea* L.) in suppressing *Rhizopus* rot; interestingly, the leaf extract of *Casimiroa edulis* Llav. *et* Lex. was not active *in vitro*, but completely inhibited disease development on fruits (Bautista-Baños *et al*., 2000).

The same authors found that powders, aqueous and ethanolic extracts of seeds and monthly harvested leaves of huamuchil (*Pithecellobium dulce* [Roxb.] Benth.) were effective against *B. cinerea*, *P. digitatum* and *R. stolonifer* and on strawberry fruit during storage; the greatest fungistatic or fungicidal effect for both *in vitro* and *in vivo* studies was recorded from extracts of leaves harvested in months having more stressful environmental conditions: the cold season (October–February) and the dry hot season (April and June) (Bautista-Baños *et al*., 2003a). Extracts from the leaf pulp of *Aloe vera* L., commonly known as *Aloe vera* gel (Saks and Barkai-Golan, 1995), have been tested *in vitro* against *P. digitatum*, *P. expansum*, *B. cinerea* and *A. alternata*, the first and the last being most sensitive. Dipping *P. digitatum*-inoculated grapefruits in solutions containing $1\,mg\,l^{-1}$ of gel delayed lesion development and significantly reduced the incidence of infection.

Extracts from stems, flowers and leaves of *Euphorbia macroclada* Boiss. were tested *in vitro* against several plant pathogenic fungi including postharvest pathogens, the strongest inhibitory activity being on *R. stolonifer*, the weakest on *A. solani*. Extracts from stems had a stronger inhibitory

activity than those from flowers or leaves (Al-Mughrabi, 2003). Aqueous leaf extracts of *Azadirachta indica* Adv. Juss., *Datura fistulosa* L., *Muraya exotica* L., *Lantana camara* L., *Ocimum sanctum* L. and *Catharantes roseus* L. almost completely inhibited the spread of soft rot diseases caused by *Fusarium scirpi* and *Helminthosporium spiciferum* on *Luffa cylindrica* L. (sponge-gourd) when applied after infection. However, activity was less evident when treatment with leaf extracts was carried out before infection with the pathogens (Ahmad and Prasad, 1995). The aqueous extract of *Acacia nilotica* L. showed pronounced antifungal activity against *P. italicum* and enhanced the shelf-life of oranges for 6 days; among various isolated compounds, kaempferol was the most active in suppressing the growth of the target pathogen (Tripathi *et al*., 2002). Recently, also extracts from lichens and lichen acids have been shown to be active against some plant pathogens including *B. cinerea*; *Evernia prunastri*, *Hypogimnia physodes* and evernic acid were the most active (Halama and Van Haluwin, 2004).

17.2.2 Propolis

Propolis (bee glue) is a resinous or sometimes wax-like compound collected by *Apis mellifera* bees from plant buds and barks. Once collected, this material is enriched with salivary and enzymatic secretions and used for the construction and maintenance of hives, as a general sealant, draught excluder, antibiotic and embalming substance to cover carcasses of hive invaders. The chemical composition, still insufficiently known, as well as its colour and aroma change according to the geographical zones (Bankova *et al*., 1998). Propolis from temperate zones, especially Europe, contains predominantly phenolic compounds, including several flavonoids (Burdock, 1998). Among the list of constituents, hydroquinones, caffeic acid and its esters, and quercetin are the most representative (Greenaway *et al*., 1991; Burdock, 1998). A study by Bankova on several propolis samples from Bulgaria, Italy and Switzerland gave evidence that most samples contained pinocembrin, pinobanksin and its 3-*O*-acetate, chrysin, galangin and caffeic and ferulic acid esters (Bankova *et al*., 2002). The substances identified in propolis up until now have been used as constituents of food, food additives and/or generally recognized as safe (GRAS) substances. The pharmacological activity of extracts of propolis as well as its antimicrobial activity are well known (Tosi *et al*., 1996; Ota *et al*., 2001). Despite the potential of propolis as a safe antifungal compound, there are few reports of its application in controlling plant pathogens. *In vitro* assays demonstrated the antifungal activity of propolis against *B. cinerea* (La Torre *et al*., 1990). In trials aimed at assessing the compatibility of post-harvest antagonistic yeasts with additives and agrochemicals, Lima *et al*. (1998) found that propolis inhibited both antagonists (*Rhodotorula glutinis*, *Cryptococcus laurentii* and *Aureobasidium pullulans*) and plant pathogens (*B. cinerea* and *P. expansum*), suggesting that it could be used as a safe, natural fungicide.

The antibacterial and antifungal activities of propolis seem to be related to the presence of polar compounds, mainly flavonoids, phenolic acids and their esters (Ghisalberti, 1979). Indeed, the flavonoids galangin, pinocembrin and pinostrobin, and the ferulic and caffeic acids are the most effective antibacterial compounds occurring in propolis (Marcucci, 1995).

Considering the data available until now on the pharmacological and antibacterial/antifungal activity of propolis, it seems worthwhile to pursue investigations of its use in controlling post-harvest pathogens of fresh fruit and vegetables as well as other food-spoilage microorganisms.

17.2.3 Jasmonates

Jasmonic acid (JA) and its volatile esterified derivative, methyl jasmonate (MeJA), are naturally occurring lipid compounds of the plant cell membranes, derived from oxygenase-dependent oxidation of fatty acids by the lipoxygenase pathway. They exert inhibitory and promotory effects, often similar to those of abscissic acid, on many plant physiological processes (Doares *et al.*, 1995; Beno-Moualem *et al.*, 2004). Among these effects is the triggering of various biosynthetic pathways associated with responses to biotic and abiotic stresses, such as wounding and infection by pathogens. In fact, application of JA or MeJA to plants induces the expression of genes involved in defensive reactions such as the gene-encoding proteinase inhibitor, and phenylalanine ammonia-lyase (PAL), the key enzyme in the phenylpropanoid pathway (Gundlach *et al.*, 1992; Sharan *et al.*, 1998). Several jasmonates have been shown to activate genes encoding antifungal proteins such as thionin (Andresen *et al.*, 1992), osmotin (Xu *et al.*, 1994) and several other genes involved in phytoalexin biosynthesis (Gundlach *et al.*, 1992; Thomma *et al.*, 1998). Few examples are reported in the literature about the application of jasmonates to control post-harvest decay. Recently, it has been shown that MeJA can be applied as a post-harvest treatment to reduce grey mould rot caused by *B. cinerea* in strawberry (Moline *et al.*, 1997). Jasmonates significantly reduced green mould in grapefruit caused by *P. digitatum* after either natural or artificial inoculation, and the most effective concentration for reducing decay in cold-stored fruit was $10\,\mu mol\,l^{-1}$. However, recently MeJA was reported as ineffective in suppressing *M. fructicola* on sweet cherry (Tsao and Zhou, 2000).

At 10 °C storage, raspberries treated with MeJA showed less decay. MeJA-treated fruit also maintained higher levels of sugars, organic acids and oxygen radical absorbance capacity compared to untreated fruit. Colour measurements showed that untreated raspberry fruit became darker and less red after storage, but fruits treated with MeJA were found to have the highest intensity of red colour (Wang, 2003). Although dipping topped radishes in solutions of MeJA (10^{-3} and 10^{-4} M) reduced sprout and root growth at 15 °C (Wang, 1998), only root development was reduced in treated garlic cloves (Cantwell *et al.*, 2003). Although jasmonates have been

reported to have direct antifungal activity against *B. cinerea in vitro*, with complete inhibition at 400 μM MeJA (Meir *et al*., 1998) neither JA nor MeJA had any direct antifungal effect on *P. digitatum* spore germination or germ tube elongation (Droby *et al*., 1999). These results suggest that jasmonates act as resistance inducers against green mould decay and the involvement of phytoalexins cannot be excluded, considering that MeJA induces the syntheses of scopoletin and scopolin in tobacco cell cultures (Sharan *et al*., 1998).

17.2.4 Glucosinolates

Glucosinolates (GLs) are sulphur-containing plant secondary metabolites occurring mainly in cruciferous crops (*Brassicaceae*) and a restricted number of other plant families, among which *Capparaceae* and *Caricaceae* are the most important (Fahey *et al*., 2001). The GL molecule consists of a β-thioglucose moiety, a sulphonated oxime group and a variable side chain derived from an amino acid. GLs may be enzymatically hydrolysed by the enzyme myrosinase to yield a variety of biologically active products, including isothiocyanates, thiocyanates, nitriles and oxazolidine-2-thiones. The nature of the original GLs present in the plant and the conditions of enzymatic hydrolysis determine the types of compounds produced and their biological activities, depending on both the substrate and reaction conditions, especially pH (Mithen, 2001).

In controlled pH conditions (near neutral), the GL breakdown products are predominantly isothiocyanates (Gil and MacLeod, 1980). These products have a wide range of biological activity, which includes both negative and positive nutritional attributes and the effects on the interactions of plants with insects and herbivores. Recent reports about the potential anticarcinogenic activity of GL degradation products (Verhoeven *et al*., 1997) have renewed interest in their possible use as food additives. Many GL breakdown products are toxic to microorganisms and it has been suggested that these compounds may play a role in plant disease resistance (Mithen *et al*., 1986; Doughty *et al*., 1996; Manici *et al*., 1997).

The activity of six GLs (glucoraphenin, gluconapin, sinigrin, glucotropaeolin, sinalbin, and rapeseed glucosinolates) against the leading post-harvest pathogens (*B. cinerea*, *R. stolonifer*, *Monilia laxa*, *Mucor piriformis* and *P. expansum*) of fruit and vegetables has been extensively tested *in vitro* and *in vivo* trials by Mari *et al*. (1993; 1996; 2002). They found that the six native GLs were ineffective in inhibiting the conidia germination of the tested pathogens, whereas all the derived isothiocyanates reduced germination with variable intensity, according to the fungal species and the compound type. The isothiocyanates from glucoraphenin, sinigrin and sinalbin totally inhibited the germination of the five pathogens tested. None of the tested compounds inhibited the mycelia growth of *M. piriformis* and *R. stolonifer*, whereas isothiocyanates from glucoraphenin proved to be the

most effective against *P. expansum*, *B. cinerea* and *M. laxa*. The volatile compounds obtained from the enzymatic hydrolysis of sinigrin and gluconapin (2-propenyl and 3-butenyl isothiocyanates, respectively) strongly inhibited conidia germination and/or mycelial growth of *M. laxa*, *B. cinerea* and *P. expansum*, thus indicating that antifungal activity is exerted by the volatile fraction of these low-molecular-weight compounds (Mari *et al*., 1993).

The same GLs and isothiocyanates were tested *in vivo* to evaluate their activity in controlling storage decay of two pear varieties (Conference and Kaiser) caused by the five fungi mentioned above. Isothiocyanates from glucoraphenin were the most effective against *M. laxa*, *B. cinerea* and *M. piriformis* after 6 days at 20 °C, determining a significant reduction of the lesion diameter in artificially inoculated fruits. The concentration of the glucoraphenin-derived isothiocyanates strongly affected the antifungal activity: the highest value tested (3.6 mg ml^{-1}) completely inhibited the lesion development by *M. laxa* even when a spore suspension at 10^6 cell ml^{-1} was used for inoculation. Moreover, the isothiocyanate was able to stop *M. laxa* infections already underway, showing a curative effect up to 40 hours after inoculation (Mari *et al*., 1996).

Allyl isothiocyanate (AITC), a naturally occurring flavour compound in mustard and horseradish, has a well-documented antimicrobial activity (Ishiki *et al*., 1992; Delaquis and Mazza, 1995). This volatile compound can be employed successfully in modified atmosphere packaging or as a gaseous treatment before storage (Tripathi and Dubey, 2004). The activity of AITC vapour from pure sinigrin or from *Brassica juncea* against the decay caused by *P. expansum* on pears has recently been tested (Mari *et al*., 2002). The best decay control was obtained by exposing fruits for 24 hours at 20 °C to an atmosphere enriched with 5 mg l^{-1} of AITCs, the extent of control depending on the inoculum density of the pathogen. Increasing *P. expansum* inoculum concentration at a constant AITC concentration resulted in increasing disease incidence, thus suggesting that inoculum density occurring on fruit surface, on packhouse working lines, in floating water, and so on, is an important parameter that needs to be known for the proper use of this compound. Moreover, AITC treatments were effective up to 24 hours after inoculation for cv Conference and 48 hours for cv Kaiser, also controlling infection caused by a thiabendazole-resistant strain of *P. expansum* and reducing by 90% the incidence of blue mould in both pear cultivars (Mari *et al*., 2002). This is particularly relevant since the availability of natural and safe compounds also possessing curative effects could allow one of the major limits of alternative methods in controlling postharvest decay to be overcome; i.e., the inefficacy against active infections. The results of analysis on the skin and pulp of AITC-treated pears indicate the extremely low concentration of residue in fruit, suggesting the absence of any implications for human health (Mari *et al*., 2002). However, further evidence is necessary to validate the effectiveness of this compound at the temperature utilized to store pome and stone fruits and for large-scale treatments.

17.2.5 Essential oils

The antifungal and antibacterial activities of essential oils (EOs) against important plant and human pathogens, as well as food spoilage organisms, has been studied extensively (Roller, 2003). Recently, there has been a renewed interest in the application of these substances to control plant pathogens and post-harvest diseases in particular (Arras and Usai, 2001; Aligiannis *et al*., 2001; Thangadurai *et al*., 2002; Palhano *et al*., 2004). The EOs are plant aromatic substances composed mainly of terpenes and other additional compounds such as aldehydes, fatty acids, phenols, ketones, esters, alcohols, nitrogen and sulphur compounds (Cowan 1999; Arras and Usai, 2001; Aligiannis *et al*., 2001; Thangadurai *et al*., 2002); the mixtures are extremely complex and vary with environmental and genetic factors. The role played by these substances in the plant has not been fully elucidated; however, it is likely that most of them are involved in chemical defence mechanisms against phytopathogenic microorganisms (Mihaliak *et al*., 1991). They also exert their activity on the external environment in producing plants, influencing insects and the microbial composition of the phyllosphere and carposphere. For these reasons, several investigators have considered them as antimicrobials in food and, in particular, against post-harvest microbial spoilage of vegetable, fruit and flower commodities. Their mode of action seems to be related to increased cell membrane permeability of microorganisms causing the contents to leak out (Piper *et al*., 2001).

A great number of EOs have been tested *in vitro* for their activity against a range of bacteria and fungi (Nychas *et al*., 2003). Among the EOs tested against post-harvest pathogens, those from plants of the genus *Thymus* have been particularly active. Thyme EOs have been tested on *P. italicum*, *P. digitatum*, *B. cinerea*, *Alternaria citri*, *A. alternata*, *F. oxysporum* and *R. stolonifer* (Arras and Grella, 1992; Arras *et al*., 1995; Reddy *et al*., 1998; Arras and Usai, 2001; Bouchra *et al*., 2003); concentrations of 200–250 ppm of *Thymus capitatus* EO completely inhibited growth of the fungi (Arras and Usai, 2001), whereas a concentration of only 100 ppm of *Thymus glandulosus* EO suppressed the growth of *B. cinerea* (Bouchra *et al*., 2003). On strawberries, *Thymus vulgaris* EO reduced decay caused by *B. cinerea* and *R. stolonifer* by up to 76% (Reddy *et al*., 1998). Vapours of thyme EO reduced grey mould development on *Botrytis*-inoculated sweet cherries (Chu *et al*., 1999) and brown rot in apricots and plums (Liu *et al*., 2002).

Generally, the fungicidal activity of EOs observed *in vitro* was not reproduced *in vivo* because of the volatile nature of the constituents. Arras and Usai (2001) have overcome this problem by combining thyme EO with a vacuum at 0.5 atm (see also Section 17.6). Carvacrol has been identified as the substance responsible for the antimicrobial activity in the EO of *Thymus capitatus* (Arras and Grella, 1992). Thymol, carvacrol and linalool were the active agents in *T. vulgaris* (Reddy *et al*., 1998). However, other minor components can also contribute synergistically to the antimicrobial

activity of an EO (Lattaoui and Tantoui-Elaraki, 1994; Cosentino *et al*., 1999; Karaman *et al*., 2001).

The EO of oregano (*Origanum* spp.), another member of the family of *Labiateae* containing thymol and carvacrol, was reported to be very active *in vitro* against several mycotoxigenic fungi (Elgayyar *et al*., 2001; Lambert *et al*., 2001) and against some citrus disease agents (Arras, 1988). Among the complex constituents of citrus EOs, the terpene citral (3,7-dimethyl-2,6-octadienal) is known to have strong antifungal properties (Rodov *et al*., 1995). Recent *in vitro* studies have demonstrated that citral inhibited *P. expansum*, *P. italicum* and *P. digitatum*, which are responsible for severe storage rot in apples and citrus fruits (Caccioni *et al*., 1995a, 1998; Venturini *et al*., 2002), and *Colletotrichum gloeosporioide* the causal agent of anthracnose of papaya fruit (Palhano *et al*., 2004). However, because of its phytotoxicity, citral may be difficult to use on fresh fruits and vegetables (Rodov *et al*., 1995).

Many other EOs are known to possess antimicrobial activity. They have been tested against a wide range of bacteria (Nychas *et al*., 2003) and other microbial contaminants of processed food (Beuchat, 2001) and also against insects (Isman, 2000), but, as reported above, very few of them have been tested against fungi and/or diseases responsible for loss during the post-harvest phase of fresh fruit and vegetables. The large body of data available in the literature can constitute a valid source for new EOs to be tested in the post-harvest environment. The strong aroma of EOs limits their application in foods; therefore, their use lies in a careful selection and evaluation at low concentration, possibly in synergistic combination with other natural products to improve the antimicrobial activity, for example a combination of carvacrol and thymol provided as great an inhibition as oregano essential oil with a lesser flavour impact (Lambert *et al*., 2001).

17.2.6 Plant phenolic compounds

Phenolics play important roles in conferring flavour and colour characteristics to plants, fruits and vegetables and serve as plant defence mechanisms against attack by microorganisms, insects and herbivores. Progress in research on the antimicrobial properties of plant phenolics has been enormous in the last few decades and many aspects have been comprehensively reviewed (Hahlbrock and Scheel, 1989; Nicholson and Hammerschmidt, 1990; Dooner and Robbins, 1991; Rhodes, 1994; Dixon *et al*., 1995; Boudet *et al*., 1995; Hammerschmidt, 1999; Dixon *et al*., 2002). In this section only the most relevant aspects of the relationships between phenolic compounds and post-harvest diseases of fresh fruits and vegetables will be covered.

All fruits and vegetables contain biologically active phenolic compounds (Spanos and Wrolstad, 1992; Shahidi and Naczk, 1995), most of which have not been fully explored as natural antifungal substances and alternative

means of controlling post-harvest diseases. The composition of phenolic constituents in fruits is influenced by both internal and external factors. These include genetic variation at the species and cultivar level, maturity at harvest, pre-harvest agrononic practices and post-harvest processing conditions (Lattanzio, 1988; Mueller Harvey and Dhanoa, 1991). Recently, antifungal activity has been found in all tissue types (viz. pith, cortex, epidermis) of strawberry fruit, and thin-layer chromatography bioassays revealed that all fruit stages yielded antifungal activity owing to the occurrence of phenolic compounds (Terry and Joyce, 2004; Terry *et al*., 2004). Several investigations confirmed that concentrations of phenolic compounds are generally higher in young fruits and tissues (Macheix *et al*., 1990; Lattanzio *et al*., 1994a). In fruits, the total phenol content ($mg\,g^{-1}$ fresh weight) falls during growth, but two distinct phenomena can be observed: the level continues to fall steadily, as in the case of white-coloured species and varieties (e.g., white grape cultivars), or it rises at the end of maturation, as in the case of red fruits in which anthocyanins or flavonoids accumulate (Macheix *et al*., 1990).

Phenolic compounds which inhibit the growth of fungi may be present in healthy, unchallenged fruits and vegetables (preformed antimicrobial compounds) or may be found only in fruit tissues that have either been infected by pathogens or weakly stressed (phytoalexins) (Kuć, 1995; Dixon, 2001). In the first group simple phenols, phenolic acids, flavonols, some isoflavones and dihydrocalchones (phloridzin) are included; the second group, of phenolics and phytoalexins, includes isoflavonoids, flavans, stilbenes, phenanthrenes, pterocarpans and furocoumarins (Lattanzio *et al*., 2001). All these compounds originate through different branches of the 'general phenylpropanoid pathway', whose core reaction is the deamination of the phenylalanine by the PAL enzyme to produce *trans*-cinnamic acid. The phenylpropanoid pathway can switch to the flavonoid biosynthesis, via the condensation of three molecules of malonyl CoA with one molecule of *p*-coumaryl CoA, to yield chalcone. This reaction is catalysed by chalcone synthase (CHS) (Hahlbrock and Scheel, 1989). However, several of the intermediates and/or derivatives of the phenylpropanoid pathway have been reported to possess antimicrobial activity (Shuen and Buswell, 1992; Snook *et al*., 1992; Tuncel and Nergiz, 1993). Material entering the general phenylpropanoid pathway leads to the formation of a series of hydroxycinnamic acids and hydroxycinnamoyl-CoA esters, which vary in their degrees of hydroxylation and *O*-methylation (Barber *et al*., 2000).

Hydroxycinnamic and cinnamic acid derivatives that have antimicrobial activity include caffeic, chlorogenic, *p*-coumaric, ferulic and quinic acids. Depending upon the botanical species, hydroxycinnamics may be present at concentrations sufficient to retard microbial invasion and delay rotting of fruits and vegetables. Moulds and yeasts responsible for food spoilage were sensitive to hydroxycinnamic acid and derivatives (Davidson and

Branen, 1981). Several studies also showed that derivatives of benzoic or cinnamic acid inhibit growth of various filamentous fungi, including *Aspergillus* and *Penicillium spp.*, and food spoilage yeasts, as well as the biosynthesis of mycotoxins (Chipley and Uraih, 1980; Tawata *et al.*, 1996; Florianowicz, 1998). A phenolic compound from walnut seed coats, gallic acid, was recently shown to prevent aflatoxin biosynthesis by *A. flavus* (Mahoney and Molyneux, 2004). Moreover, it has been demonstrated that cinnamic acid, vanillin and veratraldehyde inhibited both hyphal growth of *A. flavus* at 5 mM and spore germination at 10 mM. Vanillylacetone, vanillic acid and three coumaric acids (*o*-coumaric, *m*-coumaric and *p*-coumaric) inhibited hyphal growth at 5–25 mM, while caffeic acid showed only limited inhibition of fungal growth even at the highest concentration tested. Vanillylacetone was highly toxic to *A. flavus* relative to the vanillic or coumaric acid derivatives at 15–25 mM. All three coumaric acids showed similar levels of inhibiting fungal growth at 5–15 mM (Kim *et al.*, 2004).

Specific *in vitro* trials have been conducted to evaluate the antimicrobial activity of intermediates in the general phenylpropanoid pathway against yeasts and bacteria. Of the three main classes of compounds tested, the hydroxycinnamaldehydes were the most effective, possessing higher antifungal and antibacterial activity than hydroxycinnamic acids and hydroxycinnamyl alcohols (Barber *et al.*, 2000).

Caffeic and coumaric acids are cinnamics widely distributed and common to apple, pear and grape. They occur naturally in combination with other compounds, usually in the form of esters. The ester of caffeic with quinic acid, chlorogenic acid, is a classic example. In contrast, benzoics usually occur as free acids (Spanos and Wrolstad, 1992).

Phenolic compound induced *in vitro* inhibition of *Botryodiplodia theobromae*, the causal agent of Java black rot in sweet potato, chlorogenic acid giving the highest *in vitro* inhibition followed by pyrogallol, pyrocatechol, phenol and resorcinol (Mohapotra *et al.*, 2000).

Among a group of cinnamic acid derivatives tested *in vitro* for their activity against several post-harvest pathogens (*B. cinerea*, *P. digitatum*, *Sclerotinia sclerotiorum*, *F. oxysporum* and *Alternaria* spp.), chlorogenic and ferulic acid were strong inhibitors of *F. oxysporum* and *S. sclerotiorum*, respectively (Lattanzio *et al.*, 1994a). Starting from the basic skeleton of the cinnamic acid, the presence of a hydroxyl group in the aromatic ring (i.e., *p*-coumaric acid, *m*-coumaric acid and *o*-coumaric acid) increased the activity against *F. oxysporum* and *Alternaria* spp. An additional hydroxyl group in the benzene ring caused no increase in antifungal activity, caffeic acid being only a middle inhibitor against *P. digitatum*. Recently, similar results have been reported (Kim *et al.*, 2004) indicating that cinnamic acid (without an –OH group) has the highest antifungal activity, whereas caffeic acid (with two –OH groups) did not affect significantly the growth of *A. flavus*. Alternatively, three coumaric acids (with one –OH group) showed moderate levels of antifungal activity. Results with the coumaric acids further

indicate that the number of hydroxyl groups in the phenolic ring might affect the level of antifungal activity.

Conversely, the presence of a methoxy group increased the activity compared to coumaric acids; ferulic acids were the better inhibitor amongst the cinnamic derivatives. Benzoic derivatives are reported to be the best inhibitors of *B. cinerea*, *P. digitatum, S. sclerotiorum*, *F. oxysporum* and *Alternaria* spp. The presence of an additional hydroxyl group in the ring of *p*-hydroxybenzoic acid improved the antifungal activity of the monophenol (Lattanzio *et al*., 1994a).

The antifungal activity of 2,5-dimethoxybenzoic acid (DMBA) in controlling post-harvest decay pathogens has been specifically tested both *in vitro* and *in vivo*. *In vitro* studies demonstrated that DMBA inhibited both spore germination and mycelial growth of *B. cinerea* and *R. stolonifer*. Starting from a 10^{-4} M DMBA concentration, spore germination and mycelial growth of both fungi were affected, *B. cinerea* being more sensitive than *R. stolonifer*. At a concentration of 5×10^{-3} M, DMBA completely inhibited spore germination of both fungi. Mycelial growth of *R. stolonifer* was completely inhibited at a concentration of 5×10^{-3} M, whereas 1×10^{-3} M DMBA inhibited radial growth of *B. cinerea* by more than 93%, the fungus being completely inhibited at a concentration of 5×10^{-3} M. *In vivo* studies demonstrated that spraying or dipping into DMBA at 10^{-2} M reduced the storage decay of strawberries stored at 20 °C or at 3 °C plus a period of simulated shelf-life. Its practical use on strawberries has also been tested and the best results were obtained when fruits were dipped for 1 min in 10^{-2} M DMBA amended with 0.05% (v/v) Tween 20 (Lattanzio *et al*., 1994b).

Studies on the metabolism of exogenous DMBA during room and low temperature storage indicated that the DMBA level rapidly decreased during the first three days of storage and this decrease was more pronounced in fruits stored at 20 °C. At the end of the storage period less than 15% of the applied phenolic was found in the strawberries. The antifungal activity seems to be associated with lipophilicity of the compounds; lipophilic compounds are deacylated in the fungal cell to yield an active phenol. Lipophilicity and/or the presence of a hydroxyl group are considered essential features for the antifungal activity, since the first characteristic permits penetration of biological membranes while hydroxyl groups may act in uncoupling oxidative phosphorylation (Lattanzio *et al*., 1994a).

Among a group of phenolics from apples cv Golden delicious, only chlorogenic acid inhibited *Phlyctaena vagabunda* spore germination and mycelial growth *in vitro*, whereas (+)-catechin, (−)-epicatechin, phloridzin and quercetin glycosides showed no activity (Lattanzio *et al*., 2001). Similarly, *in vitro* bioassay of catabolic phloridzin derivatives (phloretin, phloroglucinol, phloretic acid and *p*-hydroxybenzoic acid) indicated no inhibitory effects on mycelial growth of *P. vagabunda*. Changes of apple phenolics and polyphenol oxidase activity during cold storage and the biological activity of these phenolics have also been analysed with reference

to the development of quiescent infections during cold storage and shelf-life at room temperature. The results suggest that phloridzin and chlorogenic acid in combination with polyphenol oxidase activity could function to arrest *P. vagabunda* in quiescent infections of immature and ripening apple fruit (Lattanzio *et al*., 2001).

Flavonoids are a large group of secondary plant metabolites, which are widely distributed throughout the plant kingdom. They are synthesized from phenylpropanoid and acetate-derived precursors, and are characterized by a common benzo-γ-pyrone structure (Pietta, 2000). Generally, flavonoids occur as glycosylated derivatives and play important roles in plant growth and development and in the defence against microorganisms and pests. Many studies suggest that flavonoids have biological activities, including antiallergenic, antiviral, antifungal, anti-inflammatory and vasodilating actions (Colerige Smith *et al*., 1980; Bors *et al*., 1990) and since they show low toxicity in mammals some are used in human medicine (Cesarone *et al*., 1992; Hertog *et al*., 1993; Pietta, 2000). The antioxidant properties of flavonoids have long been recognized (Schijlen *et al*., 2004). They have been reported to inhibit lipid peroxidation, to scavenge free radicals and active oxygen, to chelate iron ions and to inactivate lipoxygenase (Pietta, 2000). Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is one of the most abundant natural flavonoids. It is present in various common fruits and vegetables (apples, grapes, lemons, tomatoes, onions, lettuce, broccoli, etc.) (Sestili *et al*., 1998). As a powerful antioxidant and metal ion chelator, it protects plant antioxidant systems, such as catalase and superoxide dismutase (SOD) activities. Some flavonoid pyrogallol derivatives, such as tea tannins, also possess antioxidant activity (Williams *et al*., 2004). However, some plant phenolics have sometimes been found to show pro-oxidant properties (Lattanzio *et al*., 1994a).

Usually, flavonoids are divided into several categories, including flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids (Shahidi and Naczk, 1995). The majority of flavonoids that are recognized as constitutive antifungal agents in plants are either isoflavonoids, flavans or flavanones. Because of their *in vitro* antifungal activity, flavonoid compounds have long been thought to play a role in plant–microorganism interactions as part of the host plant's defensive arsenal (Harborne and Williams, 2000).

Stilbene phytoalexins, as flavonoid-type phytoalexins, are formed on the phenylalanine/polymalonate pathway, the last step of this biosynthesis being catalysed by stilbene synthase (STS). Real-time monitoring of STS transcript levels indicates that it accumulates selectively in grape skin in response to both biotic and abiotic stresses (Soleti-Ligorio *et al*., unpublished). The skeleton of these substances is based on *trans*-resveratrol structure (3,5,4′-trihydroxystilbene) (Jeandet *et al*., 2002). Among the most studied compounds is *trans*-resveratrol, which possesses an unspecific antifungal character, thus representing a good candidate as a 'natural pesticide'

against pathogens (Jeandet *et al*., 1995). In addition, resveratrol is known to possess antioxidant properties that can have positive effects on fruit conservation during storage. It has long been recognized that *trans*-resveratrol enhances the resistance of vine plants to pathogens such as *B. cinerea*, *Phomopsis viticola* (Hoos and Blaich, 1990), *Plasmopara viticola* (Dai *et al*., 1995) and *R. stonifer* (Sarig *et al*., 1997). Several *in vitro* investigations have also been conducted, demonstrating the antifungal activity of this compound (Paul *et al*., 1998). Recently, direct exogenous application of *trans*-resveratrol to grapes and apples maintained their post-harvest quality for weeks or months, with clear differences from the untreated ones regarding the health and quality of the fruit. In addition, it has been demonstrated that the resveratrol application does not alter the fruit organoleptic and biochemical properties (Gonzalez Ureña *et al*., 2003).

In the recent past, intense research has also been devoted to the role of resveratrol in human health because of its protective effects against cardiovascular diseases and cancer (Doraia and Aggarwalb, 2004; Fulda and Debatin, 2004). The valuable therapeutic effect of resveratrol has stimulated investigations into the occurrence of this compound in grapes, other berry fruits (Lyons *et al*., 2003; Rimando *et al*., 2004) and various herbs (Cai *et al*., 2004). Because of its capacity to confer disease resistance in the grapevine, as well as its biological properties, most interest has now centred on STS gene transfer from grapevine to numerous plants such as rice (Stark-Lorenzen *et al*., 1997), tomato (Thomzik *et al*., 1997), apple (Szankowski *et al*., 2003) and kiwifruits (Kobayashi *et al*., 2000), with the objective of increasing their tolerance to pathogens and improving the nutritional quality of their derived food products. Moreover, increasing levels of flavonoids have recently been reported in tomatoes by overexpressing the petunia gene for chalcone isomerase, leading to an 80-fold increase in the flavonoid content of the tomato peel and a corresponding 20-fold increase in the flavonoid level in tomato paste (Muir *et al*., 2002). In addition, chalcone synthase and flavonol synthase transgenes were found to act synergistically to upregulate flavonol biosynthesis significantly in the flesh of tomato fruits (Verhoeyen *et al*., 2002).

Galangin (3,5,7-trihydroxyflavone) extract from *Helicrysum aureonitens* was active against *P. digitatum* and *P. italicum* (Afolayan and Meyer, 1997), two important storage pathogens of citrus fruits. Four polymethoxylated flavones (3,5,6,7,3′,4′-hexamethoxyflavone, 3,5,6,7,8,3′,4′-heptamethoxyflavone, 5,6,7,8,4′-pentamethoxyflavone and 5,6,7,8,3′,4′-hexamethoxyflavone) from cold-pressed orange oil were characterized and evaluated for their antifungal activities against *C. gloeosporioides* (Penz.) Penz. & Sacc., a major pathogen of fruits that causes damage to crops in tropical, sub-tropical and temperate regions. Methoxylated flavones were effective in inhibiting mycelial growth of the fungus. As flavone concentration increased, mycelial growth decreased. 5,6,7,8,3′,4′-

hexamethoxyflavone completely inhibited the growth of *C. gloeosporioides* at a concentration of 100 μg ml^{-1} (Almada-Ruiz *et al*., 2003).

The antifungal activity of biflavones from *Taxus baccata* and *Ginkgo biloba*, namely amentoflavone, 7-*O*-methylamentoflavone, bilobetin, ginkgetin, sciadopitysin and 2,3-dihydrosciadopitysin, towards the fungi *Alternaria alternata* and *Cladosporium oxysporum* has recently been evaluated; bilobetin exhibited a significant antifungal activity, completely inhibiting the growth of germinating tubes of *C. oxysporum* at a concentration of 100 μM. The activity of ginkgetin and 7-*O*-methylamentoflavone towards *A. alternata* was stronger than that of bilobetin. Moreover, slight structural changes in the cell wall of *A. alternata* exposed to ginkgetin have also been reported (Krauze-Baranowska and Wiwart, 2003).

Specific trials of the activity of some synthetic flavonoids against postharvest pathogens have been conducted by Lattanzio *et al*. (1994a). On the whole, the results indicate a low activity at the concentration range tested (10^{-4}–10^{-5} M). The highest mycelial inhibition was observed with apigenin-7-glucoside and kaempferol-3-rutinoside. All the flavonoids tested, except for kaempferol-3-rutinoside, showed an appreciable activity against *Penicillium* spp. However, these results, far from demonstrating that flavonoids are not important in the resistance against the tested fungi, seem to indicate that the combination flavonoid/fungus/host is of fundamental importance. To date, the major role of flavonoids has been related to the resistance mechanisms in the host, acting as phytoanticipins or phytoalexins. Among the constitutive secondary metabolites, those occurring in citrus fruits have been widely investigated. Tangeretin and naringin may act as antifungal compounds in the resistance mechanism against fungal attack, acting as first and second defence barriers, respectively, since polymethoxylated flavones (tangeretin) are mainly localized in the outermost tissue of the fruit, the flavedo, whereas flavanones (naringin) are located in the albedo (Kanes *et al*., 1992).

Other secondary compounds induced after infection, such as coumarins, also act in the defence mechanism of citrus fruits (Angioni *et al*., 1998; Arcas *et al*., 2000). Coumarins are phenolic substances containing a fused benzene and alfa-pyrone rings. It is known that some citrus species accumulate coumarins such as xanthyletin, seselin and scoparone when infected by phytopathogenic fungi (Afek and Sztejnberg, 1993; Stange *et al*., 1993). The nature of the coumarin biosynthesized in this process varies within a species according to the pathogen. For example, *Citrus limon* L. accumulated scoparone after inoculation with *P. digitatum* (Kim *et al*., 1991); however, there was no significant accumulation of any antifungal compounds in the tissues of lemons inoculated with *Geotrichum candidum* (Baudoin and Eckert, 1985). Although coumarin inhibits the germination of spores of *A. niger*, *P. glaucum* and *R. nigricans*, other 4-hydroxycoumarins are generally

ineffective against fungi. Antibacterial and antifungal effects have been found for umbelliferone and scopoletin (Jurd *et al*., 1971a,b; Recio *et al*., 1989; Kwon *et al*., 1997). *In vitro* tests indicated that pure coumarins have a very modest activity against *A. niger*. In contrast, scopoletin promoted the growth of *B. cinerea* and *Fusarium* proved to be the most sensitive among the pathogenic fungi (Ojala *et al*., 2000).

Limettin (5,7-dimethoxycoumarin), 5-geranoxy-7-methoxycoumarin and isopimpinellin (5,8-dimethoxypsoralen) were shown to be effective antifungal compounds (Rodov *et al*., 1995). 7-Geranoxycoumarin showed inhibitory activity against *P. italicum in vitro*, which was comparable to other naturally occurring compounds like scoparone and scopoletin. Tests *in vivo* indicated that only when applied to grapefruit at $500\,mg\,l^{-1}$ 7-geranoxycoumarin has antifungal activity against *P. italicum* comparable to that of scoparone and scopoletin. However, its activity was much higher against *P. digitatum*, the major post-harvest pathogen of citrus fruit (Angioni *et al*., 1998). Scoparone and scopoletin applications are reported as phytotoxic, causing browning and necrosis to the rind at a very low concentration ($50\,mg\,l^{-1}$). In contrast, 7-geranoxycoumarin had no adverse effects on the citrus peel when applied at a higher concentration (Rodov *et al*., 1995; Angioni *et al*., 1998). For the citrus industry all these compounds have potential as alternative means of controlling post-harvest decay since it has been demonstrated that they can be induced by a number of physical and biological treatments (Ben-Yehoshua *et al*., 1988; Kim *et al*., 1991; Wilson *et al*., 1994).

In conclusion, despite the large body of evidence concerning the antifungal activity of numerous phenolic compounds, little data exist about their practical application as alternative compounds in controlling post-harvest diseases of fresh fruit and vegetables. However, their antifungal activity and low or absence of mammalian toxicity (Singleton, 1981) make these natural compounds interesting candidates for application as surface disinfectants for fresh fruit and vegetables.

17.3 Volatile compounds

Volatile compounds from plants, defined as those compounds with a relatively high vapour pressure capable of approaching an organism through the liquid and the gas phases, can be either inhibitory or stimulatory to fungal growth and/or spore formation and germination (Fries, 1973). Acetaldehyde, a volatile compound accumulating in fruit during ripening, has shown a fungicidal effect against various post-harvest pathogens. Among nine low-molecular-weight aliphatic aldehydes produced by sweet cherries, acetaldehyde, together with propanal and butanal, significantly reduced decay of *P. expansum*-inoculated sweet cherries (Mattheis and Roberts, 1993), acetaldehyde being the most active. Resistance of straw-

berry fruit to rot in high-CO_2 storage has been ascribed to the production of high levels of acetaldehyde and ethyl acetate by the fruit in response to these conditions. Fumigation with acetaldehyde at 0.1%–1% has resulted in inhibition of spore germination and reduced mould development on strawberries (Prasad and Stadelbacher, 1974; Pesis and Avissar, 1990), raspberries (Prasad and Stadelbacher, 1973), apples (Stadelbacher and Prasad, 1974), grapes (Avissar and Pesis, 1991) and sweet cherries (Mattheis and Roberts, 1993). However, in some cases acetaldehyde induced phytotoxicity symptoms (Stadelbacher and Prasad, 1974; Stewart *et al*., 1980; Avissar and Pesis, 1991; Perata and Alpi, 1991; Mattheis and Roberts, 1993) and altered fruit sensory traits (Pesis and Avissar, 1990; Avissar and Pesis, 1991), depending on the concentration and exposure duration. The mode of action of acetaldehyde has not been fully elucidated; however, there is evidence that it causes membrane disruption followed by leakage of electrolytes, reducing sugars and amino acids in the cells (Avissar *et al*., 1990). In spite of the large number of trials carried out against post-harvest pathogens and diseases, no commercial application of acetaldehyde is reported in the literature and no recent applications have been tested.

Hinokitiol, a natural volatile oil extracted from the root and bark of some *Cupressaceae* such as the Hinoki tree (*Hiba arborvitae*), known for its high degree of resistance against wood decay, showed antimicrobial properties at a low dosage and had a wide antimicrobial spectrum against general bacteria and fungi. At a dosage of 15–30 $\mu g\,ml^{-1}$ it was effective in reducing the spore germination of *M. fructicola*, *R. oryzae* and *B. cinerea* by 50% and prevented decay of commercially harvested peaches (Sholberg and Shimazu, 1991). In *in vivo* trials, hinokitiol at 750 $\mu l\,l^{-1}$ behaved better than prochloraz in suppressing *B. cinerea* and *A. alternata* on eggplants and peppers (Fallik and Grinberg, 1992). Although these are interesting results and a large body of experiments have been conducted in the pharmaceutical field, no other tests have been carried out against plant or post-harvest pathogens.

Volatiles from 'Isabella' (*Vitis labrusca* L.) grapes revealed a strong inhibitory action on the sporulation and sclerotia formation of *B. cinerea* and significantly limited the incidence of infection on 'Roditis' grapes and 'Haiward' kiwi fruits by reducing both the inoculum density and the activity of the pathogen (Kulakiotu *et al*., 2004a,b); mycelium of *B. cinerea* grown in the presence of volatiles showed endolysis, deformation of the cell wall and formation of chlamydospores. Studies are in progress to identify the active component/s of the 'Isabella' volatiles related to antifungal activity (Kulakiotu *et al*., 2004b).

In vitro trials using 16 volatile compounds from peach and plum have demonstrated the high efficacy of ethyl benzoate, methyl salicylate and benzaldehyde in inhibiting the growth of *B. cinerea* and *M. fructicola*; benzaldehyde totally inhibited spore germination of *B. cinerea* at 25 $\mu l\,l^{-1}$ and germination of *M. fructicola* at 125 $\mu l\,l^{-1}$ (Wilson *et al*., 1987). Tonini and

Caccioni (1990) reported similar results for stone fruit. Caccioni *et al.* (1995b) reported that, among eight volatile compounds forming the characteristic aroma of ripe stone fruit, benzaldehyde was, at 5000 ppm, one of the most active in reducing decay by *M. laxa* and *R. stolonifer* in inoculated peaches, nectarines and plums.

Archbold *et al.* (1997) have reported that hexanal, 1-hexanol, (*E*)-2-hexenal, (*Z*)-6-nonenal and (*E*)-3-nonen-2-one, and the aromatic compounds methyl salicylate and methyl benzoate had potential as post-harvest fumigants for the control of *B. cinerea* on strawberries, blackberries and grapes at concentrations as low as 2–12 µl/250 ml. Later, the same authors (Archbold *et al.*, 1999) showed that one of the compounds, (*E*)-2-hexenal, was effective against grey mould on seedless table grapes but complete mould suppression was not achieved as the level of hexenal declined during the course of the trial. Headspace analyses have shown that (*E*)-2-hexenal concentrations below 0.5 µmol l^{-1} stimulated *B. cinerea* mycelial development *in vitro*, while concentrations above it inhibited growth of the mould (Fallik *et al.*, 1998). (*E*)-2-hexenal is the major volatile compound (accounting for 70%–74% of the total peak areas) in extract of *Poligonum cupsidatum* S. et Z. leaves, a plant commonly used in human medicine for centuries, which is very effective against bacteria but still not tested against plant pathogenic fungi (Kim *et al.*, 2005). The antifungal activity of hexanal, whose effectiveness seems to be related to its vapour pressure (Gardini *et al.*, 1997), has been studied on several host–pathogen combinations (Nandi and Fries, 1976; Hamilton-Kemp *et al.*, 1992; Caccioni *et al.*, 1997) and has proved to be active on stone fruits against *R. stolonifer* and *M. laxa*. At 2500 ppm, hexanal produced the same fungistatic effect as 5000–10 000 ppm of benzaldehyde, but at higher concentrations it was phytotoxic (Caccioni *et al.*, 1995b). Methylsalicylate demonstrated some fungistatic activity, but it also gave an unpleasant odour to the fruit (Caccioni *et al.*, 1995b). The use of natural volatile aroma compounds as antimicrobial fumigants is an interesting field of investigation still not fully explored. In general, these substances have limited toxicity in mammals and a degree of volatility that allows their application in fumigation of cold storage rooms or in 'active packaging' (Toray Research Center, 1991).

Acetic acid, the principal organic compound of vinegar, could also be used in the vapour phase to control post-harvest pathogens. Early studies demonstrated its efficacy against conidia of *M. fructicola* on peaches (Roberts and Dunegan, 1932). As little as 1.4 mg l^{-1} of acetic acid vapour prevented decay of peaches inoculated with conidia of *M. fructicola* or *R. stolonifer*; fumigation with 2.0 or 4.0 mg l^{-1} acetic acid before wounding prevented decay in apples contaminated with *B. cinerea* or *P. expansum*, respectively (Sholberg and Gaunce, 1995, 1996). Acetic acid was lethal at 0.1% and 0.15% to *B. cinerea* and *P. expansum*, respectively, while 0.7% and 2% acetaldehyde were required to achieve the same effect (Avissar *et al.*, 1990; Stadelbacher and Prasad, 1974). At concentrations of 0.18%–0.27%

(vol/vol), acetic acid controlled *Botrytis* and *Penicillium* decay on two Canadian table grape varieties, to the same extent as SO_2, with no adverse effects on fruit composition (Sholberg *et al*., 1996). However, acetic acid, like other short-chain organic acids, can be extremely phytotoxic in the vapour form, depending on temperature, concentration and exposure (Sholberg, 1998). After repeated trials with acetic acid vapours on d'Anjou pears, Sholberg *et al*. (2004) suggested that the fruit be fumigated as soon as possible after harvest, at a rate not over $200\,\mu l\,l^{-1}$ and at a temperature of 1 °C, to reduce effectively stem infection and fruit rot, with any phytotoxic effect.

A phytotoxic effect on stone fruits, strawberries and apples has also been avoided using heat-vaporized vinegar, but the volume needed to reduce decay in these fruits was high, at $36.6\,\mu l\,l^{-1}$ of air (Sholberg *et al*., 2000). Since vinegar and its active component, acetic acid, do not penetrate into the fruits, they do not control latent or quiescent infection (Sholberg and Gaunce, 1996). Although informal tasting of the treated fruits has not identified any off-odours, rigorous sensory evaluation of acetic acid-treated fruits has not been undertaken to date (Sholberg *et al*., 1996, 2000, 2004). Vapours of ethanol to control post-harvest decay have not been investigated extensively but aqueous ethanol has been used as a dip treatment to control brown rot and *Rhizopus* rot of peaches (Ogawa and Lyda, 1960; Feliciano *et al*., 1992) and table grapes (Lichter *et al*., 2002) or in combination with hot water to control post-harvest decay of table grapes, lemons and stone fruits (Smilanick *et al*., 1995; Margosan *et al*., 1997; Karabulut *et al*., 2004a,b) with varying degrees of success. Ethanol vapours inhibited decay of oranges by *P. italicum* and *P. digitatum* after five days of continuous exposure (Yuen *et al*., 1995). No other use of ethanol vapours has been reported, probably because of concern about its inflammability and explosive potential under high pressure.

17.4 'Oxidative stress' and the control of post-harvest pathogens

In early phases of infection, upon sensing the invading microorganism, plants can evoke diverse defence mechanisms in an attempt to restrict pathogen growth and, finally, to destroy it. In numerous incompatible plant–pathogen interactions, cell wall reinforcement, phytoalexins and antimicrobial protein production are often associated with the death of a small number of cells at the site of infection, known as the hypersensitive response (HR) (Hammond-Kosack and Jones, 1996). Initiation of the HR is thought to signal molecules called elicitors, which could activate pre-existing cellular components, like the release of huge amounts of reactive oxygen species (ROS) (superoxide anion [$O_2^{\bullet -}$], hydrogen peroxide [H_2O_2] and nitric oxide [NO]) in plant tissues by generating a so-called oxidative burst (Wojtaszek, 1997). These highly reactive compounds react quickly with

organic matter provoking a number of diverse radical molecules which strengthen the entire process better known as oxidative stress (Halliwell and Gutteridge, 1991).

In general, programmed cell death (apoptosis) and tissue necrosis around the infections sites are the most important results of oxidative stress (Lamb and Dixon, 1997). However, the plant cells are able to suffer or partially overcome the oxidative damage, because they exhibit both enzyme- and non-enzyme-based scavenger systems. The cellular antioxidant substances are represented by a large number of protoplasmic water and fat-soluble compounds (see also Section 17.2.6 'Plant phenolic compounds') and are involved in lowering the oxidation state of other potentially pro-oxidant molecules. The functional meaning of antioxidant substances is strictly linked to cellular enzyme systems such as dehydroascorbate reductase or glutathione reductase, which regenerate ascorbic acid and glutathione, respectively (Halliwell-Asada pathway) (Halliwell and Gutteridge, 1991). Other important antioxidant enzymes in the cell metabolism are SOD and catalase (CAT), catalysing the convertion of H_2O_2 to H_2O and $O_2^{\bullet -}$ to H_2O_2, respectively. These enzymes, largely widespread in the cytosol and cellular organules of many aerobial organisms, efficiently detoxify ROS and the respective by-products, reducing oxidative stress (Jamieson, 1998).

Although much experimental evidence about the key role of oxidative burst during HR has been produced, only some studies have been carried out on the utilization of natural exogenous antioxidants to control plant pathogenic fungi. Prusky (1988) demonstrated that the application of flavonoid epicatechin delayed the onset of anthracnose and stem end rot by *C. gloeosporioides* in avocado fruit after harvest. Subsequently, Elad (1992) tested 17 free radical scavengers at different concentrations and in plants, leaves and fruits of various hosts (tomato, pepper, groundsel, bean, eggplant, grapes, rose) to control the rots produced by *B. cinerea*, *S. sclerotiorum*, *R. stolonifer* and *Aspergillus* spp.

Butylated hydroxytoluene (BHT), tannic acid, ascorbic acid and dimethyl sulphoxide at concentrations of 1 mM reduced to 50% grey mould rot of tomato fruits, while when amended with thiourea, polygalacturonase and ascorbic acid, the incidence of *R. stolonifer* in bunches of grapes was controlled in the range of 72%–77%. Combined application of antioxidants was found to be more effective than either compound alone on pepper or tomato. Ascorbic acid (25 mM) combined with benzoic acid (1 mM) or tert-butylhydroquinone (1 mM) buffered with citric acid (5 mM) completely prevented grey mould rot on tomato for 25 days. Interestingly, application of 1 mM BHT challenged the colonization of *B. cinerea* on groundsel and lettuce leaves pre-treated with ethephon or H_2O_2 by reducing necrotic symptoms to 50%.

Following these findings, Prusky *et al*. (1995) showed that a dip or spray of avocado fruits with antioxidant butylated hydroxyanisole (BHA), alone

or combined with the fungicide prochloraz, consistently reduced the incidence of anthracnose caused by *C. gloeosporioides* in semi-commercial experiments. When BHA (5 mM) was combined with imazalil (0.45 mM), better control (almost 80% inhibition) of simulated crown rot was achieved than when imazalil (1.78 mM) or thiabendazole (2.46 mM) was applied singly (58% and 54% inhibition, respectively). The results suggest that antioxidants like BHA, which is a food grade chemical, might have the potential to enhance the activity of fungicides currently used to control fungal post-harvest disease, allowing lower concentrations of fungicide to be used (Khan *et al*., 2001).

The relationship between antioxidants and biocontrol agents has been investigated by Castoria *et al*. (2003). These authors analysed a model system consisting of two yeasts with high (*Cryptococcus laurentii* LS_{28}) and low (*Rhodotorula glutinis* LS_{11}) antagonistic activity against the post-harvest pathogens *B. cinerea* and *P. expansum*. The combined application of biocontrol yeasts with CAT and SOD solutions in apple wounds prevented cell dropping in LS_{11} and significantly enhanced colonization and antagonistic activity of the two biocontrol yeasts against both pathogens. These results demonstrated that the resistance to oxidative stress by antagonist yeasts could represent a pivotal mechanism of action involved in wound competence of biocontrol yeasts. Indeed, the molecular characterization of biocontrol agent (BCA) genes switching on during the resistance to oxidative stress could represent the next challenge to improve BCA efficacy.

17.5 Compounds of microbial and animal origin

Examples of decay control achieved using compounds of microbial or animal origin are very few in comparison with those obtained with compounds of plant origin. Antibiotics secreted by antagonistic bacteria are also among the natural compounds which may suppress post-harvest pathogen development. Iturin, an antibiotic produced by several strains of *Bacillus subtilis*, has been effective in controlling brown rot of peaches (Gueldner *et al*., 1988). Similarly, pyrrolnitrin, purified from a strain of *Pseudomonas cepacia*, has provided effective control of grey mould on raspberry (Goulart *et al*., 1992), blue mould and grey mould on apples and pears (Janisiewicz *et al*., 1991) and has delayed rot on strawberries (Takeda *et al*., 1990). However, the potential for development of antibiotic resistance precludes a more widespread use of these compounds. 6-Pentyl-2-pyrone, a secondary metabolite of *Trichoderma* fungi, applied topically at 0.4–0.8 mg/fruit controlled *B. cinerea* rot on kiwi fruit (Poole and Whitmore, 1997). This compound occurs naturally in ripe peaches and nectarines (Horvat *et al*., 1990) and is an approved food flavouring additive (Oser *et al*., 1984). Fusapyrone and deoxyfusapyrone, two α-pyrones originally isolated from

cultures of *Fusarium semitectum*, but also produced by *Alternaria*, *Aspergillus*, *Penicillium* and *Trichoderma* spp., inhibited the growth of post-harvest pathogens as well as mycotoxigenic and human filamentous fungi *in vitro* (Altomare *et al*., 2000). Another compound that may have potential as an antimicrobial for fresh fruit and vegetables could be Ascopyrone P (APP) produced by the fungi *Anthracobia melanoma*, *Plicaria anthracina*, *Plicaria leiocarpa* and *Peziza petersi*. APP was shown to inhibit bacteria but not yeasts (Thomas *et al*., 2004), therefore its use can be considered in combination with antagonistic yeasts in the biological control of post-harvest diseases. Many other antifungal compounds from fungi reviewed by Ng (2004) can be of interest and importance in combating post-harvest diseases.

Xanthan gum (XG) is a high-molecular-weight polysaccharide produced industrially by fermentation of *Xanthomonas campestris* (Kennedy and Bradshaw, 1984); it is a GRAS compound and is commonly used as a stabilizer and thickener, suspending agent, bodying agent or foam enhancer in foods, and has been suggested as an adjuvant in commercial wax-based formulation for easy peeler citrus fruit (Cohen and Nussinovitch, 2000). XG reduced the sour rot of grapes when applied prior to harvest at a concentration of 0.05% (w/v) (Ippolito *et al*., 1998). Against sour rot, a disease even more severe than grey mould in southern Italy, XG was more effective than the antagonist *A. pullulans* (10^7 cells/ml), $CaCl_2$ (1% w/v) and the fungicide procymidone at $10\,g\,l^{-1}$. Since no synthetic fungicide is currently available to combat sour rot, XG could be an interesting compound to be validated in large-scale trials. The mode of action of XG has not been elucidated but it is conceivable that the nature of the polysaccharide and its coating properties are involved.

Among the animal-derived compounds, chitosan (poly-*N*-acetylglucosamine), a biodegradable polymer made commercially by alkaline deacetylation of chitin, has gained particular interest for controlling post-harvest diseases. Chitin is an abundant constituent of crustacean shell (e.g. shrimp and crabshell) and fungi (e.g., *A. niger*, *Mucor rouxii*, *Penicillium notatum*). Chitosan has been shown to be useful in many different areas: as a flocculating agent in wastewater treatment, an additive in the food industry, a hydrating agent in cosmetics, a pharmaceutical agent in biomedicine and, more recently, in agriculture as an edible coating and natural antimicrobial compound capable of inducing plant defence response (Muzzarelli and Muzzarelli, 2001; Rabea *et al*., 2003). Because of its film-forming ability, chitosan delayed ripening by acting as a barrier to gas diffusion. Tomatoes, bell peppers, cucumbers, strawberries, lettuce and peach coated with chitosan had reduced weight loss and respiration rates, improved appearance and extended shelf-life (El Ghaouth *et al*., 1992a; Li and Yu, 2001; Devlieghere *et al*., 2004). The polymer was effective in reducing decay in other fruits including table grapes, mangoes, sweet cherries, papaya, oranges and in carrots (El Ghaouth, 1994; Bégin *et al*., 2001; Romanazzi *et al*., 2001; Bautista-Baños *et al*., 2003b; Molly *et al*., 2004). The range of chitosan

application generally varied from 0.1%–2%; the higher the concentration, the greater the control of various diseases.

In the above examples, chitosan has been applied as a post-harvest treatment; however, the few examples of its application in pre-harvest treatment of strawberries, sweet cherries and table grapes to control post-harvest decay have also been very promising (Romanazzi *et al*., 1999b, 2000a,b, 2001, 2002; Reddy *et al*., 2000). Pre-harvest spraying of chitosan to reduce storage decay of table grapes seems to be the best way to apply this compound since exposure to post-harvest liquid-based treatments is not advisable, as it could damage the bloom (Ippolito and Nigro, 2000). The only effect on grape berry appearance was a slight shining when using the highest concentration of chitosan (1%), whereas no effects were visible at lower doses (0.5%–0.1%), which were as effective as the higher one (Romanazzi *et al*., 2002). Pre-harvest application of chitosan is also advisable against *Botrytis* rot on strawberries. Field application of the polymer during flowering can avoid infection via senescent floral parts that later develop into active rot on ripe fruits (Romanazzi *et al*., 2000a). On strawberries, pre-harvest application of glycolchitosan, a water-soluble form of chitosan, gave similar results to chitosan (Romanazzi *et al*., 1999a). Enzymatically hydrolyzed chitosan showed a greater effect than the high-molecular-weight chitosan on carrots (Molly *et al.,* 2004). In *in vitro* tests, chitosan-Zn complexes showed up to 8 and 16 times higher antimicrobial activity than those of chitosan and zinc sulphate, respectively (Wang *et al*., 2004), but up to now no reports are available for control of post-harvest rots of fruits and vegetables.

Compounds based on chitosan have never been reported to induce symptoms of phytotoxicity on treated fruits and vegetables. The mode of antifungal action of chitosan and its derivatives is still not well understood, but different mechanisms have been proposed. In *B. cinerea, R. stolonifer* and *F. oxysporum*, chitosan caused cellular leakage and morphological alterations consisting of excessive branching and cell wall damage (Benhamou, 1992; El Ghaouth *et al*., 1992b). In tissues of chitosan-treated bell peppers, *B. cinerea* hyphae displayed various levels of cellular disorganization, ranging from wall loosening to cytoplasm disintegration (El Ghaouth *et al*., 1997). Leakage of proteinaceous and other intracellular constituents has been ascribed to interaction between positively charged chitosan molecules and negatively charged microbial cell membranes (Rabea *et al*., 2003), presumably mediated by chitosan action on chitin deacetylase (El Ghaouth *et al*., 1992c).

The eliciting property of chitosan has been demonstrated in several post-harvest commodities. Induction of antifungal hydrolases such as beta-1,3-glucanase, chitinase and chitosanase have been observed in strawberries, tomatoes and bell peppers (Wilson *et al*., 1994); in tomato and bell pepper, the activity of these enzymes remained high up to 14 days after treatment. On table grapes, chitosan enhanced the activity of PAL (Romanazzi *et al*.,

2002). Moreover, it elicited phytoalexin formation in carrot roots, thus restricting *B. cinerea* infection (Reddy *et al*., 1999). The induction of lytic enzymes, PAL and phytoalexins in harvested tissues by pre-storage treatment with chitosan could supply the tissue with weapons capable of restricting fungal colonization; this could be important in retarding the resumption of quiescent and latent infections which typically become active when tissue resistance declines. Chitosan treatment also stimulates various structural defence barriers in host tissues such as thickening of the cell wall, formation of papillae and deposition of electron opaque materials in the intercellular spaces, presumably being antifungal phenolic-like compounds (Wilson *et al*., 1994). As the endogenous microflora on fruit surfaces may play an important role in antagonism to pathogens (Wilson and Wisniewski, 1994), treatments of fruits and vegetables should avoid any negative effect on the naturally occurring microflora (Nigro *et al*., 1998, 2000). Chitosan applied as pre- and post-harvest treatment on table grapes did not impair the naturally occurring yeasts and yeast-like fungi, among which antagonistic microorganisms are common (Ippolito *et al*., 1997). On the contrary, chitosan treatment reduced the propagules of filamentous fungi naturally occurring on berries (Romanazzi *et al*., 2002). As naturally occurring filamentous fungi on table grapes include decay-causing species such as *B. cinerea*, *Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp., it has been hypothesized that their reduction can cooperate in lowering rot incidence during storage (Ippolito *et al*., 1998).

17.6 Additive and synergistic combinations

The commercial control of post-harvest diseases of fruits and vegetables must be extremely efficient, in the range of 95%–98%, unlike the control of tree, field crop or soilborne diseases (Droby, 2001). None of the natural antimicrobial systems investigated to date offers post-harvest disease control comparable to that obtained with synthetic fungicides. Attempts to surmount the variable performance and enhance the efficacy of natural compounds have led to the development of combined approaches based on additive and synergistic effects. While many synergistic combinations of antimicrobials have been identified *in vitro*, relatively few investigations of multifactorial systems have focused on organisms or conditions relevant to post-harvest storage of fruits and vegetables.

Synergistic effects have been reported for a mixture of *Allium sativum* (0.25%) and *A. cepa* (0.75%) bulb extracts in inhibiting the *in vitro* growth of *Alternaria* spp. (Bokhary, 1985). Similarly, mixtures of *Allium* plant extracts and acetic acid were more active against *Aspergillus flavus*, *A. niger* and *A. fumigatus* than single treatments, especially when treatment was further combined with high temperature (Yin and Tsao, 1999). On citrus a

remarkable increase in the activity of garlic extracts was observed when extracts were mixed with vegetable (sunflower) cooking oil (Obagwu and Korsten, 2003). An interesting combination of different approaches has been the use of sub-atmospheric pressure with EOs. The fungitoxic activity of *Thymus capitatus* EOs (75, 150 and 250 ppm) on oranges inoculated with *P. digitatum* and placed in 10-litre desiccators was weak at atmospheric pressure (3%–10% inhibition), whereas, under vacuum (0.5 bar), conidia mortality on the exocarp reached 90–97% (Arras and Usai, 2001). Under sub-atmospheric conditions (0.2–0.8 atm) two phytoalexins (scoparone and scopoletin) were elicited on orange and mandarin fruits; the biosynthesis of these compounds was also stimulated in fruits treated with thyme oil vapours at a concentration of 50–100 ppm. The simultaneous use of thyme oil and hypobaric pressure on citrus fruit had a synergistic effect eliciting five times as much scoparone (Arras, 1999). This was attributed to increased contact between the EO, the pathogen's conidia and the host tissue.

A synergistic effect was observed in sweet cherries under sub-atmospheric pressure in combination with chitosan. The extent of decay inhibition was, on average, 20% with sub-atmospheric pressure alone, 65% with chitosan treatment alone and 83–89% when both treatments were applied (Romanazzi *et al*., 2003). The machinery for rapid vacuum cooling of fruit and vegetables is already in use in some packinghouses. Therefore, it might be feasible to add some of the natural antifungal compounds reported above to improve the effectiveness of the treatment. Another synergistic effect was obtained against grey mould on 'Thompson Seedless' grapes treated by combining ethanol (20%) and 0.5% or 1.0% potassium sorbate, with an equal efficacy to commercial SO_2 generator pads (Karabulut *et al*., 2005). Limpel's formula, as described by Richer (1987), was used to determine synergistic interactions between chitosan and sub-atmospheric pressure and ethanol and potassium sorbate.

Another promising combination has been reported by Palhano *et al.* (2004) in which the activity of lemongrass EO against *C. gloeosporioides* spores significantly increased when applied in combination with a high hydrostatic pressure (150 MPa); as hypothesized by these authors the enhanced effect could be explained by a higher uptake of oil constituents into the spore owing to the high pressure, leading to an increase in the number of molecular targets affected. 'Bio-Coat', a biocontrol product under commercial development, is a preparation consisting of a water-soluble form of chitosan (glycolchitosan) and the antagonistic yeast *Candida saitoana*. This product was superior to the yeast and glycolchitosan alone in controlling decay of several varieties of sweet orange, lemons and apples, and the control level was comparable to that achieved with imazalil or thiabendazole (El Ghaouth *et al*., 2000). Interesting results were also obtained with pre-harvest application of the product on table grapes (Schena *et al.*, 2004).

Xanthan gum has also been evaluated in combination with *A. pullulans* to control post-harvest table grape and strawberry rot; on both commodities, the activity of the antagonist was significantly improved when applied in combination with the polysaccharide at 0.5% (w/v) (Ippolito *et al.*, 1997, 1998). The higher activity of *A. pullulans* combined with XG on table grapes and strawberries has been related to its greater survival on the fruit surface, probably because of its coating properties. Another natural gum, locust bean gum, extracted from the seed of the carob tree (*Ceratonia siliqua* L.), applied in combination with antagonistic yeasts improved their activity with results comparable to the antagonists applied alone but with a 100-fold higher concentration (Lima *et al.*, personal communication).

Ethanol applied at concentrations between 8% and 20% was not effective in reducing grey mould incidence and severity on apples and pears. Likewise, several strains of *Saccharomyces cerevisiae* (10^8 cfu ml^{-1}) were not effective. However, a combination of the two reduced disease incidence by over 90% (Mari and Carati, 1997). Similar results were obtained against green mould on lemon, combining a heated solution of ethanol (10%, 45 °C) with curing, a physical treatment consisting of keeping fruits at a relatively high temperature and humidity (e.g., 32 °C, 95%–98% relative humidity), and combining ethanol with the biocontrol yeast *Candida oleophila*. Infections were reduced from 82% (control), 17% (ethanol alone) and 40% (yeast alone) to 3.5% (ethanol-curing) and 3.3% (ethanol-yeast), with no appreciable differences compared with the fungicide imazalil (Lanza *et al.*, 1997). The above examples clearly show the advantages of using combination strategies to control post-harvest diseases of fresh fruit and vegetables. Many other possible combinations could be explored, such as the use of antagonistic microorganisms, low doses of fungicides, physical means (UV radiation, modified atmospheres, etc.), organic and inorganic salts, nutrients, mixtures of natural substances with different modes of action, and so on. The complexity of the mode of action that combined alternatives can display should also make the development of pathogen resistance more difficult.

17.7 Extent of take-up by industry

Chemical fungicides have been used to reduce storage losses for a long time and in many situations, but even with a substantial increase in chemical use, the overall proportion of fruits and vegetable losses and their absolute value appear to have increased over time. Despite this perverse relationship, an increase in fungicide use still appears to be profitable for the chemical industry. Some of these fungicides are persistent enough to be detected after several weeks in fruit, vegetables and soil; moreover, as reported in the introduction, inappropriate and excessive fungicide use has increased the development of multiresistant fungal strains, thus requiring

greater use to protect products. Progressively, over the 1990s environmental and health impact issues, both because of the direct and indirect impact of chemicals, became increasingly important, resulting in pressure for reduced pesticide use and the loss of previously useful chemicals. In spite of widespread public concern about the negative effects of synthetic fungicides, especially in Europe and North America, natural fungicides from plants, animals and microorganisms at present have scarce impact in the marketplace.

The success of large-scale studies on microbial antagonists generated interest by several agrochemical companies and currently some antagonistic microorganisms for controlling post-harvest diseases of fresh fruit and vegetables are commercially available (http://www.oardc.ohio-state.edu/apsbcc/productlist2003USA.htm). Considering natural insecticides, a very active field in developing and applying alternative control methods *Bacillus thuringiensis*- and pyrethrum-based products command 1%–2% of the global insecticide market (Isman, 2000). Although the commercial formulation of natural compounds to fight post-harvest diseases is still in an early phase of development, it seems they have the potential for market expansion. An additional feature favouring commercial development of several natural compounds with antioxidant properties (essential oil, flavonoids and other phenolic compounds) is their potential efficacy in protecting against the toxic effects of mycotoxins (Atroshi *et al*., 2002). For certain substances the take-up by industries should be relatively easy, as for those already tested in other systems, especially human medicine and the food industry. For example, chitosan, owing to its lipid-binding capacity and hypocholesterolaemic action (Maezaki *et al*., 1993), is an edible and safe compound widely used in human medicine for slimming diets; a wide variety of plant extracts, essential oils, mixtures or single plant compounds and so on, are available without prescription through health food stores, herbalists, vitamin retailers, and so on and are commonly used as culinary herbs and spices; XG is a compound commonly used in the food, cosmetic and pharmaceutical industries. It is worth mentioning that in the case of essential oils, because of their long history of global use by the food and fragrance industries and, recently, in the field of aromatherapy, are readily available at low moderate cost; moreover, in some countries some of these compounds are exempt from the usual data requirements for registration. American companies taking advantage of this situation have been able to bring essential oil-based pesticides to market in a far shorter time period than would normally be required for a conventional pesticide. This is the case for Cinnamite™ and Valero™; both of these are a miticide/fungicide for glasshouse and horticultural crops, based on cinnamon oil with cinnamaldehyde as the active ingredient (Isman, 2000).

Possible barriers to the commercial development of natural compounds as antimicrobials for controlling post-harvest diseases of fruit and vegetables are: (i) expensive procedures for extracting the active compounds; (ii) the need for chemical standardization, stability and quality control; (iii)

extended studies on toxicological aspects for specific compounds; (iv) production at competitive costs to existing pesticides; (v) the lack of studies on the development of resistance; (vi) difficulties in registration as pesticides; (vii) efficacy sometimes not consistent and acceptable unless in complex integrated approaches; (viii) restricted market confined to the post-harvest environment; and (ix) scarce interest by companies in testing for botanicals since it is still unclear whether proprietary claims can be made. The above reported issues should not deter the search for effective antimicrobials of animal, plant and microbial origin. Current 'economic' and 'biological' assessment upon withdrawal of most of the conventional pesticides registered for post-harvest disease control may change radically, providing new perspectives for the development and commercialization of future pesticides based on natural substances. In this picture, the possibility of using marker-assisted selection or genetic engineering to select or introduce resistant genes from other plant species cannot be discarded.

17.8 Concluding remarks

What is needed in applying natural antimicrobials is a change in the philosophy of companies and growers, who are still rooted to the concept that a 'stand-alone' treatment has to control a disease completely. In addition, consumers also have to consider the benefits deriving from the intake of sound products without residues and with improved quality.

A more sustainable approach against post-harvest diseases needs to be based on the use of multifaceted control strategies, including the use of natural compounds, microbial antagonists, physical methods, induced and genetic resistance, low doses of fungicide, and so on. Some evidence has been reported in this chapter demonstrating the effectiveness of the integrated approach to reach a level of efficacy comparable to that provided by synthetic fungicides.

It has also been demonstrated that some compounds such as isothiocyanates hold an interesting curative effect against post-harvest diseases. The ability to control previously established infections in the post-harvest environment is of crucial importance, considering that under commercial conditions the application of a post-harvest treatment may be delayed for hours or even days after harvest, leading the pathogen to penetrate the flesh where control becomes very difficult. Therefore, compounds with a mode of action able to control incipient, latent and quiescent infections should be preferred. As an alternative, for compounds with no curative activity, application before events that open infection pathways (e.g., during harvest and post-harvest handling operations) are suggested (Ippolito and Nigro, 2000). Another interesting feature for some of these compounds, is the possibility of acting as vapour-phase substances, for example essential oils, vinegar, allylisothiocyanates, and so on. Apart from the high efficacy, their use

against post-harvest diseases seems promising considering their capability to penetrate easily inside the mass of staked commodities in the cold room without any further manipulation. This characteristic, exploitable only as a post-harvest milieu in a confined environment, seems the most appropriate treatment for those products where post-harvest handling reduces their market appeal.

A concept to debunk is that the effectiveness of newly emerging alternative biocontrol methods are still evaluated in comparison with that of synthetic pesticides. Natural antimicrobials, like other biocontrol alternatives, may require application at different times, depending on the intrinsic characteristics and the mode of action of the compound. Currently, considering that the majority of natural antimicrobials have no toxic effects on mammals and low phytotoxicity, it should be possible to apply such compounds in a wider range of concentrations and application schedules.

Although scientists have always demonstrated an interest in searching for alternative methods to control post-harvest diseases, more effort has been devoted to this field since the late 1980s. Among natural compounds, many have been tested and some are very promising; however, there is an inestimable number of other substances yet to be discovered in nature's store. Many substances that are effective in apparently unrelated systems could be a source of new compounds to be tested against post-harvest diseases. In this regard, it is worth mentioning that a rational way of obtaining results at a lowest cost/benefit ratio should be a collaborative effort between plant pathologists, physicians, chemists and companies to develop safe commercial products; their public acceptance could be anticipated since, as stated before, most of them are well known and already used for many other purposes. The possible beneficial effect on human health, for example phenolics are recognized as being antioxidant and anticarcinogenic, should further increase their approval and speed up demand.

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18

Consumer risk in storage and shipping of raw fruit and vegetables

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18.1 Introduction

A recent World Health Organization (WHO) report noted that countries with reporting systems have documented 'significant increases' in the incidence of foodborne illnesses over the past two decades. Each year, according to WHO, unsafe food causes approximately 76 million incidences of illness, 350 000 hospitalisations and 5000 deaths in the USA and 2 366 000 illness cases, 21 138 hospitalisations and 718 deaths in England and Wales (Anonymous, 2004). Thus, food risk will be 'a defining issue of a 21st century marked by globalisation and foodborne illnesses' experts said at the Annual Meeting of the American Association for the Advancement of Science.

Motarjemi and Kaferstein (1999) emphasised, notwithstanding that the HACCP process has become mandatory in most countries, foodborne diseases were increasing instead of decreasing. Several reasons were reported in the article:

- the food supply system (mass production and distribution, long food chain, more complex processed food, catering)
- the health and demographic situation (increase of vulnerable people, strong migration, rapid urbanisation)
- the social situation (increase of out of home consumption, increase of ready to eat foods, poverty, lack of education)
- the health system (inability of health organisations to control the huge amounts of food, poverty, lack of education)
- environmental conditions (pollution, climatic changes).

Some other considerations must be added, such as a lack of ethical behaviour among the producers (growers, processors, distributors), increasing interest in care of the home and of the body (manifest in the increasing use of skin cream, shampoo, shower soaps, ethanol-based perfumes – which all reduce or eliminate the skin defences – and detergents for cleaning) in developed countries, consumer habits of food consumption that are dependent on a large distribution chain and on fast food companies who use aggressive marketing programmes, a great variety of retail goods/foods and huge food and non-food storage facilities. Finally, the certification system has become distorted, mainly owing to the fact that the company that needs certification draws up a legal contract with the certification body and pays that body to certify its accounts (e.g. the Parmalat scandal) (Cusani and Tripodi, 2004).

Among the huge number of food items marketed, whole fresh fruits and vegetables are considered less risky from the viewpoint of microbiological disease but they are still risky from the point of view of pesticide residues. At the beginning of 2004, monitoring Alert Notifications (which are sent when a food or feed presenting the risk is on the market and when immediate action is required) and Information Notifications (concerning a food or feed for which a risk has been identified, but for which the other members of the network do not have to take immediate action, because the product has not reached their market) reports for food commodities that are imported in EU Countries, for a period of three weeks, revealed (Table 18.1) that the percentage referring to fresh fruits and vegetables was low compared to other foodstuffs. The contamination of fruits and vegetables

Table 18.1 Foodstuffs alerted or notified during three weeks (three weeks in February) divided by category and as a percentage of the total (91 items) (Mencarelli, 2004)

Commodity group	Percentage	Reason for notification and related product	Provenance (country)
Fresh meat	9		
Processed food	7		
Dairy products	2		
Fish	27		
Dry fruits	3	Sulphites in dry apricots	Turkey, China
	20	Aflatoxin in pistachios	Iran
Fresh horticultural commodities	7	Ochratoxin in currants	Uzbekistan
		Moulds in peppers	Egypt
		Methomil and thiocarb in grapes,	Greece
		Dithiocarbammates and procymidone in lettuce,	France
		GMO papaya	USA

can be due to different pathogens, including bacteria such as *Salmonella* (sprouts, melons), *Shigella* (parsley), *Escherichia coli* (lettuce, sprouts, carrots), *Vibrio cholerae*, *Listeria monocytogenes* or parasites such as *Giardia lamblia*, *Cryptosporidium parvum* (cider, onions), *Entamoeba hystolitica*, *Ascaris lumbricoides* and *Cyclospora cayetanensis* (raspberry), the source of which can be human or animals (Silva *et al*., 2004). The problem of contamination is more important in developing countries where contaminated water may be used for irrigation or manure used as fertiliser (Beuchat, 1995; WHO, 1998). However industrialised countries have experienced an increase in the number of produce-associated foodborne illnesses. In the USA in the decade 1988–1998, 35% of outbreaks of produce-associated foodborne illnesses in fresh horticulture products were detected in a salad bar, about 21% and 17% in fruits and lettuce, respectively, mainly caused by *Salmonella* and *Escherichia coli*. More recently, a sampling programme carried out in the USA of 1000–2000 units of each commodity (melon, strawberry, raspberry celery, lettuce) revealed contamination of 0.6% and 0.14% caused by *E. coli* and *Salmonella*, respectively (Silva *et al*., 2004). In Japan in 1996 an outbreak of *E. coli* O157 on radish sprouts provoked the death of 12 children and affected 5700 school children (Molins *et al*., 2001).

A classification of fruits and vegetables based on identified risks is reported in Table 18.2. Thus, today, attention to food safety is much greater than in the past and even for fresh fruits and vegetables, which are stored and shipped all over the world, precautions must be taken and, borrowing the terms and codes of the Quality Certification ISO 9000-2000, 'preventive action (3.6.4), corrective action (3.6.5) and correction (3.6.6) must be taken into account'. In April 2004, the European Union adopted the 'hygiene package' composed of five legislative texts. EC Regulation N. 825/2004, referred to as 'hygiene', is one of the texts of this package, focusing mainly on the responsibility of the operator for food safety, on maintaining the cold chain and on improving the HACCP (Hazard Analysis and Critical Control Points) and the good practice guide.

In this chapter the main storage and shipping technologies will be discussed, taking into account the safety aspects of the product for the consumer.

Table 18.2 Classification (unofficial) of fruits and vegetables based on identified risks (hazard, exposure) (Silva *et al*., 2004)

High risk	Melons, leafy vegetables, berries
Medium risk	Tomato, garlic, papaya, mango
Low risk	Nuts, grapes, apples, blueberries

18.2 Precooling technology

The three basic rules of refrigeration are wholesome food, early refrigeration and permanent refrigeration. The third rule is commonly known as the 'cold chain', which is defined as the means successively employed to ensure the refrigerated preservation of perishable foodstuffs from the production to the consumption stage. If a break in the cold chain occurs, the consequences may provoke quality loss of plant products but above all favour the development of spoilage flora, or even worse, pathogenic flora or toxins causing foodborne diseases (Billiard, 2003). Instantaneous removal of heat from the commodity as it leaves the field (sensible or field heat) in order to reduce the temperature down to storage or shipping temperature is not possible in a regular cold storage room, although the quality and safety of the product are strongly dependent on the state of health of the commodity. Turgor loss, which is the main worry for the distribution of several fresh fruits and vegetables, is related to the psychrometric characteristics of the air surrounding the product. Decrease of cell turgor, which means to decrease the water potential by a few bars, makes the cells more susceptible to microorganism infection and to gas permeability changes (Kays, 1997) which deteriorate the fruit during storage and distribution, compromising the quality and even allowing toxin contamination. Once a fruit is picked in a hot or warm climate (i.e. 30 °C and 50% relative humidity RH) with a water vapour pressure of 21 mbar, its internal water vapour pressure (WVP), considering a fruit temperature of 30 °C and high turgor pressure of the cells, will be equal around 42 mbar. The difference between the WVP of the fruit and the external environment will be 21 mbar. If the fruit is stored immediately in a cold room at 0 °C and 85% RH (water vapour pressure = 5 mbar), the difference between the WVP of the fruit cell and the WVP of the cold room will be 37 mbar, which is higher than the previous difference. This means that the fruit will rapidly lose water as gas when it is placed in the cold room, until it reachs the cold room storage conditions.

The reason for adopting a precooling technique is just to avoid this water loss and to shut down the temperature rapidly to match the storage or shipping thermohygrometric conditions immediately. Thus, precooling techniques are technologies used to reduce rapidly the temperature of horticultural commodities, removing field heat and decreasing respiration heat. Five techniques are mainly used: room cooling, forced air cooling, hydrocooling, vacuum cooling and ice cooling. Another technique, especially valid when primary sources like capital and electric power are not available, mainly in poor areas, is evaporative cooling. These techniques will be analysed from the point of view of the safety of the product.

18.2.1 Room cooling

The room cooling technique consists of the use of a simple cold room with a typical refrigeration plant, compressor and condenser outside the storage

room, and expansion valve and evaporator (coil) inside the cold room. In comparison with the cold storage room, the cold air flow across the packed containers and the refrigeration capacity have to be increased in order to guarantee adequate air circulation.

In the precooling room, the distribution of pallet and container determines the air flow. Pallets and containers should be aligned in order to avoid obstruction of the air flow between and across the container. Adequate precooling rates are obtained with a cold air speed close to $0.5\,m\,s^{-1}$ and refrigeration power inside of the cold storage room of more than $0.28\,kW\,t^{-1}$ assuming a product average density of $0.20\,t\,m^{-3}$ (Mencarelli *et al.*, 2004). It is sometimes necessary to improve the air movement with a mobile fan or/and to optimise the amount of product to be cooled to match the installed cooling power (generally this amount ranges between 10 and 20% of the total storage capacity of a cold storage room).

Once stacked, the pallets may be cooled and subsequently stored in the same place without further movement. The design and subsequent use of the plant is very simple but the cooling time is very long (20–100 h) and the energy efficiency is low (Thompson *et al.*, 2002a).

A long precooling time imposes a long delay in production and rapid shipping is not possible. This means that there is an increase in the potential deterioration of the product owing to the development of fungi such as *Penicillium expansum*, which can release mycotoxins such as patulin, which has recently been considered by US and EU legislation (EU Regulations 472/2002 and 1425/2003) especially in relation to contamination of apple-based food products destined for children, or of *Aspergillus carbonarius*, which produces ochratoxins in grapes.

Moreover, the high ventilation in the room can favour the diffusion of spores and contaminate all the precooled product and, if an adequate cleaning procedure is not performed, even later loading of cold storage (Jett, 2004). Finally, spores of fungi or bacteria such as *Listeria*, which are resistant at low temperature, can nest in the evaporative coil, especially when the temperature used is not below 0 °C. Thus, an adequate cleaning and hygiene programme is strongly required. In quality certification, such as ISO 9000-2000, or in Retail Consortium certification, such as BRC or IFT, which include HACCP, this step can be identified as a critical control point (CCP) to be maintained under control.

18.2.2 Forced air cooling

The problem of contamination, owing to the flow rate mentioned above for room cooling, affects even a forced air cooling system if the product, which is going to be cooled, has already decayed. But if the product does not show any symptoms, the cooling speed of this technique does not permit decay to develop. The use of a precooling tunnel reduces the cooling time (1–10 h) depending on the system used and the type of product, since the cooling

time is related to volumetric air flow and product diameter (Thompson, 2003). The cooling time greatly affects the quality of product as well as the appearance of disorder (Jooste and Khumalo, 2004). Using an air speed over 1 m s^{-1} it is possible to cool much more product each day than by room cooling.

In tunnel cooling, heat is carried away primarily by air flow through the product inside the containers rather than by flow circulating outside the containers, as in the room cooling. Using high air speeds and adequately vented containers, more than one row of pallets can be cooled at the same time. The plant and energy costs are greater than for room cooling, but considering that cooling plants are specialised, the space required for a specific volume of product and the refrigeration losses are much lower than for room cooling. Adequate package design is required in relation to the fixed air speed and cooling time (Mencarelli *et al.*, 2004). Different systems are proposed but all of them are performed inside the cold room. The tunnel-type is the most common, based on two rows of packages, bins or palletised product placed on both sides of an air-return channel in order to create a plenum. A tarpaulin is placed over the product and channel and a fan draws air away from the channel, creating a slight depression in the channel. The pressure gradient between the channel and the air in the cold room forces the cold air to pass through the product to reach the channel, where it is drawn away by an exhaustive fan. Another system works by pressing the cold air (tunnel precooler) through the packages, such as for table grapes: the fan produces a high pressure level on one side of the tunnel (about 20 mm H_2O) so that the air is forced through the products. The air speed around the product is generally maintained at around 2.5–3.5 m s^{-1}. In the cold wall system the plenum is created by a double wall with openings to accommodate the palletised product. The fan directs the air up from the plenum, thus the cold air of the cold room is forced through the product, entering the plenum through the openings. A variation of this system is serpentine cooling, especially for bins, where up to four bins are placed one over the other and the slots in the bins that accommodate the forks of the forklift truck are alternately sealed with a wooden board in order to create a sinuous flow (serpentine) through the product inside the bin.

Forced air cooling is widely used even though some products need to be packed (e.g. flowers) to avoid high water loss.

18.2.3 Hydrocooling

Hydrocooling technology has the greatest energy efficiency owing to the high heat transmission coefficient of the water in contact with the product (Thompson, 2003). The product can be immersed into or sprayed with cold water; in the latter case the product has already been packed. The time of cooling ranges from 0.1–1 h. Today both systems are automated by using moving conveyors. The water is cooled by a refrigeration plant. Apart

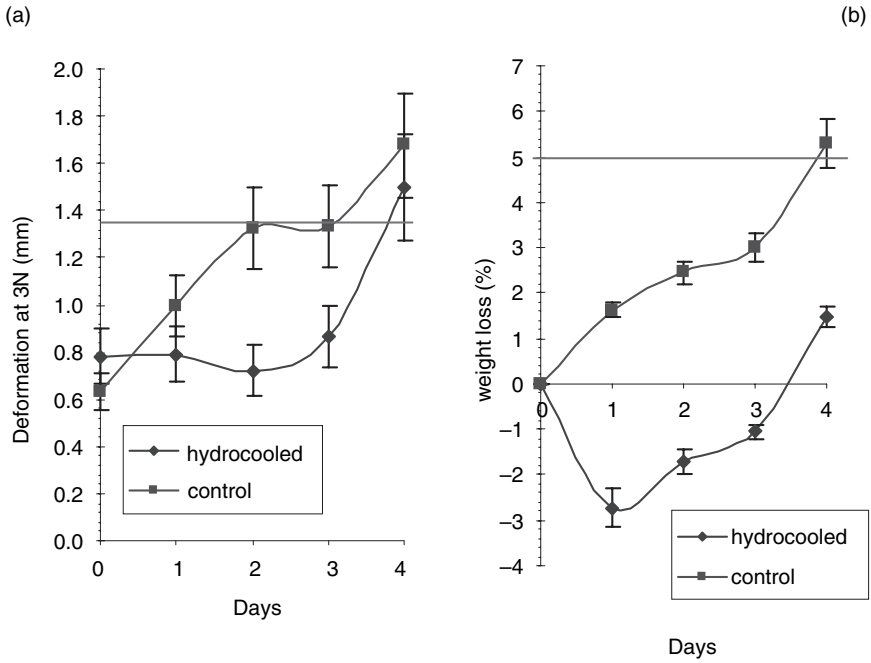

Fig. 18.1 Influence of hydrocooling on quality aspects of artichokes. The horizontal lines indicate the tolerance limits beyond which the product is no longer saleable. Hydrocooling was performed after immersion for 1 h in water at 1 °C. Artichokes were maintained at 0 °C covered with plastic film for 2 days and then at 20 °C for 1 day. (a) Firmness or compactness, (b) weight loss (Vizovitis *et al*., 2003).

from the rapid cooling time, the advantage of this technique is that the product does not lose weight and in some cases, such as artichokes, gains weight (Fig. 18.1). In terms of quality and freshness, this is a good result since we increase the turgor and thus the compactness of the artichoke (Fig. 18.1) but in terms of contamination risk, it is very detrimental. If the cooled water is contaminated with microrganisms, the potential for product contamination is very high. For this reason a lot of attention has been paid to water sanitation. Moreover, even if the fruits or vegetables have a waxy surface, when they are immersed or come in contact with cold water, the volume tends to contract, owing to pressure reduction and their natural apertures, stomata and lenticels are exposed (Vigneault, 1998). This effect gives a further risk of contamination.

In the process of quality certification such as ISO 9000-2000, the hydrocooling step must be marked as CCP and actions to prevent contamination must be taken. These preventive actions can refer to use of water disinfectant such as chlorine and other chemicals, or physical treatments used to sanitise the water, which are discussed in other chapters. We have to remem-

ber that most of these treatments used at consumer-safe doses do not sterilise the water. Chlorine sanitises the wash water and maintains a low microbiological count in the water so that it does not become a reservoir for mould spores and bacteria which may infest produce (Gorny and Zagory, 2003); moreover microrganisms adhere strongly to the surface of the product, especially in natural and accidental apertures like scratches (Zhuang *et al*., 1995). Safety problems related to the use of chlorine are its corrosive action on common metals which can release ions that may be absorbed by the product, and the formation of trihalomethane (THM) from the reaction of chlorine with organic matter, a carcinogenic compound. Recent regulations for drinking water have introduced restrictive limits for oxidation/disinfection by-products (DBPs) particularly total trihalomethane concentrations (TTHMs), chlorite and bromate. The Italian standards are respectively for TTHMs, chlorite and bromate 30, 200 and $10\,\mu g\,l^{-1}$ (Collivignarelli and Sorlini, 2004). These compounds are not just produced with chlorine but also with chlorine dioxide and ozone treatments; thus continuous surveillance of the right dose and analyses of these by-products must be maintained.

Another preventive action is harvest and handling care of the product. In the case of hydrocooling, the presence of mechanical injuries following poor care at harvest and handling of the product enhances the potential for contamination much more than for other precooling methods. Even injuries like impact bruising in apricot fruit, which does not appear at the time of impact, provokes great internal and superficial disorganisation of cells making them more susceptible to microorganism contamination (Fig. 18.2).

Finally the use of a water disinfectant must be seen as a final solution because the presence of chlorine or other chemicals residues, even food grade ones, in fruits and vegetables is not very acceptable to the consumer;

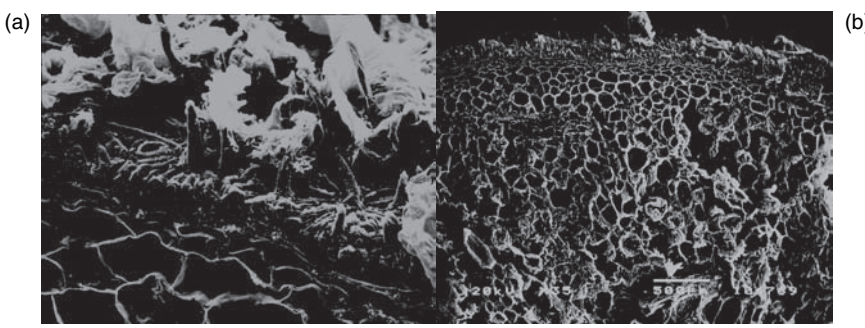

Fig. 18.2 SEM images of surface (a) and internal tissue of apricot after impact injury (b). It is possible to observe the cells squeezing (a) and the cells disorganisation (b). The white deposit on the apricot surface in (a) is flour powder used to mark the impact area (Mencarelli, 2004, personal images).

thus the absolute purity and safety of chemical additives must be continuously controlled together with the concentration and treatment time.

18.2.4 Vacuum cooling

Vacuum cooling is considered very efficient in terms of energy used but the equipment is very expensive and purchase can only be justified for long-term use all around the year. The principle of this technique is to allow water to evaporate at low temperature by reducing the pressure. Product is placed in a steel vessel and vacuum pumps reduce pressure in the vessel, from atmospheric pressure at 760 mm Hg to 4.6 mm Hg. Water boils at a pressure of 20–30 mm Hg depending on its temperature, causing rapid water evaporation and product cooling. Usually during the vacuum cycle, the pressure reaches 4.5–4.6 mm Hg and water boils at 0 °C. It is a very rapid, uniform way of cooling especially for product with a high surface/mass ratio, like leafy vegetables or small diameter fruits. The cooling time for these products can be between 20 and 30 min even in perforated plastic film wrapped product (Cheyney *et al*., 1979). Weight loss is the main disadvantage of this technique and, for this reason, the product is sprayed several times with water before the vacuum treatment (hydrovac cooling). For product with a low surface/mass ratio, before the pressure reaches the value of 4.5 mm Hg, the vacuum pump and the condenser are turned off to permit the tissue to recover its structure, avoiding excessive compactness, and then are switched on again to complete the cycle (bouncing technique) (Anelli and Mencarelli, 1990).

In terms of food safety, the very low pressure negatively affects fungi and bacteria and, in addition, water evaporation completely removes the boundary layer (water film tightly bound to the fruit surface) around the product hindering the survival of microorganisms. Indeed a spore of *Botrytis cinerea* needs a 10 μm boundary layer and a bacterium only needs 1–2 μm (Jarvis, 2003). When water is used in a hydrovac system, special attention must be taken to use sanitised water.

18.2.5 Package icing

Package icing is the most-used precooling technique although it is mainly used for fresh fish, not for fruit and vegetables. Two systems are used for fruits and vegetables which are tolerant to ice contact: body icing and top icing. The former is a uniform distribution of crushed or flaked ice inside the package surrounding the product; in the latter, ice is spread only on the surface.

Cooling of the product is due to ice contact more than to ice melting. Indeed, 1 kg of ice melting absorbs 80 kcal (335 kJ) although ice melting is not required during the maintenance and shipping of the product. Shipping containers or cold rooms are maintained at 0 °C just to avoid ice melting.

Unfortunately ice melting occurs during product cooling (i.e. broccoli) and ice injection into the cardboard box in the packinghouse, thus the amount of ice required is greater than the amount really needed (14.5 kg of ice per 9 kg of broccoli) (Thompson *et al.*, 2002a). Heat absorption from the product during melting can cause surface burning in most fruits, while in other products, such as broccoli or green onions, injury does not occur. After ice melting, dripping water causes technical problems during transport, especially during air transport and, moreover, increases the risk of micro-organisms contamination on the product. For this reason, the efficiency of this cooling system depends more on ice contact with the product, as the thermal conductivity of the ice is four times higher than that of water. Thus, if the ice packaged product is kept in an environment at 0 °C, product cooling is due to heat transfer from and through the ice.

The CCP of this technique is the quality of the ice. In developed countries safety regulations require the use of drinkable water to produce ice but in developing or poor countries, product contamination caused by the package ice melting is frequent, especially when the surface of the product is not sound. Use of drinkable water is absolutely required to produce ice.

18.3 Storage technology

Dealing with storage technologies is very challenging because the main result of using an efficient technology to store perishable commodities is a reduction of their losses, which means controlling or inhibiting the development of decay and delaying ripening or senescence. Other objectives of the storage technology are the maintenance of appearance, aroma, flavour, although the importance of these objectives depends on the type of market and the primary need. In poor countries where low income families face challenges in their every day life just to survive, the storage technology must permit them to have a reasonable, minimum amount of food, regardless of the appearance and aroma. In developed countries where the family income, even though not evenly distributed, is higher, the objective of the storage technology is still loss reduction but, at the same level of importance or sometimes at an ever greater level, the objectives are external quality attributes (appearance, colour, size, shape), attributes required by the regulatory quality standards and flavour and odour characteristics. In any case, the efficiency of a storage technology must be evaluated in terms of maintenance of product safety, wherever the technology is applied and at whichever level of applied technology.

18.3.1 Refrigerated storage

Fresh horticultural commodities are living organisms characterised by a respiratory metabolism and thus consume oxygen and substrate, and release

carbon dioxide, water vapour and heat. The production of 1 mg of CO_2 consuming 1 mg of O_2, releases 1 mg of water vapour and 2.55 cal (1 cal = 4.187 J) energy. Depending on the species, the rate of respiration changes significantly, passing from values of 200–400 mg CO_2 $kg^{-1}h^{-1}$ in cut flowers down to a few milligrams in tubers, bulbs and dry fruit. The rate of respiration is governed by temperature and follows the van't Hoff rule fairly closely. The rule states that the rate of most chemical and biochemical reactions increases/decreases two or three times with every 10 °C rise/drop in temperature.

Thus reduction in temperature is a great tool to decrease the respiration rate of fresh horticultural commodities, maintaining the product substrate and diminishing the heat release (vital heat), although it is not possible to reduce indiscriminately the temperature to the cryoscopic limit of the commodity because, depending on different factors, but mainly on the geographic origin of the species, the tolerance of fresh horticultural commodities to temperature reduction changes significantly. Chilling injury is a well-known disorder which appears as different symptoms (pitting, black stains, decay increasing, uneven ripening, etc.) on commodities originating in tropical and subtropical regions, but sometimes appears even in fruits of temperate origin such as peaches and apricots, which display internal breakdown symptoms. However, even for tropical and subtropical fruits reduction of the temperature to a reasonable limit is possible. For instance, in bananas the lowest limit is 13 °C, permitting bananas to be shipped over a 20 day-period from South America to Europe without any significant changes in ripening.

An efficient cold room for storage of fresh horticultural commodities, which generally require high relative humidity, is strongly dependent on correct calculation of heat loads in order to equilibrate the components of the cooling plant, compressor, condenser, expansion valve and evaporator. Together with an efficient cooling plant, thermal insulation of the walls, floor and ceiling is necessary to avoid heat leakage and excessive working of the compressor especially in hot geographic areas. For use in these areas, cold equipment is usually 'tropicalised', which means significantly increasing the condenser surface to permit efficient condensation. Heat loads that have been calculated erroneously result in undersized cooling plants which are not able to maintain high relative humidity or to reach the correct temperature, thus requiring higher energy consumption and compromising the quality of the product. A high relative humidity is not easy to reach and maintain; it is strictly dependent on the evaporator surface following the equation:

$$S = q/k \times \Delta T$$

where S = evaporator surface (m^2), q = heat load per hour, k = coefficient of heat transmission and ΔT = temperature difference between evaporator air and room air.

The bigger the evaporator, the higher is the relative humidity in the cold room (Anelli and Mencarelli, 1990), so accurate evaporator design both permits energy saving and reduces weight loss of the fresh commodity (Yun *et al*., 2004).

There are two types of refrigerated storage: direct and indirect refrigeration. The difference between the two systems depends on the method of cooling. In the former, the evaporator is sited inside the storage room, while in the latter the evaporator is outside the cold room and cools a water–ethylene glycol solution which is pumped into a heat exchanger inside the cold room. The first system is widely used at different levels of food distribution, from the large storage room to the domestic refrigerator. The second system is used for large storage rooms, especially for long-term storage (i.e. a controlled atmosphere storage room) because it can maintain a very high relative humidity in the storage environment since the evaporator is placed outside of the cold room. In addition, different products can be stored in the same packinghouse but in different rooms (i.e. pears and apples) with different thermal needs.

Apart from the technical aspects of the evaporator, it is important to note that the evaporator (or the heat exchanger in the case of an indirect refrigeration system) and the expansion valve are the only parts of the cold equipment that are located inside the cold room where the commodities are stored. Owing to the nature of the construction, with serpentine pipes and fins, which constitute the heat exchange surface, and fans, which drive out cold air and draw in warm air, there are a lot of dust particles, as well as chemical residues (i.e. antiscald chemical residues in apples) present, and fungi and bacteria spores stick to the fins, making cleaning very difficult. For this reason, for organic apples in long-term storage, storage facilities must be thoroughly cleaned. In many case different storage facilities are provided in order to avoid cross-contamination (Mencarelli *et al*., 2003). There have been cases where wooden bins used for storing conventional apples have released chemical residues (diphenylamine, DPA) to organic apples (Pertner *et al*., 1996). The problem can be easily solved with the use of plastic bins as it has been done in Bozen in North Italy, one of the major areas of apple production.

Organic produce must be handled separately from conventionally grown produce to avoid cross-contamination with pesticide residues. An EU organic agriculture normative was issued in 1991, n.2092/91 (http://europa.eu.int/comm/agriculture/qual/organic/brochure/abio_en.pdf). National organic standards in the USA became effective in October 2002 and are available electronically at http://www.ams.usda.gov/nop/.

Cold storage facilities, and in particular refrigeration coils, refrigeration drip pans, forced air cooling fans, drain tiles, walls and floors should be cleaned and sanitised on a regular basis. The human pathogen *Listeria monocytogenes* can multiply at refrigeration temperatures in moist conditions and may contaminate produce if condensation, from refrigeration

units or ceilings, drips on to the produce. The common environmental pathogen, *L. monocytogenes* may get onto walls, or into drains and cooling systems. Comprehensive sanitation programmes that target these areas are instrumental in preventing the establishment of this pathogen (Gorny and Zagory, 2003). Thorough cleaning and sanitation, especially after fumigation or thermonebulisation, should be carried out. Cleaning of evaporators can be performed by using water spraying or vapour. Anyway, the abundant use of water (8–10 $m^3 h^{-1}$) to defrost (twice a day, each time for half an hour) the evaporator guarantees adequate washing (Nardin, 2004). In apple handling, water is used for feeding and packinghouse transport from one quality control station to another. In Bozen in North Italy, chlorine is not used in the water because it was noticed that the total contamination, from the time of arrived from the field at the packinghouse to the time of leaving, does not vary. The water cleaning programme in this case is performed by using a continuous three-step filtration: first a mechanical filtration, then a quartz dust filter and finally activated carbon to remove pesticides residues. Every 7 days the water is completely changed (Nardin, 2004). Longer intervals between water change provoke the formation of mucilage film. Comprehensive sanitation programmes that target these areas are instrumental in preventing the establishment of this pathogen (Gorny and Zagory, 2003).

Smoothness of the walls, ceiling and floor, without any cracks, pipes or frames, which can be potential nests for pathogens and chemical residues, is very important. This must be taken strongly into consideration especially at the wholesale and retail level where short-term storage is used and the maintenance conditions of the cold room are often insufficient. In this distribution step the risk of microorganism contamination is even worse owing to the cooling and warming cycle when the fruits are moved out from the cold room to be displayed for sale and, successively, placed back into the cold room. This 'in and out movement' can occur several times and it is usual for water to condense on the fruit surface when the temperature of the product falls below the dew point of the surrounding atmosphere (Linke *et al*., 2004). Pathogens infection such as of *Penicillium expansum* on apples or *Botrytis cinerea* in kiwi fruits or table grapes can develop with the formation of toxin metabolites or spores released into the air, which can be breathed by operators.

Water condensation inside the cold room can be very critical especially in the bulk storage of some commodities such as grains, maize and nuts (chestnuts, pistachio, hazelnuts, pinoles, pine seed) where there can be uneven ventilation and erroneous stacking, or particularly when there is scarce insulation or heat leakage, common situations in developing countries (Mencarelli, 2004). Hazelnuts picked from the trees together with their green calyx and then packed from into plastic bags before being transported to the packinghouse, as has been done in some Turkish areas, or chestnuts that have been harvested with the burr and then bulked (i.e. in China) can

develop *Aspergillus* spp which can produce aflatoxins. This development can occur when dry nuts are stacked inside the cold room and there are droplets of condensed water and a pocket of heat where the temperature is higher than the rest of the room, caused by uneven air circulation. Most items for which alerts or notifications were issued by the National Health Organisation in Europe were pistachios from Iran and dried figs from Turkey. In 2002, the EU alerted European countries to import dry fruit from Turkey only if the batches were guaranteed by certified analyses for mycotoxins (European Union, 2002). Among the aflatoxins (B1, B2, G1, G2), B1 is the most dangerous to the consumer and its proportion of the total mycotoxins is, respectively, 70–100% (almonds) 66–93% (pistachios), 50% and less (hazelnuts) and 40–60% (brazil nuts), results provided from the analyses of the Rapid Alert Notification 1999–2002. EU Rapid Alert Notifications between 1998 and 2002 indicated 572 rejections of tree nuts which exceeded EU limits of 4 ppb total/2 ppb B1 (Esposito, 2004).

Exposure to microorganism contamination today is very high, especially at the retail level, in huge hypermarkets where food and non-food products are in the same environment. Most of the time, raw fruits and vegetables are neither packed nor maintained at low temperature, thus exposing them to cross-contamination. Special attention should be devoted to the ceiling and the aeration system. The presence of visible piping and frames must be avoided.

Contamination occurs frequently at the catering level during commercial refrigeration as shown by a survey carried out in 236 inspections of 27 catering extablishments in the 2001–2002 period in Italy. In 8% of cases raw vegetables were contaminated by *E. coli* and *Listeria* mainly due to cross-contamination by other food commodities (Legnani *et al.*, 2004).

18.3.2 Controlled atmosphere (CA) storage and gas treatments

Controlled atmosphere storage is widely applied to apples, pears and kiwi fruits for long-term storage. The advantage of this technique compared to refrigerated storage is the combination of low temperature with a modification of the atmosphere surrounding the fruits. Several acronyms are used to identify the different methods of CA storage: RCA (regular CA), LO (low oxygen), ULO (ultra low oxygen) and LECA (low ethylene CA). Reduction of oxygen to extremely low values (close to the anaerobic compensation point) or increase of carbon dioxide to the limit of phytotoxicity for the commodity are currently used for insect disinfestations as well as for storage. Several CA formulas have been tested on most fruits and vegetables, including flowers, and the best formula, the lowest limit for oxygen and the highest limit for carbon dioxide have been reported (Saltveit, 2003; Kupferman, 2003; Kader, 2003), as well as insect and mite control by CA (Mitcham, 2003).

From the technological viewpoint, compared to a regular cold room, the CA room must be airtight in order to isolate the internal atmosphere of the room from the outside atmosphere. This is usually performed with elastic acrylic resin, which is spread over the surface of the internal walls, ceiling and floor. Special attention is addressed to the efficiency of the refrigeration plant in order to maintain a continuous constant temperature in the CA room, without too great a temperature difference between the on and off modes of the compressor. Today, this difference is usually around 0.5 °C. Moreover the use of breathers (3–5% of room volume), especially when low oxygen is required, is absolutely necessary to avoid pressure reduction during the initial cooling (cool down), which can create room structure problems (implosion) and compromise the maintenance of the fixed atmosphere. Indeed, when the room is loaded, a decrease of 1 °C in temperature provokes a depression of 37 mm of the water column, which means a pressure on the wall of 3.7 kg m^{-2}. For a 560 m^2 cold room, the pressure force on the wall and ceiling will be 2 tonnes for a 1 °C temperature decrease; to bring down the temperature of the product from 25 to 0 °C, the external walls of the room will experience a pressure of about 52 tonnes (51 800 kgf).

In terms of innovations in technology, in the last few years particular attention has been addressed to atmosphere purging. The most up-to-date and used technique for reducing oxygen is non-cryogenic separation which is based on two systems: membrane and adsorption. In the former, compressed air is fed to the membrane separator(s) where oxygen preferentially permeates across a polymeric fibre membrane. Permeation occurs continuously and no regeneration or purging of the membrane is required. In the latter, a molecular sieve has a finite capacity to absorb oxygen and thus must be periodically regenerated by depressurising the molecular sieve (pressure–swing–adsorption PSA system) (Malcolm, 2004). The pressure of air feeding the equipment in both systems ranges between 8 and 13 bar. In the PSA system, the use of a vacuum pump (VPSA) has permitted the operative pressure to be reduced to 1–2 bar during the adsorption and to 0.1 bar for regeneration.

A dynamic control system has been developed in Holland (van Schaik and Verschoor, 2003). The principle is continuous information exchange between the product and the room atmosphere mediated by an accurate sensor (oxygen, carbon dioxide, ethanol, ethylene) and a computer, in order to modulate the atmosphere as a function of the metabolic behaviour of the commodity.

In terms of quality, the great advantage of the use of CA is the possibility of using a higher temperature than the regular refrigerated storage temperature since the effect on the metabolism is provided, apart from the low temperature, by a combination of oxygen reduction and carbon dioxide increase. From the point of view of safety, several papers have shown the efficiency of CA in controlling the development of diseases (Sugar, 2002). In particular high CO_2 controls the development of *B. cinerea* and *Monilia*

fructicola in different fruits such as cherry, strawberry, peaches, red chicory and raspberry (Adaskaveg *et al*., 2002; Choi *et al*., 2004; Bertolini *et al*., 2004; Mencarelli *et al*., 1993) and CO_2 is used in commercial transport of strawberries in several countries.

Actually, the most recent concern of consumers of fresh fruits and vegetables is the use of SO_2 to control *B. cinerea* in table grapes (Mencarelli *et al*., 2004). In particular, when table grapes are packed in plastic bags with a small package of potassium metabisulphite which releases SO_2, in a high relative humidity environment, the concentration in the atmosphere surrounding the product is not precise and sometimes exceeds the legal limit (EU SO_2 residues: $10\,mg\,kg^{-1}$ or per litre as sulphurous acid and its derivatives). These packages are forbidden by EU legislation but are used commonly. Special attention must be paid to the gas treatment in the storage room and in the transportation package. As with wine labelling in the USA, the consumer should be informed about the possibility of sulphur compound residues in fruit.

An interesting result is the use of ethanol vapour on table grapes as a substitute for sulphur dioxide: $2\,ml\,kg^{-1}$ of grapes controlled *B. cinerea* without affecting the quality of the stem (Chervin *et al*., 2004; Karabulut *et al*., 2004).

A recent addition to the storage atmosphere for fruits and vegetables is 1-methylcyclopropene (1-MCP) (Sisler and Blankenship, 1996) which is marketed as SmartFresh® by AgroFresh, Inc. The great advantage of this gas, which is released when the commercial powder (α-cyclodextrin) comes into contact with water, is the ability to work at very low levels. It has a non-toxic mode of action and is chemically similar to naturally occurring substances. The rat inhalation LC_{50} is greater than $2.5\,mg\,l^{-1}$ (or 1.126 ppm v/v active ingredient in air). In tests for acute toxicity of 1-MCP, no death or clinical signs of systemic toxicology were seen (EPA, 2002). Experimental work has been carried out on most fruits and vegetables (Blankenship and Dole, 2003), but actual use is authorised on ornamental crops (this application is very important because it is will be a substitute for silver thiosulphate which is banned in several countries because of its high risk as a water pollutant), in apples in several countries (USA, New Zealand, Australia, Brazil, The Netherlands, Austria, South Africa, Argentina, Canada) and in pears, avocado, kiwi fruits, papaya, tomato, melon and plums in the USA, New Zealand, Brazil and Australia. 1-MCP acts by competing with ethylene for the membrane receptors and this competition depends on the concentration of the 1-MCP as well as the ethylene production rate (Klee and Tieman, 2004). The treatment is applied inside an airtight room, such as that used for CA storage and can last for 12–24 h at room or low temperatures. The maximum dose applied is 1 ppm with residues in the fruits of less than 0.1 ppm (Regiroli, 2004). The treatment can be repeated periodically during storage depending on the progress of fruit ripening or can be used together with CA storage.

18.3.3 Heat treatments

In a time of increased awareness among consumers that many chemical treatments of fruit and vegetables to control insects, diseases and physiological disorders are potentially harmful to humans, there is an urgent need for physical alternative treatments to the use of chemicals (Lurie, 1998). Hot water treatments first applied in 1922 (Fawcett, 1922) have widely been used for several fruits only in the last decade, especially for insect disinfestation. Prestorage heat treatments can be used as hot water dips, vapour heat or hot dry air (Lurie, 1998) or by hot water rinsing and brushing (Lichter *et al*., 2000; Fallik *et al*., 2001).

Hot water immersion (HWT) has a great advantage compared with hot vapour treatment in the efficiency of heat transfer and thus in the rapidity of the treatment. Moreover, the ability to monitor water temperature and to kill skinborne decay-causing agents, together with less expensive plant (10% less), are the other advantages. An innovative technology is hot water rinsing and brushing (HWRB), which consists of rinsing the product using pressurised hot water sprayed by nozzles while it is rolled on brushes made from medium-soft synthetic bristles. The temperature and the time of rinsing and rolling are 48–63 °C for 10–25 s respectively, depending on the produce type and cultivar (Fallik, 2004).

HWT for quarantine purposes usually consist of dipping fruit in water at 43–49 °C for a few minutes or a few hours. This treatment provides Probit 9 level (99.99683%) quarantine security against Mexican fruit fly. A HWT for 20 min at 49 °C was approved by the USDA-APHIS for tropical fruits such as papaya, litchi or carambola from Hawaii (APHIS, 1997).

Notwithstanding the high temperature of the water, sanitisation of water is always required since these temperatures are not effective against bacteria. In 2000, mangoes treated with hot water before export from Mexico to the USA, caused 78 infections with the same pulsed field gel electrophoresis (PFGE) profile of *Salmonella* in 13 states (Sivapalasingam *et al*., 2000).

18.3.4 Biocontrol agents (BCA) and natural compounds in management of post-harvest diseases

Biocontrol agents in management of post-harvest diseases have been widely tested (Janisiewicz and Korsten, 2002). The pioneering biocontrol products Biosave and Aspire were registered by the EPA in 1995 for control of post-harvest rots of pome and citrus fruits, the former containing *Pseudomonas syringae* and the latter *Candida oleophila*. Avogreen is another commercial product containing *Bacillus subtilis* and just recently another BCA named Arabesque (*Muscodor albus*) has been registered. *C. oleophila* has inhibited the spore germination of *Penicillium* spp and *C. oleophila* and *Pichia guilliermondii* have reduced the conidia production of *Aspergillus carbonarium* diminishing the formation of OTA (ochratoxin) (Castoria *et al*., 2004; Favilla *et al*., 2004; Droby *et al*., 2004). Arabesque has

been shown to have an effect on the reduction of *E. coli* O157:H7, *Shigella sonnei*, *S. boydii*, *S. flexnerii* and *L. monocytogenes* (Suslow *et al*., 2004).

As an alternative to the use of post-harvest chemical treatments such as azadirachtin and pyrethroids, the search for natural products, which are comparatively biodegradable and almost non-residual in nature, has become very wide. Natural flavour compounds such as acetaldehyde and other aldehydes have been tested to control development of several fungi in post-harvest fruit treatment and acetic acid has been tested for control of *B. cinerea* in grapes. Potential antimicrobial activity is expressed by glucosinaloates produced by Cruciferae. Hydrolysis of these compounds produces isothiocyanate, thiocyanate and nitrile derivatives. Fusapyrone and deoxyfusapyrone extracted by *Fusarium semitectum* has been reported to have strong efficiency in controlling *B. cinerea* in grapes. Hundreds of essential oils have been tested *in vitro* with interesting results for controlling fungi development, as reviewed recently by Tripathi and Dubey (2004). Thymol has been seen to be effective in cherry in controlling *B. cinerea* and the US Food and Drug Administration lists this product as a food for human consumption as well as a food additive. Carvone, the essential oil of *Carum carvi* has been shown to inhibit potato sprouting during storage and it has been introduced commercially under the trade name of TALENT in The Netherlands.

However, even for these products, extensive knowledge of potential mammalian toxicity is required (Essers *et al*., 1998) and the concept of hormesis, the unexpected or paradoxical effect of a chemical product or radiation used at low dose, must always be kept in mind (Calabrese and Baldwin, 1998). In fact, it has been recently demonstrated that the majority of toxic compounds exhibit hormesis whenever test designs are used that allows this to be detected (Calabrese and Baldwin, 2003). Carvone, carvacrol, cinnamaldehyde and thymol have been seen to have genotoxic effects and for *in vitro* toxicity they belong to the mild/moderate toxicity class (Stammati *et al*., 1999). In general, the health benefits from supplementation of low concentrations of compounds like β-carotene in cases of malnutrition have been widely proved, but it has been even seen to promote lung cancer in prolonged used when its use is not strictly necessary (Omenn *et al*., 1996). Vitamin C can behave as a pro-oxidant when used in high doses and the frequent use of capsicin, increasing the Ca^{2+} permeability of membranes, can produce neural problems (Brandt *et al*., 2004). Inherent food plant toxicants are defined as 'plant constituents which might give rise to adverse effects in humans when the plant or plant products are ingested'. A list of these compounds was produced by EU-project NETTOX (Gry *et al*., 1998).

18.3.5 Irradiation

Irradiation is an effective control measure for eliminating pathogenic bacteria and parasites from the surface of fruits and vegetables (WHO, 1998) and in 1980 a Joint FAO/IAEA/WHO Expert Committee on Food

Irradiation (JEFCI) declared 'Irradiation of any food commodity up to an overall average dose of 10 kGy causes no toxicological hazards; hence, toxicological testing of food so treated is no longer required'. Irradiation is approved for imported products in the USA for controlling plant pests (APHIS, 2002). Papaya from Hawaii is regularly irradiated (APHIS, 1997). Information is requested for import of irradiated fruits in the USA, such as approved doses, location of facilities, compliance agreements, certification of facilities, monitoring and interagency agreements, packaging, dosimetric systems, records, certification and inspection of the facility and any denial on withdrawal of certification. In 1986, based on irradiation data for many tephritid fruit fly species and a limited number of other insect pests, a dose of 150 Gy for fruit flies and 300 Gy for other insects was proposed (ICGFI, 1991). An irradiation dose of 150 Gy applied to 62 400 artificially inoculated and an estimated 31 266 naturally infested melon fly third instars (93 666 total) resulted in no survivors to the adult stage and no pupae ever partially emerged (Follet, 2004). Approving lower doses may be advantageous to lower the costs of treatment and increase product throughput by decreasing the required time for treatment. Although most fruits are tolerant of irradiation (Follett and Sanxter, 2003), lowering doses may also permit the treatment of radiation-sensitive fruits such as avocado. Following the quarantine treatments, the use of irradiation has been seen to control the development of different fungi species such as *Aspergillus, Penicillium, Botytis, Rhizopus*, *Alternaria* and mycotoxin production in different fruits by using 1.5–3.5 kGy during refrigerated storage (Aziz and Moussa, 2002). Notwithstanding, today 40 countries permit the irradiation of one or more foodstuffs and 29 are applying it commercially (ICGFI, 1997), although consumer concern about the use of this technology for food is still great. Molins *et al*. (2001) suggested that irradiation for minimally processed foodstuffs including fruits and vegetables can be considered a critical control point since critical limits (minimum and maximum doses) can be established and monitored, and process control is well documented.

18.4 Transport

Transportation is an important part of the food chain, often the most important factor in the marketing of fresh produce and involves transport of fresh produce from the field to the consumer. Consumers expect products to be of the best quality. Product quality can only be maintained, not improved, during transportation, and moreover safety has to be guaranteed. From 1985 until today the commercial exchange of horticultural commodities has increased by 80%, passing from almost 25 to 40 million tonnes for fruit and from 10 to 20 millions tonnes for vegetables (Della Casa, 2000). The greatest increase in the export of fresh horticultural commodities from different continents between the end of the 1980s and the end of the 1990s has been

for Asia, Europe and Latin America, rising respectively from 7 to 11, from 19 to 32 and from 2.5 to 4 million tonnes (Della Casa, 2000). Today, in the USA, each pound of food travels for an average of 2000 km and in Germany, to distribute fresh vegetables every year 170 millions litres of diesel are consumed, producing 500 000 tonnes of CO_2 emissions (Geyer, 2004).

In this panorama it is clear that the precautions related to sanitary aspects of transportation have become more and more important.

The transportation step in the food chain represents the most risky step because the product is moved from the packinghouse, loaded into the means of transport, shipped, unloaded at arrival, sometimes in several locations, all with continuous temperature changes. In all these points, contamination can occur depending on the commodity (perishable, sensitivity to temperature, sensitivity to mechanical injury), shipper professionalism (knowledge of shipped product, punctuality) and the organisation of shipping (time of departure and arrival, loading and unloading facilities, occurrence of legal procedures). For instance, some chemical contamination from fuel engine discharge can contaminate the product during loading and unloading. Thus, special attention must be reserved for this distribution step especially for fresh horticultural commodities which, most of the time, are not tightly packed and have a porous structure which easily absorbs gaseous or liquid contaminants. Transportation of food presents different types of hazard:

1 physical hazards such as pieces of metal, wood or glass;
2 chemical hazards from residues of cleaning agents, from previous food and/or non-food cargoes, from food and/or non-food cargoes mixed in the same load, from refrigerant leaks or from the external environment during loading and unloading operations or during inspections;
3 biological hazards from contamination by bacteria, insects, yeast, moulds, rodents and from growing of contamination microorganisms at improper temperatures.

In order to plan transport of perishable produce it is necessary to know the produce's characteristics which are affected by:

- temperature
- water loss
- mechanical damage
- decay in storage.

Each product has an optimum storage temperature and products with different optimum storage conditions should not be transported together in the same load (mixed load) and should also be compatible for ethylene sensitivity and relative humidity requirements (Welby and McGregor, 2004; Thompson, 2002; Anonymous, 1989). Moreover produce should have enough post-harvest life for the trip and subsequent marketing at the destination.

Table 18.3 Pre-shipping factors which can affect the shipping quality (Snowdon, 2004)

Health status of planting materials
Weather during the growing season
Crop husbandry
Harvesting and handling techniques
Post-harvest treatments
Packaging
Precooling
Shippers' carriage instructions
Design and function of ship
Stowage
Interpretation of carriage instructions
Duration of voyage

Mechanical damage should be avoided using proper handling and packaging facilities. Corrugated fibreboard boxes have to be strong enough for high humidity conditions, which could cause warping. Wood and plastic boxes are not affected by humidity and maintain their strength in high-humidity conditions. The size and design of packages should provide adequate levels of ventilation to the contained product without compromising the mechanical resistance of the package; 5% over the total box surface is considered to be the correct venting surface (Thompson *et al*., 2002b). In Table 18.3 pre-shipping factors that can affect the shipping quality are reported. These are intended to be 'the ability to maintain the same level of the produce quality from the departure point to the arrival point'.

Sanitation is essential for protecting the products and the following principles must be followed (IIR, 1995):

- take special precautions against chemical contamination when the equipment being used has previously been used to carry chemicals;
- prevent microbial contamination (both pathogenic and non-pathogenic) through routine and efficient cleaning of the walls and floor, including nooks and crannies, and joints;
- monitor the transported products closely for their initial bacteriological quality, their initial temperature, the quality of packaging;
- take the necessary precautions against tainting.

These principles include transport equipment (choice of materials, avoidance of nooks and crannies, use of special paints), cleaning materials, packaging materials, refrigerating equipment, especially the evaporators, air ducts and defrosting system, routine treatment for pests and rat elimination measures, staff hygiene, and awareness of all these by all personnel involved.

18.4.1 Trucks

Trucks are the main system used to transport fresh vegetables and fruits. Positive characteristics are that they are extremely flexible compared to others shipping facilities like trains, ships and aircraft, they allow the destination to be modified rapidly and they carry a limited load. Nevertheless, there are also some negative aspects like increasing road traffic, international trade rules and the growing cost of fuel (Anelli and Mencarelli, 1990).

Trucks used for transporting perishable foods such as fresh fruits and vegetables have refrigerated trailers. Very often today, a multimodal refrigerated container (reefer) is loaded over the flat trailer. Refrigerated trailer and reefer containers have the same construction characteristics: a refrigeration unit with a ventilated evaporative coil, a chute to address air movement hanging at the ceiling, a T-bar floor and a bulk-head under the evaporative coil. Uniform distribution of air is essential to obtain uniform temperature of the load; the design of the container, the efficiency of air circulation fans, the packaging and the load stowage influence the air distribution (Anelli and Mencarelli, 1990; Thompson *et al*., 2002b; Hill, 2004). Heat loads in a refrigerated trailer are mainly due, as for the cold storage room, to respiration of the produce (Welby and McGregor, 2004), but in the summer or in hot locations, a large amount of heat is transferred from the asphalt on the road to the container (Anelli and Mencarelli, 1990). Moreover, the ratio between the sum of the linear measurements of the container (connections between the walls, and with the floor and the ceiling) and the surfaces of the walls, roof and the floor of a standard 12 m ($12 \times 2 \times 2$ m) long container, is much greater (0.62 vs 0.16) than that of a larger volume cold storage room ($20 \times 10 \times 10$ m). This means that heat infiltration into the shipping container is much easier than into the cold storage room.

Cold air circulates longitudinally from the evaporative coil (evaporator) along the ceiling inside the chute, up to the opposite door and returns to the evaporative coil, passing through the products and through the T-bar floor. The bulk head, positioned vertically under the coil 20 cm away from the floor, creates a plenum with a lower pressure which permits the evaporator fans to draw up the warm air. Each barrier which could interfere with air circulation lowers the air circulation volume limiting the amount of cooled air passing through the commodities. Erroneous pallets stacking and too tight stowage can hinder the cold air circulation through the commodities. In truck transport of ice-packaged commodities such as broccoli, ice placed over the commodities (top icing) can melt and freeze back creating a uniform ice layer over the box surface which hinders the cold air from passing through; a loading too high in front of the evaporative coil could obstruct the chute, reducing air flow; the presence of frost on the evaporator coil hinders the cold air leaving the evaporator. To facilitate

produce cooling, pallets must be placed away from the walls and rear. Packages should be stacked in a way that allows air to circulate between them without creating short circuits that could make cooling uneven or the stacks unstable (Anelli and Mencarelli, 1990; Thompson *et al*., 2002b).

Another very important aspect of the refrigerated trailer or refrigerated container is insulation. The insulated body of the refrigerated trailer is composed of 'sandwich panels'. A sandwich panel is obtained by assembling two main elements (IIR, 1995):

- sheathings with good tensile and compressive strength, made of different materials: 1 mm steel (thermal conductivity, λ, $W m^{-1} °C^{-1}$, = 52) or aluminium ($\lambda = 204$) alloy sheet; 2–3 mm fibreglass reinforced polyester resin sheet ($\lambda = 0.20–0.30$); marine-quality plywood ($\lambda = 0.14$) minimum 5 mm thick covered by gel coat;
- the core, which serves as a brace, made of a rigid, closed-cell insulating foam with mechanical qualities inferior to those of the sheathings.

Accurate management of stowage, efficient running of the refrigeration unit, and care in insulation maintenance should be marked as a CCP in a quality management system. For instance, the heat transmission coefficient increases by 25–26% over 5 years depending on the insulated layer thickness. The presence of a 'heat bridge' in the container structure reduces the efficiency of the insulation system creating condensation problems and the potential formation of microorganism nests which can contaminate the fresh fruits and vegetables which are usually unprotected (Agrotrans, 1982; Anelli and Mencarelli, 1990). Market requirements for thinner walls to increase the shipping volume and pallet loadings hasten the ageing of insulation materials; moreover the banning of Freon compounds because of their high ODP (ozone depletion potential) has decreased the quality of insulation even though a vacuum technique that creates a sandwich panel can partially solve the problem (Panozzo, 1999).

Before loading, perishable produce should be cooled to the desired transport temperature as, in a refrigerated trailer, the amount of air flow is limited to enable the air to be forced through the boxes. Truck refrigeration units have a little more cooling capacity than that required to remove the heat coming from outside and, if correct precooling has been achieved, removal of heat released by produce is not a problem (Welby and McGregor, 2004; Thompson *et al*., 2002b; Hill, 2004). In very perishable products such as strawberries, precooling and shipping procedures greatly affect the quality (Massantini *et al*., 1994).

The shipping company should inspect the trailer interior to ensure that it is clean, dry and odour free before shipping (Welby and McGregor, 2004; Thompson *et al*., 2002a). Very often truckers, carrying fresh fruits and vegetables to the destination point, load other products (food or not food) on the return journey and, if a sanitation programme is not performed thoroughly (complete disinfection, not only of the walls, ceiling and floor, but

also of the coils and fans), the contamination risks for fresh product are very great.

Any damage to the interior walls should be repaired and doors should close tightly in order to avoid contamination or loss of insulation (Welby and McGregor, 2004). It is important to emphasise that anyone involved in loading and unloading operations, inspectors and produce buyers too, should use good personal hygiene and sanitation practices (Gast and Holt, 2000).

Loading docks and load assembly areas should be refrigerated. Perishable foods should not stay in the sun before loading; on the other hand, in very cold weather if the produce is not managed at the correct temperature, chilling injury could occur. During loading and unloading operations a potential hazard can occur from cross-contamination from other food or non-food sources and exposure to contaminated surfaces (Gast and Holt, 2000). In these areas, the presence of rodents is frequent and the contamination of the product by excrement or urine is very easy. This is a very critical point (CCP). There was one case of death caused by the contamination of beverage cans by rodent urine in Switzerland in 2002 and several cases of salmonellosis in Italy (Caramello, 2004). The risk is even greater for fresh fruit and vegetables as most of them are unwrapped. Sanitation of load and unload assembly areas, applying good sanitary and cleaning procedures, is essential to reduce chemical hazards and biological hazards from contamination by bacteria, moulds, yeasts, parasites, rodents and animals.

A frequent practice is to wet the pallets of some vegetables with water before loading into the truck when the outside temperature is high, with the objective of cooling down the temperature and maintaining the cell turgor. In this case, water must be drinkable otherwise contamination can occur. Legal authorities do not permit the use of water which does not conform to the sanitary norms, although sometimes, especially at farm level, a farmer may use water from a well that does not always conform to sanitary standards. The risk is even higher when fruits or vegetables have been brushed, for example kiwi fruits, because brushing provokes small wounds which as well as accelerating ripening can provide a natural aperture for fungi penetration (Massantini *et al*., 1995). Brushing can be a very critical operation. There has been a case of *Salmonella* contamination of dried milk because brushes were contaminated by this bacteria (Kane, 2001). A case of dried nuts being contaminated by *E. coli* because well water was used for washing was reported (Esposito, 2004).

During transport, air temperature within the refrigerated trailer should be regularly monitored. Temperature recorders are usually placed on the tops of the pallets but is necessary to note that data can be influenced by the heat generated from the product. Sometimes even if the refrigeration unit is producing adequately cooled air, the recorder may indicate warm conditions because the produce was warm at loading (Thompson *et al*., 2002b). Accurate calibration of these devices is required and at least once every 6 months a calibration must be run.

Temperature and time–temperature indicators (TIs and TTIs) attached as labels have been tested in order to monitor the distribution chain (Selman, 1995), providing an interesting tool to guarantee the maintenance of quality and safety of food (Riva *et al.*, 2001). Commercially available TIs and TTIs based on colour changes (enzymatic hydrolysis, polymerisation of diacetylene), diffusion of chemical substances (viscoelastic polymers) and radiofrequency (enzyme incorporated in a passive radio frequency unit) (Bøgh-Sørensen and Londahl, 2004) should be encouraged in order to enhance cold chain monitoring. Micro and nanotechnology sensors (Smart Dust) development can represent a great step forward in the control of temperature and other atmospheric characteristics during distribution (Warneke, 2003).

18.4.2 Ships

Marine transport is largely used for long distance transport (intercontinental) of fresh vegetables and fruit which have a long life under controlled temperature conditions (IIR, 1995). When considering marine transport, the extreme atmosphere conditions should be considered. A ship, in the route from one hemisphere to the other, can move from a low temperature to a very high temperature, and the relative humidity is always very high. Moreover, the presence of storms puts the load under mechanical stress which very often results in product losses. Since marine transport is usually used for intercontinental transport of food that has a long life under controlled temperature conditions, boxes should maintain their mechanical characteristics for all the time of the transport. A high humidity environment can affect the strength of fibreboard; moreover moisture absorbed can increase box dimensions and cause warping. Humidity does not affect the strength of plastic and wooden boxes. Boxes should be vented to allow vertical airflow, but vents should not account for more than 5% of the box wall's surface and should be located away from the vertical edges of the box because they reduce box strength. Boxes should not extend beyond the pallet and should be well supported by deck board so as not to fail (Thompson *et al.*, 2000).

Marine transport today is carried out by two different systems (IIR, 1995):

- **containerised transport** – by porthole-insulated containers or integral reefer containers (mechanically refrigerated containers);
- **conventional refrigerated cargo ships** – are used mainly for bananas, then by citrus fruit and frozen foods such as meat and fish.

Containerised transport

This is carried out in specialised container ships where containers are held in stacks below deck in specially designed holds.

Porthole-insulated containers

Porthole-insulated containers do not have an autonomous refrigeration unit but are cooled collectively and are generally placed in the hold. A central refrigeration unit produces cold air and distributes it to the containers through insulated ducts which supply stacks of containers in the hold. Every container has two front openings: the lower is used for blowing and the upper one for aspiration (IIR, 1995).

There are some potentially hazardous critical points in this system that need to be controlled continuously. Insulation of this type of container is very important, even more than for truck transport, because it must protect against extreme temperatures. Internal accurate inspection of the container should ensure the absence of any internal damage that could allow loss of insulation. Products inside connected containers in a porthole stack must have the same requirement for temperature, sensibility to ethylene and they must be compatible for odour. Monitoring the temperature is extremely important to avoid chilling injury or deterioration caused by improper temperatures. Temperature abuse together with other stresses and/or mechanical damage make the commodity susceptible to decay-causing pathogens and favour growth of microorganisms that are potentially dangerous to human health. In this system it is also important to keep filters clean to avoid potential dissemination of pathogens spores from one container to another. Temperature monitoring during transport is obtained through a temperature sensor located at the point of entry and exit of air from the porthole-insulated container.

Integral reefer containers

Integral reefer containers have an independent refrigeration unit. Today it is the most common method of marine transport. In the 1980s, there was a shift from breakbulk ships where the boxes were placed singularly one over the other, to pallet-friendly ships. In the 1990s there has been the boom in the use of reefer containers for fresh horticultural commodities shipping: from 320 000 TEUS (twenty feet equity unit) the number has increased up to 800 000 TEUS by 1999 (Della Casa, 2000).

The great advantage is the autonomy of the reefer containers; they are safer in terms of product quality and contamination than the porthole container. Generally they are placed on a deck where they are connected to electricity supply outlets. Sometimes to protect reefer containers from the sun it can be useful to place an upper layer of ordinary non-refrigerated containers. The presence of salt in the atmosphere makes the containers susceptible to rapid deterioration. Reparation procedures and regulations are reported below in Section 18.4.5 on Multimodal transport.

At loading docks and load assembly areas the same conditions should be observed for truck transport: they should be refrigerated and thoroughly cleaned to avoid chemical and/or biological contamination from other

loads. People involved in loading or inspection operations should respect sanitation and hygiene practices (Thompson *et al*., 2000, 2002b; Gast and Holt, 2000).

Perishable produce like fruit and vegetables must be cooled to the required transport temperature prior to loading. The container should be cooled before loading and the refrigeration unit must be turned off before the doors are opened for loading to avoid condensation (Thompson *et al*., 2000). In order to obtain a uniform temperature inside the container and to avoid refrigerated air short-cycling (by-passing most of the load), the container's floor must be completely covered with pallets or boxes. In this way, refrigerated air is forced up through, around and under the load to protect it from heat from the outside air during hot weather (integral reefer containers are usually placed on the deck), from heat produced by vegetables and fruits through respiration, and from heath loss when the outside temperature is extremely cold (Thompson *et al*., 2000; IIR, 1995). Moreover efficient air circulation protects produce from ethylene and chilling injury or freezing that might occur during refrigeration. Produce loading to minimise physical damage and maximise flow of cold air helps to reduce the potential for contamination. In integral reefer containers the temperature is usually controlled by temperature recorders installed on the top of the pallet (Thompson *et al*., 2000). New technologies have been developed for monitoring temperature inside the reefer container in real time (KlaxonIQA technology; Morris *et al*., 2003): a container sensor unit (CSU) with sensors is included inside the refrigerated container. The sensors measure commodity temperature in the load, in the delivery and return air temperature. The CSU reports the readings to a base station unit (BSU) on board the ship or at the terminal. Through a wireless satellite link, the BSU transmits regular reports of the temperature inside the reefer container to KlaxonIQA. The data can be continuously controlled over the internet and if there is a container malfunction or if the condition of the load is outside the desired limits, an alarm is sent to KlaxonIQA. This is really important in order that prompt action can be taken to minimise any quality loss to the load (Morris *et al*., 2003).

Conventional refrigerated cargo ships

These carry the majority of temperature-controlled produce which is often packed into cartoons and loaded on pallets. Cargoes are carried frozen, chilled, cooled or increasingly at controlled temperatures that can be varied to ensure that the produce reaches the market in an optimum condition, often ripening on the voyage.

Conventional refrigerated cargo ships have some positive characteristics:

- because they have on-board cranes, they can operate in ports with limited handling facilities;
- the load (long-life produce) can be cooled during transport;

- this is a versatile system that allows chilled or frozen food to be carried one-way transport and normal goods at ambient temperature to be carried as return freight;
- all or part of the holds or 'tween decks can be refrigerated or frozen depending on the load.

The insulation is provided by different materials, like polyurethane, polystyrene and expanded polyvinyl chloride. Insulation thickness is generally calculated to obtain a coefficient of heat transmission, K, of 0.5–0.4 $W m^{-2} K^{-1}$. These ships, in order to be more competitive and attractive to a range of cargoes, tend to be divided into several 'tween decks so that different products can be separated and shipped at different temperatures. Usually refrigerated ships are equipped to provide shipping temperatures between +12, 0 and –25 to –30 °C.

Different materials can be used to cover insulation materials: stainless-steel lining is fire resistant, easy to clean, salt resistant, but care must be taken with joints and nooks to avoid fouling and contamination of the insulation material, either by the cargo or by cleaning materials; plywood linings coated with fibreglass-reinforced polyester are less resistant to fire and should be and remain smooth to facilitate cleaning.

Air circulation is the main problem in these ships because the huge volume and the typical shape of the ship with curved walls create pockets of heat caused by a lack of uniform ventilation. This represents a problem especially in fresh fruits and vegetables such as bananas where even a few degrees more or less than the required shipping temperatures (13 °C) could accelerate ripening or provoke chilling injury.

For this reason auxiliary fans are placed or special stacking is carried out. Air circulation is important not only for cooling but also to remove carbon dioxide and ethylene. When products are loaded on the pallet, air circulation must be possible in the gaps between pallets especially for produce that is sensitive to ethylene and carbon dioxide. Air short circuiting must be avoided in all cases in order to obtain a uniform temperature of the load.

In these ships, the loads may be cooled on board. At the start of the cooling, it could be necessary to keep the delivery air temperature a few degrees above the required temperature, for example when the air circulation rate is high (60/90 air changes per hour) or when the fruit or vegetables are wet or sensitive to transpiration (IIR, 1995).

Since huge amounts of product are shipped, the risk of cross-contamination by microorganisms is very high as is the risk to the operators' health caused by breathing in pesticide residues or microorganisms spores. Special attention must be paid to the cleaning programme and to storage conditions management (temperature, relative, humidity, ventilation). Thermometers, hygrometers and windmeters, all calibrated periodically, must be placed at several points on the storage decks to guarantee uniform and required conditions.

Controlled atmosphere (CA) can be used in long-distance transport whether in conventional refrigerated cargo or in refrigerated containers, especially reefer containers which are the most used. The limitation on the use of CA in transport is gas tightness (hermetic seal). The Tectrol system (TransFresh Corp.) is based on a reduced oxygen environment achieved by nitrogen flushing, or CO_2 removal by using bags of fresh hydrated lime in the transit vehicle and breather bags to compensate for barometric pressure fluctuations. Liquid nitrogen or a nitrogen-separator unit can be used to reduce the oxygen concentration. Scrubbers for CO_2 or ethylene can be used in marine containers. CA shipping requirements for fruits and vegetables are reported in the CA transport guide (Serek and Reid, 1999)

18.4.3 Aircraft

Air freight is the most rapid shipping system over long distances. It is the most expensive transport system and only perishable high-value products or early-season products, including vegetables, exotic fruits, flowers, ornamental produce, fresh-cut fruits and vegetables, are commonly shipped by air, together with chilled and frozen meats, dairy products and seafood (Thompson *et al*., 2004). Commodities are commonly exposed to high temperature, low pressure, and a large vapour pressure deficit (VPD) during air shipment, which can increase their transpiration rate and result in undesirable loss of moisture.

Freight aircraft are equipped with pressurised cabins and holds but pressurisation is at around 0.7 atm and temperatures range from 4–12 °C. Temperature control is the most important critical point in air shipping. Products must be cooled before transport, loading and handling operations should be quick, but cold storage is not always possible at airports and even if it is present it may not be available for use by vegetables and fruits. Moreover, commodities should be delivered to the airports 6 or 8 h before the flight because they have to be weighed and loaded onto freight containers or pallets, called ULDs (unit load devices), and temporary holding areas are not refrigerated in most airports (Thompson *et al*., 2004).

Before loading the load is positioned outdoors near the plane. There are two reasons to consider this step as a CCP: first, they may wait for some hours at ambient conditions such as high temperature, direct sun or chilling temperatures or in rain, all conditions which can injure the products; second, hazardous substances sprayed as fumes from aircraft like aluminium, barium, biological organisms, pathogens and so on, can be absorbed by fresh fruits and vegetables which are often unwrapped and in some cases, such as strawberry or mushrooms, are very spongy.

On the plane, cargo compartments can vary considerably in temperatures. Both passenger and freight aircraft are maintained near-room temperature because animals and product that require warm temperatures are shipped there. Together with the high temperature, the low pressure inside

the cabin greatly increases the VPD (vapour pressure deficit) of the commodity resulting in great quality loss especially for highly perishable product (Laurin *et al*., 2004a) like strawberry and asparagus.

At the destination airport, an internationally shipped product has to be cleared by the local authorities and this can require long time periods, creating the same problems that apply for departure loading. To maintain quality it has been shown that re-cooling the product as soon as it arrives is worthwhile (Laurin *et al*., 2004b).

Selection of the best packaging system is more important for air shipping than for other means of shipping. In fact the reduced pressure and the high temperature require the product to be protected by plastic film in order to avoid water loss. This is the reason that flowers are very well packaged with plastic film in cardboard boxes. Cardboard is very useful because it is light and telescopic cardboard (half, HTC or full, FTC) are especially resistant and in an aircraft there is no problem of wetting. A problem in packaging with plastic film is the formation of condensed water when periods of temperature oscillation occur at the time of loading and unloading. Microorganisms can develop depending on the product. An airtight container to prevent water loss has been tested on cucumbers with good results in term of weight control. Fresh truffles are shipped in a vacuum package to maintain freshness and avoid odour contamination in the shipping environment. This is a very risky method of transport because truffles, like other commercial mushrooms, have a high pH and in anaerobic conditions can develop *Clostridium botulinum*. Usually the package is labelled 'Open on arrival' because it is considered that the time of shipping is short enough to avoid the development of anaerobic conditions. For this reason modified atmosphere packaging has been proposed for shipping fresh truffles (Massantini *et al*., 2002).

Unit load devices (ULDs, aircraft pallets and containers) are designed to maximise the aircraft contour and are available in different shapes and sizes to fit various locations inside the aircraft; they are usually designed with an overhang for the lower deck or an igloo shape for the main deck. Aircraft containers are of lightweight construction made from aluminium alloy, glass fibre or plastic. Insulated containers are made from reinforced fibre-glass panels and doors (IIR, 1995) and have a pressure release valve which is fitted on the container to equalise internal pressure during rapid decompression. Of course, people involved in container inspection should control the valve efficiency. Since mechanically refrigerated containers are seldom used because they are heavy, have reduced volume availability and have logistical problems, shippers prefer to use an expandable refrigerant. Expandable refrigerants like water ice, dry ice and liquefied gases absorb heat by changing phase.

Dry ice has a heat-absorbing ability 70% greater than that of water ice. The main problem with dry ice is that it may cause chilling injury on fruit and vegetables in contact with it; therefore dry ice should be packaged. To

ensure that the effect of ice is generally diffused and not local, the stow must be located away from the walls of the containers to permit the gas/air mixture to circulate by natural convection, carrying heat from the walls to the dry ice carton. When using dry ice, the cargo should be protected because its low temperature creates an atmosphere of low relative humidity. The sublimation of dry ice produces carbon dioxide which might be released directly into the container or into a jacket around the walls; since CO_2 is denser than air, a fan is necessary to create air circulation and maintain uniform temperature. Carbon dioxide gas is hazardous to the occupant of the aircraft (threshold limit value = 5000 ppm, $9\,g\,m^{-3}$) and may be harmful to animals and phytotoxic to fruit, vegetables and plants sensitive to high CO_2. For these reasons, airlines must be notified that dry ice is being used, and the ventilation must be able to ensure a concentration of CO_2 below the threshold limit value. Of course sensitive chilled commodities should be protected.

Water ice protects chilled produce against temperature rise and against desiccation by maintaining a high relative humidity. It cannot cause food freezing as its temperature is 0 °C. The main negative aspect of water ice is the melt water that could damage packaging materials, or other load and can corrode the structure of the aircraft. The use of water-resistant fibreboard cartons, enclosing the package in a plastic bag, using absorbent, sealing the water in sachets or immobilising the water in gel before freezing, could prevent the appearance of melt water. The use of water ice can be considered as a CCP because if it is not produced from drinkable or sanitised water, it can contaminate the product by contact during ice melting (IIR, 1995; Welby and McGregor, 2004).

Liquified gases are rarely used because the transport of compressed gas is regulated and only qualified personnel are able to handle the apparatus (IIR, 1995).

ULDs must be odour-free and thoroughly cleaned before loading because previous loads may have contained toxic chemicals or products that could harbour human pathogens. At the destination the contents of the container must be transferred to refrigerated trucks or storage facilities (Thompson *et al*., 2004). Owing to their low mechanical resistance, air containers are difficult to use in intermodal transport (IIR, 1995).

Aircraft open pallets must be cross-stacked carefully to prevent product damage and to help prevent the load from shifting. Pallets loads should also be stabilised with corner boards and straps or net wrapping. Balancing is necessary too, to avoid shifting during turbulent flight. In order to prevent warm or excessively cold air from penetrating the load, it is necessary to wrap the load; for a load of grocery pallets, this should be done in a refrigerated room at the packing facility. Usually plastic sheet is used to cover the load, and a reflective cover is more effective than an opaque one when the load is exposed to the sun. Covers protect produce from low-humidity conditions in the aircraft and reduce moisture loss. They also

Table 18.4 Specification of some mechanically refrigerated railway wagons (adapted from *Guide to Refrigerated Transport*, IIR, 1995)

Equipment	Type	*K* coeff. ($W m^{-2} K^{-1}$)	Max number of pallets	
			80 × 120 (cm)	100 × (120)
Refrigerated (non-mechanically) cars	LC	0.4	20	
	VLC	0.2	25/26	20
	SC	0.25	37/40	30/32
Mechanically refrigerated cars	VLC	0.25	24	18
		0.13	28	22
	SC	0.20	39/42	30/34
		0.23	42/44	34

protect the load from rain and reduce exposure to ethylene from neighbouring produce.

To prevent risks to human health, loading of fruits and vegetables near human remains, live animals, toxic or infectious products must be avoided. These should be stowed away from food or in a separate cargo if possible or, at least, each type of cargo should be in closed ULDs separated from each other (Thompson *et al*., 2004).

An interesting technique developed for use in transport is the Passive Refrigeration System (PRS™), developed by NOMOS (Ghiraldi, 2004). The principle involves storing a quantity of 'cooling power' needed over a predetermined period of time (5–20 days) in high efficiency thermal accumulators. Cold charge is done by an independent or integrated charging unit and is repeatable.

18.4.4 Trains

Train transportation provides high-capacity and low-energy consumption but has the negative aspect of being less flexible than a trailer. It is mainly used for trips of over 300 km or for more than 2 days. Perishable produce can be transported in insulated railway cars or mechanically refrigerated cars and non-mechanically refrigerated cars. Insulated cars have only insulation without refrigeration equipment, and are mainly used for precooled fruits such as bananas, citrus fruits, pineapples, avocados, and so on, which do not need low temperatures. Thermal transmission coefficients of train wagons are reported in Table 18.4.

In Europe all refrigerated railway cars must meet the requirements of the UIC (International Railway Union). The UIC classifies refrigerated cars based on area (Anelli and Mencarelli, 1990):

- less than 22 m^2, normal capacity (NC)
- from 22–27 m^2, large capacity (LC)

- from 27–39 m^2, very large capacity (VLC)
- more than 39 m^2, super capacity (SC).

Refrigerated cars are cooled by water ice contained in tanks situated at the ends of the car and equipped with fans that circulate air through the ice and the load (IIR, 1995). Sometimes ice is mixed with solid CO_2 which has greater refrigerating power (150 kcal kg^{-1}) and enriches the atmosphere in CO_2 (useful in prolonging the shelf-life of some kinds of fruits and vegetables) (Anelli and Mencarelli, 1990).

Mechanically refrigerated cars can be elements of a general traffic train or a part of a block train; in this case some cars can be used as isolated units and are equipped with an individual self-contained generator. The thermal motor of mechanically refrigerated cars has 10–13 h of autonomy. It maintains the required temperature during the trip and is equipped with continuous circulation devices, a thermostat sensor on the evaporator outlet, and fine-tuning devices for refrigerating capacity and evaporating temperature, making the mechanically refrigerated cars suitable for transport of fresh fruit and vegetables (IIR, 1995).

18.4.5 Multimodal transport

Multimodal transport allows goods to be switched from maritime to land transport and vice versa, strengthening the links of the cold chain (IIR, 1995). Containers are the fundamental element of multimodal transport able to take advantage of the high cargo capacity and low costs of marine transport, the velocity, low cost and cargo capacity of train transport and high flexibility and capillarity (transporting door-to-door) of trailer transport.

The integral reefer container (or mechanically refrigerated container) is the most common type of equipment used in multimodal transport. The characteristics of this container have been reported in Section 18.4.1 on Trucks. An energy supply can be provided by an on-board electricity network or, in some cases, an independent diesel generator.

Reefer containers are designed for an air circulation rate of 30–40 volumes per hour when frozen goods are transported; at least 60 volumes per hour are necessary for chilled cargoes. This rate is also necessary when the container is waiting on the ground before shipping in the parking areas, considering that the ambient temperature in a tropical climate can exceed 30 °C and may even reach 50 °C in parking areas.

Most common stowage systems have a longitudinal channel, a chimney and uniform permeability (Fig. 18.3 and Fig. 18.4). It is clear that the stowage system must match the container requirements to maintain a uniform temperature and relative humidity environment and to optimise the volume occupied (Anelli and Mencarelli, 1990).

Before loading, containers should be thoroughly cleaned and sanitised to avoid risks of contamination from the previous load, to eliminate resid-

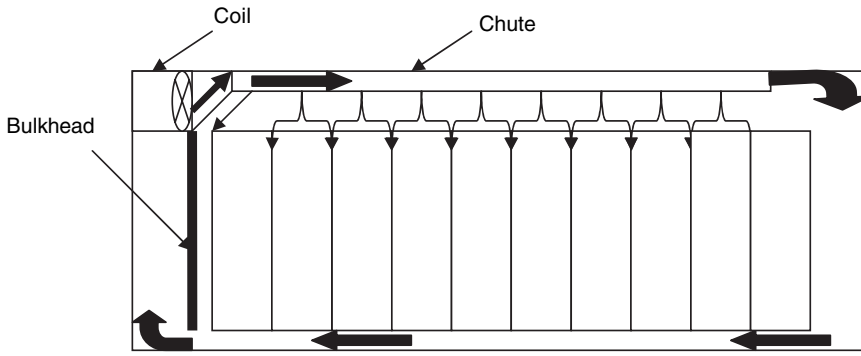

Fig. 18.3 Stowage of boxes with uniform permeability in the shipping container. The arrows represent the air movement from the coils on the top and back to the coil from the bottom. The chute is perforated. Boxes are separated by 1 cm wide channels so the air circulates around the boxes and the container volume is filled to capacity (Agrotrans, 1982).

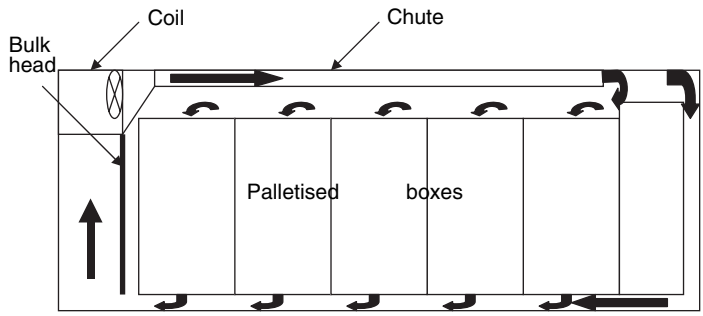

Fig. 18.4 Chimney stowage system for palletised boxes in the shipping container. The black arrows represent air movement. A vertical channel is left in the middle of the palletised load (Agrotrans, 1982).

ual toxic chemical residues that can represent a hazard to human health, to eliminate insects, the decaying remains of agricultural products and odours from other shipments; rinsing and drying should follow disinfection. Of course containers must be free of toxic materials. Workers who load and unload products, inspectors, produce buyers and other people involved should practice good hygiene prior to inspecting the load (Gast and Holt, 2000; Welby and Mc Gregor, 2004).

Damage to floors, walls and ceilings can affect various features of the container, from insulation to residual contamination. Damage like holes may allow heat to enter from outside with consequent loss of refrigeration efficacy (Welby and McGregor, 2004). Holes may also allow toxic material (from insulation material, paint) to come into contact with food and

microorganisms could proliferate in gaps in the floor or in other holes that are difficult to clean thoroughly. Both these could successively contaminate fresh fruit and vegetables by direct contact or via ventilation. With regard to this, the IICL (Institute of International Container Lessors) has divided damage into three categories in order to help inspectors to reveal conditions that may be considered unsafe for human health or which may reduce the useful life of the container:

1 Damage is one or more physical defects in a container caused by a single event or a series of single events, such as impact, abrasion, contamination, etc.
2 Wear is one or more physical defects caused by continuous deterioration in the physical condition of the container occurring under normal-use conditions (for example exposure to sea water and the elements).
3 Non-conforming repair is a condition resulting from a repair not being performed in accordance with IICL criteria.

Table 18.5 shows a list of the types of damage and wear (IICL, 1989). Damage and wear that exceed some limits must be repaired, for example:

- a bottom side rail dented more than 40 mm,
- a corner post dented more than 20 mm,
- the outboard corrugation of a side panel dented more than 25 mm,
- a top side rail holed,
- a door panel holed,
- a roof panel deformed by more than 25 mm into the interior,
- splintered boards on the floor,
- the painted surface burned, etc.

However, a series of small amounts of damage that do not require repair on their own may accumulate enough to require repair. Moreover, a damage that initially does not need repair may eventually lead to the development of repairworthy defects due to wear. In addition to damage and wear, inspectors should also inspect previously performed repairs. Non-conforming repairs can be considered unacceptable, requiring correction, or acceptable, depending on the condition observed.

18.5 Final remarks

The complexity of the distribution system of fresh horticultural commodities today is very great and with the complexity, the contamination risk is increased dramatically. Handling, storage and transport represent key steps in the distribution of these products. Thus professionalism and honesty (ethical behaviour in general terms) are required of the operators in the chain. Too many accidentally contaminated foods are marketed, and for

Table 18.5 Types of damage and wear adapted from *Guide for Container Equipment Inspection* (IICL, 1989)

Types of damage	Types of wear
• Bends • Bowing • Breaks • Burns • Cargo debris or dunnage accumulation inside the container • Compression line dented • Contamination due to infestation, stains or objectionable odour • Corrosion or other defect due to contact with foreign substances • Cracking • Cuts or tears • Dents • Dimension beyond ISO and IICL tolerances • Dirtiness requiring sweeping or cleaning • Failure in door operation due to racking • Floor warping, expansion or delamination due to excess moisture • Holes or pin-holes • Loss of removable components • Miscellaneous labels, port stickers, graffiti or other markings not appearing on the container when originally received and requiring removal • Scratches or gouges • Vandalism-related defects	• Corrosion of metal components not due to contact with foreign substances • Delamination or rot of wooden components such as general deterioration of floors including expansion, shrinkage or warping arising from normal use • Colour fading or adhesion failure of decals • Loose or missing parts of markings (except those that are normally removable), in the absence of evidence of accompanying damage • General paint failure or fading not due to contamination • General wear and deterioration at corner fittings • General deterioration at door gaskets and fittings, including loose and corroded fittings or loose fittings arising from normal deterioration of doors

every 100 food items that are identified and removed from the market by the legal authorities, probably there are ten times more contaminated products which remain on the market commonly; this is an underestimation. In today's market, with food and non-food items being transported all over the world and originating in developed and undeveloped countries, control of every load is almost impossible. This is due to sampling limits, insufficient numbers of public authorities for food control, insufficient numbers of specialised laboratories for controlling food items, inadequate (slow) analytical (chemical and microbiological) tests and insufficient public investment to enforce all the necessary control systems at the borders and to provide suitable numbers of food inspectors, chemical analysts, analytical equipment and laboratories. Traceability is the most recent keyword attributed to the system and a continuous update of quality certification is required along the distribution chain to the production companies. There is no way

to solve the problems mentioned above and all the quality certification programmes and traceability codes, which are required of the companies, represent higher costs but do not guarantee food safety. Extreme competition in the market and the oligopoly of the distribution chains controls the sale price and the only possibility for growers to make profit is to invest in innovative technology and marketing or to reduce the costs. In this economic context, investment in innovative technology, such as non-destructive equipment for quality measurements, requires the assumption of a high risk for ROI (return on investment) and a strong marketing programme, which only big cooperatives or large private companies can afford; the alternative is to reduce production costs by decreasing labour costs (the black economy, which today means illegal immigrants) or moving production to places where workers have less legal protection, taxes are not paid, or illegal, lower cost materials are used, for example Sudan colorant for tomato paste or paprika. Ethical behaviour and profit are usually in inverse proportion to one another.

For raw fruits and vegetables, handling, storage and the method of shipping, the CCP is always considered in the quality certification of the products from the field; this means the pesticide residues risk. As we showed, today, more critical points must be identified because contamination can occur anywhere. *E. coli*, *Salmonella*, *Listeria* and mycotoxins can be found in these products even in unlikely situations (heat-treated fresh mangoes). The following is a list of the main points which must be controlled accurately to maintain safety in the system:

- quality of water in any situation;
- handling equipment (cleaning and disinfection) especially brushes because they can produce surface injuries and contaminate the products;
- handling area: a slight overpressure must always be maintained in the working area;
- storage facilities: beyond the cleaning and disinfection of the walls, ceiling and floor, special attention must be devoted to cleaning coils and the load frame;
- shipping: beyond the cleaning and disinfection of the means of transport (trailer, decks, wagon) special attention must be paid to the protection of the product in the loading and unloading areas (port, airport, truck and wagon loading and unloading areas)
- package materials: cardboard and wood can be easily contaminated by pesticide residues and especially when wet, from microorganisms; jute sacs are even worse;
- retail: hypermarkets can represent a contamination source for raw fruits and vegetables because of the presence, in the same environment, of other raw foods such as meat and fish and non-food items; detailed attention to the architecture of ceiling and walls is required.

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19

Combined preservation techniques for fresh fruit

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19.1 Introduction

The increasing popularity of minimally processed fruit and vegetables has been attributed to the health benefits associated with fresh produce, combined with the ongoing consumer trend toward convenience of distribution and preparation, high quality and safety standards, and variety of choice. To meet these expectations, the food industry and its researchers have developed a range of strategies and minimal processing techniques that allow better retention of product flavour, texture, colour and nutrient content than comparable conventional treatments. The widely accepted concept of minimally processed fruit and vegetables involves the idea of living respiring tissues. But since the mid-1990s, this concept has evolved, giving a wider approach than those terms used earlier by Rolle and Chism (1987), Shewfelt (1987), Huxsoll and Bolin (1989), Wiley (1994), Ohlsson (1994, 1996) and Welti-Chanes (1997). According to Manvell (1997), a minimal process is 'the least possible treatment' to achieve a purpose that allows food to be safely distributed under specified storage conditions. In the same vein, Snyder (2003) considered that minimally processed foods are foods in which the biological, chemical and physical hazards are at a tolerable level. He divided them into three groups: (1) those that need no intervention cooking (i.e. raw foods that need refrigeration to minimise microbial damage or can be frozen to extend shelf-life); (2) foods with a mild disinfection (i.e. fresh cut fruit and vegetables, berries, etc., that

require washing with or without chemicals and/or blanching); and (3) foods that are formulated to be safe by pasteurisation, fermentation, drying, salting or acidification.

The expansion of the minimal processing concept has been reflected in new, renewed and improved products and processes formulated and designed to produce a greater diversity of minimally processed foods (Ahvenainen *et al*., 1994; Ahvenainen 1996, 2002; Singh and Oliveira, 1994; Wiley, 1994; Oliveira and Oliveira 1999; Alzamora *et al*., 2000a; Ohlsson and Bengtsson, 2002). Three major initiatives are currently being proposed by the research community and the industry to make minimally processed foods with improved quality (Alzamora *et al*., 1998):

1 Optimisation of traditional preservation methods to enhance sensorial, nutritional and microbiological quality of foods, yield and energy efficiency (e.g. aseptic packaging of thermally processed foods, semi-aseptic processes, ohmic heating, radiofrequency heating, microwave heating, inductive electrical heating, infrared heating, microfiltration, osmotic dehydration, vacuum dehydration, microwave and dielectric drying, cryogenic freezing, freezing in a dynamic dispersion medium, etc).
2 Development of mild processes by novel combinations of traditional physical and chemical preserving factors, each one applied at low intensity, to obtain products with quality attributes reminiscent of the fresh or native state of a given food but with longer shelf-life (e.g. *sous vide* and cook–chilled processing, modified/controlled atmosphere packaging, active packaging techniques, 'ready-to-eat foods').
3 Development of new techniques to obtain novel foods with fresh quality attributes by using combinations of emerging preservation factors or combinations of emerging factors with traditional ones, all of them applied at low doses (e.g. high hydrostatic pressure, ionising radiation, high electric field pulses, ultraviolet light, light pulses, natural antimicrobials, ultrasound, biopreservation).

In particular, maintenance of fresh-like attributes, microbial stability and sensory quality of most foods is nowadays mainly based on a combination of sub-lethal intrinsic, extrinsic and implicit inhibiting factors for the control of undesirable organisms in foods. Multitarget technologies have proved useful in the optimisation of traditional foods and development of novel products, as well as providing tools to increase shelf-life in the store and at home.

Water activity (a_w) continues to be one of the main factors to be manipulated. The use of combinations of extrinsic and intrinsic preservation factors, together with lowered a_w levels, are common in the food industry to control the growth and to limit the survival of both spoilage and pathogenic organisms. Generally, as the minimal a_w for growth of a microorganism is approached, changes in other environmental factors will have a greater impact on death or survival.

This contribution considers the use of water activity in combination with temperature (high or low) and chemical treatments (synthetic and/or natural antimicrobials) in the context of minimal preservation treatments to maintain the quality and safety of fresh-cut fruits.

19.2 Water activity: microbial growth, death and survival

Water activity is a major factor in preventing or limiting microbial growth and for this reason has been extensively studied by food microbiologists. The response of microorganisms to lowered water activity is essentially a response to osmotic stress, and is therefore often referred to as 'osmoregulation' or 'osmoadaptation' (Gould, 2000). The control of water content is essential for all types of cells. Growth restriction caused by hyperosmolarity is a common situation found by microorganisms in nature. To cope with the deleterious effects of this stress, cells have evolved very sophisticated and rapid molecular responses to repair the damage and protect against further exposure to high osmolarity and other forms of stress (Leitsner and Gould, 2002; Gould, 1996).

Although the specific details of how each organism responds to a hyperosmotic shock are different, several common features, both physiological and genetic, have arisen (O'Byrne and Booth, 2002). For instance, bacterial response to hyperosmolarity encompasses two aspects. The first one (the most readily observable) is specific, permitting survival in hypertonic environments, and mainly concerns the ability of bacteria to accumulate osmoprotective compounds for turgor and growth restoration. The second one concerns the osmotic induction of general stress systems, with the consequent development of multitolerances towards other environmental stresses when subjected to hypertonic environments (Pichereau *et al.*, 2000; O'Byrne and Booth, 2002). The internal osmotic pressure of growing microbial cells is higher than that of the surrounding medium to ensure that the direction of water flow is into the cell. As a consequence, the high turgor pressure exerted outwards on the wall provides the mechanical force necessary for expansion of the cell and growth (Gutierrez *et al.*, 1995; O'Byrne and Booth, 2002). When the organism is put into an environment with higher solute concentrations (i.e. lower a_w) than are found in the cytoplasm, water is extracted from the cytoplasm of the cell (in a passive way or possibly mediated by water channels). Plasmolysis is observed and membrane turgor is lost. The homeostasis (or internal equilibrium) is disturbed and the organism will not multiply but will remain in the lag-phase until the equilibrium is re-established.

A universal and major response of cells to reduced a_w is the accumulation of low molecular weight solutes in their cytoplasm at concentrations sufficient just to exceed the osmolarity of the external medium. In this way the cells regain, or avoid loss of, water by osmosis, and restore the turgor

that is essential for proper functioning and growing. The general reaction therefore appears to be a homeostatic mechanism with respect to cell water content (Gould, 1989).

Compatible solutes (so called because, even at very high relative concentrations, they do not appreciably interfere with the metabolic and reproductive functions of the cell) are generally non-ionic solutes, since many enzymes will start to lose activity in the presence of a high salt concentration (Gutierrez *et al*., 1995). While amino acids (proline, α-keto glutarate, γ-amino butyric acid, glutamic acid), quaternary amines (betaine, carnitine) and sugars (trehalose) appear to be the most common compatible solutes in bacteria, polyols of various types (mannitol, cyclohexanetetrol, arabitol, sorbitol, glycerol, erythritol, etc.) are the predominant protoplasmic solutes in many fungi (Troller, 1987). These compatible solutes have the following common properties (Gutierrez *et al*., 1995): (a) they are soluble to high concentration and can be accumulated to very high levels in the cytoplasm of the cells; (b) they do not modify enzyme activity and can even protect enzymes from denaturation by salts; (c) they are small and usually neutral or zwitterionic molecules; (d) the cell membrane exhibits controlled permeability to them.

Depending on the nature of the growing media, compatible solutes can be either transported from the environment or synthesised *de novo* in the cytoplasm. Some solutes are only available from the environment (e.g. choline, betaine and ectoine); others can be either synthesised or transported (e.g. proline) whilst others are only available by synthesis (e.g. trehalose). Accordingly, the availability of these compounds in the environment can influence the growth rate of organisms under conditions of hyperosmotic stress. In particular, many foods contain a wide range of substances that will act as compatible solutes or are their precursors (e.g. glycine, betaine, carnitine and proline in plant materials and various types of meat; taurine in fish and crabs, etc.) and thereby facilitate growth at lowered a_w, increasing the limit of tolerance to hyperosmolarity of the organism.

The pool of accumulated solutes is also influenced by the degree of osmotic stress. Salts (usually potassium glutamate) are accumulated at low osmolarity of the environment while, as the osmolarity increases, the initial response is also the accumulation of glutamate. But this accumulation is only transient and the cell then initiates the accumulation of other compatible solutes since high concentrations of potassium glutamate or other salts are inimical to enzyme activity. As stated by O'Byrne and Booth (2002), despite the fact that organisms differ widely in the range of osmolarity over which they will grow and the compatible solutes that they accumulate, osmoregulation involves control over the influx and efflux of solutes from the cell and water transfer (usually passive) across the membrane.

The genetic basis of osmoregulation has been a major topic in basic research (Abee and Wouters, 1999; Gould, 2000; Estruch, 2000; O'Byrne and

Booth, 2002; Pichereau *et al.*, 2000). Adaptive strategies involve the osmotic regulation of the expression of a number of genes to optimise growth under stress conditions, allowing cells to modulate the synthesis or uptake of compatible solutes. Many of these genes are under the control of alternative stress and stationary phase global sigma factors, σ^S in the Gram-negative and σ^B in the Gram-positive species (Pichereau *et al.*, 2000). For example, the growth of *Escherichia coli* at high osmolarity in the absence of other compatible solutes from the growth medium occurs by accumulation via its synthesis of trehalose. Trehalose synthetic enzymes are under the control of the σ^S subunit of RNA polymerase (RpoS), which accumulates when cells are growing at high osmolarity and other diverse environmental stresses (O'Byrne and Booth, 2002). Moreover, in *E. coli* the σ^S regulon includes over 50 different genes and the products of these genes confer resistance to a wide range of stress conditions, such as osmotic stress, oxidative stress, starvation and low pH stress.

The analysis of gene expression under stress conditions in *Saccharomyces cerevisiae* also reveals that a large number of proteins are induced by one or several types of stress. Some protein functions lead to protection of cell structures, repair of damaged components or counteract cell dehydration, while others are metabolic enzymes, indicating that a re-organisation of metabolic fluxes is required to ensure survival and adaptation to the stress conditions (Estruch, 2000). Thus, more than 1300 genes involved in very different biochemical processes are induced after a short exposure of *S. cerevisiae* to 0.4M NaCl. As presently known, there is a general response mechanism (the so-called global response) underlying many of the apparent distinct responses of microorganisms to different stresses (e.g. low a_w, low pH, low or high temperature, oxidative stress, starvation, etc.) and mediated by the stationary-phase regulator RpoS, which regulates the expression of many important stationary-phase stress resistance genes linked to survival under starvation conditions in the stationary phase. This fact would explain the cross-resistances to non-homologous stresses that have usually been found to occur in response to a single sub-lethal stress (Gould, 2000).

The ability of a microorganism to grow and survive depends on its capacity to adapt to changing environments. Osmoregulation capacity, and so the a_w limits for growth, differs between microorganisms. The optimum a_w for growth of the majority of microorganisms is in the range 0.99–0.98. Every microorganism has limiting a_w values below which it will not grow, form spores or produce toxic metabolites (Beuchat, 1987). Considering a_w in relation to microbial stability, the minimum a_w values that permit microbial growth for different types of microorganisms are important. Extensive tables with minimum a_w values for growth and toxin production of several pathogenic and spoilage microorganisms have been reported by many authors (Corry, 1973; Beuchat, 1983, 1987; Gould, 1989).

Table 19.1 summarises minimal a_w values for growth and production of toxins, mostly determined in laboratory media, for some microorganisms at

Table 19.1 Minimal water activity for growth of selected microorganisms under optimum pH, temperature and nutrient availability (compiled from various sources by Alzamora *et al*., 2003)

	a_w
Infectious pathogens	
Campylobacter jejuni	0.99
Aeromonas hydrophila	0.97
Shigella spp	0.96
Salmonella spp	0.94
Yersinia enterocolitica	0.95
Escherichia coli	0.93–0.95
Listeria monocytogenes	0.90–0.92
Vibrio parahaemolyticus	0.94 (glycerol) 0.95 (NaCl) 0.96 (sucrose)
Toxinogenic spore-forming pathogens	
Clostridium perfringens	0.93–0.95
Clostridium botulinum A & proteolytic B strains	0.94 (TP: 0.94)
C. botulinum E & non-proteolytic strains B and F	0.96 (TP: 0.96)
C. botulinum G	0.96 (TP: 0.96)
Bacillus cereus	0.90 (glycerol) 0.94 (NaCl)
Toxinogenic pathogens	
Staphylococcus aureus (anaerobic)	0.91
S. aureus (aerobic)	0.86
S. aureus (aerobic)	0.93 (xylitol)
S. aureus (aerobic)	0.95 (erythritol)
S. aureus (aerobic)	0.89 (glycerol)
Moulds and yeasts	
Aspergillus flavus	0.80 (TP: 0.83–0.87)
Aspergillus parasiticus	0.82 (TP: 0.87)
Botrytis cinerea	0.93
Byssoclamys nivea	0.84
Aspergillus ochraceus	0.77 (TP: 0.80–0.88)
Penicillium citrinum	0.80
Penicillium cyclopium	0.81
Penicillium patulum	0.81 (TP: 0.85–0.95)
Eurotium spp	0.66–0.73
Monascus bisporus	0.61
Saccharomyces cerevisiae	0.89 (glucose) 0.90 (sucrose) 0.92 (NaCl)
Zygosaccharomyces bisporus	0.70
Zygosaccharomyces rouxii	0.65
Torulopsis candida	0.65

TP = toxin production.

their optimal conditions of pH, nutrient availability and temperature. It can be observed that a_w limits for growth differ between microorganisms. In general, common spoilage bacteria are inhibited at a_w about 0.97, clostridial pathogen at a_w 0.94 and most *Bacillus* species at a_w 0.93. *Staphylococcus aureus* is the most a_w-tolerant pathogen, and can grow in aerobiosis at a_w 0.86 and in anaerobiosis at a_w 0.91. Many yeasts and moulds are able to proliferate at a_w values below 0.86, with some osmophilic yeasts and xerophilic moulds capable of slow growth just above 0.6. So, to preserve a food by using only a reduction in a_w as a stress factor, its a_w should be at least lowered to 0.6. More also can be observed, minimum a_w for growth is always equal to or lower than a minimum a_w for toxin production.

The a_w of the medium is not the only determining factor regulating the biological response, but the nature of the a_w-controlling solute also plays a role (Christian, 1981; Ballesteros *et al.*, 1993). Gould (1989) recognised that in some instances solute effects may depend on the ability of the solute to permeate the cell membrane, as in the case of glycerol, which readily permeates the membrane of many bacteria and therefore has a lower inhibitory water activity (an exception is *S. aureus*, an important foodborne pathogen, for which the reverse is true). Chirife (1994) discussed in detail the 'specific solute effect' for *S. aureus*. He concluded that the inhibitory effects of solutes most often present in low a_w-preserved foods, such as NaCl and sucrose, are primarily related to their a_w lowering capacity. But for other solutes such as ethanol, propylene glycol, butylene glycol and various polyethylene glycols, antibacterial effects (attributed mainly to the effects of these molecules on the membrane enzymes responsible of peptidoglycan synthesis) are important.

19.3 Combinations of water activity reduction with other preservation factors

If fresh-like fruit is the goal, reduction of a_w by addition of humectants should be employed at a minimum level to maintain the product in a high moisture state. On the other hand, high-moisture foods have a_w values well above 0.90. Thus, in this category, the reduction of a_w is a hurdle of less relative significance because most microorganisms are able to proliferate (Leitsner and Gould, 2002). To compensate for the high moisture left in the product (in terms of stability), other preservation factors placed in context with the hurdle technology principles, make up an interesting alternative for decontamination of fruits.

Homeostasis or internal media stability (composition and volume of fluids) is vital for survival and growth of microorganisms. Preservation procedures are effective when they overcome, temporally or permanently, the various homeostatic reactions that microorganisms have evolved in order

to resist stresses (Gould and Jones, 1989). Homeostatic mechanisms that vegetative cells have evolved in order to survive extreme environmental stresses are energy dependent and allow microorganisms to keep functioning. In contrast, homeostasis in spores is passive, acting to keep the central protoplast in a constant low-water level environment, this being the prime reason for the extreme metabolic inertness or dormancy and resistance of these cells. It seems likely that the structural arrangement of the cortex of the spore is responsible for the dehydration of the protoplast (Gould, 1977; Gould *et al*., 1983).

In foods preserved by combined methods (so called hurdle technology or multitarget preservation), the active homeostasis of vegetative microorganisms and the passive refractory homeostasis of spores are disturbed by a combination of gentle antimicrobial factors at a number of sites or in a cooperative manner (Leitsner, 1995a,b). For vegetative cells (where homeostasis is energy dependent), the goal is to reduce the availability of energy (removing O_2, limiting nutrients and reducing the temperature) and/or to increase the demand for energy (reducing water activity, reducing pH and adding membrane active compounds). Placing a number of sub-lethal stresses (i.e. preserving factors) on a microbial cell potentially results in metabolic exhaustion and death. For spores (where homeostasis is non-energetic and depends on the structures of the organism), the goal is to damage key structures or to release spores from dormancy. Antimicrobial preservation of foods by combined methods should be considered not only as interference in the homeostasis by additive or synergistic hurdles on the same microorganism, but also as the selective application of preservation factors that may be effective against a specific organism or group of organisms, while not against others (Leitsner, 1995b).

Different approaches have been explored for obtaining shelf-stability and fresh-likeness in fruit products. Commercial, minimally processed fruits are fresh (with high moisture) and are prepared for convenient consumption and distribution to the consumer to sustain freshness. Minimum processing includes minimum preparation procedures like washing (conventional or with sanitising agents), peeling and/or cutting, packing, and so on, after which the fruit product is usually placed in refrigerated storage where its stability varies depending on the type of product, processing and storage conditions. The fruit product has a very short life, as short as 1–7 days at chilled temperatures (Ahvenainen, 2002). However, product stability without refrigeration and/or greater shelf-lives are important issues, not only in developing countries but in industrialised countries as well. Many simple and inexpensive processing methods based on the rational combination of hurdles can be used to obtain fresh-like cut fruits with high moisture but with greater shelf-lives and/or stored at room temperature.

Selected technologies based on a combination of a_w reduction with other inhibiting and inactivating factors to combat the deleterious effects of

microorganisms in fruits, including additional factors to diminish major quality loss, will be considered next.

Since the mid-1980s, use of this approach led to important developments of innovative technologies for obtaining shelf-stable 'high-moisture fruit products' storable for 3–8 months without refrigeration. To compensate for the high moisture left in the product (in terms of stability), a controlled blanching and/or a mild heat treatment can be applied without affecting the sensory and nutritional properties; pH reductions can be made that will not impair flavour; and preservatives can be added to alleviate the risk of potential spoilage microflora. In conjunction with the above mentioned factors, a slight reduction of a_w (a_w 0.94–0.98, usually adjusted with glucose, sucrose, fructose, maltodextrins, corn syrups and/or some polyols), control of pH (pH 3.0–4.1, usually adjusted with citric or phosphoric acid), addition of preservatives (in doses legally approved and/or sensorily compatible, usually weak acids) and antibrowning additives were the factors selected to formulate the preservation procedure (Alzamora *et al*., 1989, 1993, 1995; Guerrero *et al*., 1994; Cerrutti *et al*., 1997; Argaiz *et al*., 1995; Tapia de Daza *et al*., 1995).

Preliminary operations consist of selection, washing, peeling and slicing (some fruits may be preserved whole) of the fruit. After washing again, the process involves fruit blanching followed by a step of a_w depression (where the fruit loses water and gains sugar or other humectants) with simultaneous incorporation of additives, achieving final values after equilibration of a_w = 0.94–0.98, pH = 3.0–4.1, 400–1000 ppm potassium sorbate or sodium benzoate and generally 150 ppm sodium bisulphite. The dewatering and impregnation process is performed at room temperature by placing the fruit in concentrated aqueous solutions of sugar or other humectants and additives (moist infusion) or by mixing the fruit, sugar and additives in the required proportions (dry infusion).

After equilibration (between a few hours and 7 days according to the size of the whole or sliced fruit and/or the agitation of the medium), fruit slices prepared by moist infusion are drained and packaged, not necessarily hermetically, leaving only enough syrup to cover them. Fruit slices (and the corresponding own juice) prepared by dry infusion are directly packaged. The processing of some fruits (i.e. banana and pomalaca) included a slight thermal treatment after packing or a hot filling stage.

The fruit can be packaged into glass or high density polyethylene jars, in tanks or in flexible high-density polyethylene bags and can be held at room temperature during storage. For developing countries, where refrigeration is costly and not continuously available, these techniques for obtaining minimally processed fruits represent an alternative of special interest (Leitsner, 1995b). This process has been successfully applied to whole peeled fruits and pieces of pineapple, mango, fig, plum, strawberry, chicozapote, passion fruit, papaya, tamarind, banana, peach and pomalaca (Alzamora *et al*., 1995).

Moist infusion procedures, however, in contrast to dry infusion, result in residual diluted syrup fairly high in sugar and additives which, if not effectively recycled, increase the cost and provoke serious effluent problems (Leitsner, 1995b). Argaiz *et al.* (1995) have found that the syrup can be re-used five times (after restoring it to the initial conditions by new additions of sugar and additives) without affecting the microbial and sensory quality of fruit products. Leitsner (1995b) stated that the re-use of the syrup may become a risk in relation to some preservative-resistant microorganisms, and recommended, before the re-use, the pasteurisation of the syrup.

It is interesting to analyse the role of each hurdle in the combined technique. Blanching in saturated vapour is a critical operation in the decontamination of fruits. Although its primary objective is the inactivation of enzymes, heating during vapour blanching inactivates yeasts, most moulds and aerobic natural flora and sensitises remaining microorganisms to other hurdles. Reductions in the microbial load from 60–99% have been reported after blanching of papaya, mango, pineapple and strawberry (Alzamora *et al.*, 1995; Tapia de Daza *et al.*, 1995).

The hurdle a_w was selected to be in the range 0.93–0.98, satisfying an emergent interest for 'fresh-like' characteristics and low sugar food. The value of pH was maintained equal to or near the pH value of fresh fruit. In those fruits with a higher pH, it was adjusted to the lowest value sensorily compatible with the natural flavour of the fruit. Foods with high a_w are susceptible to the growth of bacteria, moulds and yeasts. But high acidity provides an unsuitable environment for the growth of most bacteria. So the low pH establishes a potential type of spoilage comprised of yeasts, moulds and acid-tolerant bacteria. The minimum growth limits for a_w and external pH for the main bacteria capable of growing in fruit products are presented in Table 19.2. Considering that a slight reduction of pH increases the lower limit of a_w for bacterial growth and, vice versa, a slight reduction of a_w diminishes the range of pH that permits growth; it is expected that interaction of pH with a_w in these ranges will be enough to suppress the growth of most bacteria of concern in fruit preservation. The ability of fungi (moulds and yeasts) to tolerate reduced a_w and pH, in contrast, demands the incorporation of antifungal (e.g. sorbic or benzoic acid) in moderate amounts (400–1000 ppm potassium sorbate or sodium benzoate).

Sulphiting agents were used in very low concentration (usually 150 ppm sodium bisulphite), when necessary, to inhibit or delay non-enzymatic browning reactions. They also act as antifungal compounds, especially against sorbate-tolerant yeasts (Tapia de Daza *et al.*, 1995).

The major goal for the design of these combined techniques was the development of simple and inexpensive techniques for bulk storage without refrigeration, which are energy efficient and suitable for preserving fruits *in situ*, which help in overcoming seasonal production constraints and reduce post-harvest losses.

Table 19.2 Minimum a_w and minimum pH for growth of certain bacteria in fruit products (with optimum values of other growth factors) (compiled from various sources by Alzamora *et al*., 1995)

Microorganism	a_w	pH
Clostridium botulinum	≥0.945–<0.965 (glucose) ≥0.935–<0.950 (glycerol)	>4.8
Clostridium pasteurianum	0.985	3.5–4.5
Bacillus coagulans	0.94 (glucose or sucrose)	3.8–4.8
Bacillus licheniformis	>0.89–<0.91 (NaCl or glucose)	4.2–4.4
Bacillus stearothermophilus	>0.97 (NaCl or glucose)	>5.0–<6.0
Lactobacillus species	>0.94 (glycerol)	3.8–4.4
Lactobacillus plantarum	0.94	
Leuconostoc mesenteroides	0.94 (NaCl)	
Streptococcus faecalis	0.94 (NaCl)	4.4–4.7
Salmonella species	0.95	3.7–4.5
Salmonella oranienberg	0.95 (NaCl) 0.935 (glycerol)	

Table 19.3 compares some characteristics and processes corresponding to minimally processed refrigerated fruits, high-moisture fruit products (obtained by these combined techniques) and intermediate moisture fruits. The parameters contrasted in this table define striking differences in the characteristics of final products (freshness), processes, shelf-life, packaging and storage conditions and, it can be inferred, in production and retail costs, too.

To optimise the stabilisation of fruits at high moisture contents by combined methods, the response to the stress factors of microorganisms was addressed using different approaches: studies in laboratory media, studies of evolution of native flora in fruit products and challenge tests of fruit products with the microorganisms of concern. Two examples are presented next.

The microbial stability of banana purées (against native and inoculated flora) with diverse treatments (a_w 0.97 adjusted with glucose, 400 ppm $NaHSO_4$, 250 ppm ascorbic acid, 100 ppm potassium sorbate, mild thermal treatment after packaging) was analysed by Guerrero *et al*. (1994) in order to evaluate the effectiveness of different hurdles. Figure 19.1(a) shows the changes in the aerobic mesophilic plate count during incubation at 25 °C of banana purées treated with different combinations of the above-mentioned hurdles. Only the combination of all hurdles proposed resulted in growth inhibition. It is interesting to note that aerobic mesophilic microorganisms seemed to be more sensitive to heat in banana with additives and pH 3.4 than in 'natural' banana, since initial counts for A and B systems were at least two orders lower than for D system. Inoculated flora comprised

Table 19.3 Comparison of three fruit preservation systems in reference to some characteristics of final products and processes (adapted from various sources by Tapia de Daza *et al*., 1996)

Fruit process/ technology	a_w	Overall quality	Shelf stability	Preservatives added	Process and preservation operations	Blanching	Packaging
IMF	0.75–0.092	Slightly modified to modified	Usually shelf-stable at room temperature	Sulphites, sorbic acid, benzoic acid, citric acid, ascorbic acid	Peeling, coring, slicing, dipping in preservative solutions, dehydration	Generally required	Required
MPRF	0.97–0.99	Fresh-like	Refrigeration temperatures required	Might include some (i.e. ascorbic acid)	Peeling, coring, slicing, dipping in preservative solutions	May be used (excluded in most descriptions)	Required MAP/CAP can be used
HMFP	0.93–0.98	Fresh-like to slightly modified	Shelf-stable at room temperature	Sulphites, sorbic acid, benzoic acid, citric acid, ascorbic acid	Peeling, coring, slicing, dipping in preservative solutions	Generally applied	Required

IMF: intermediate-moisture fruits; MPRF: minimally processed refrigerated fruits; HMFP: high-moisture fruit products; CAP: controlled atmosphere packaging; MAP: modified atmosphere packaging.

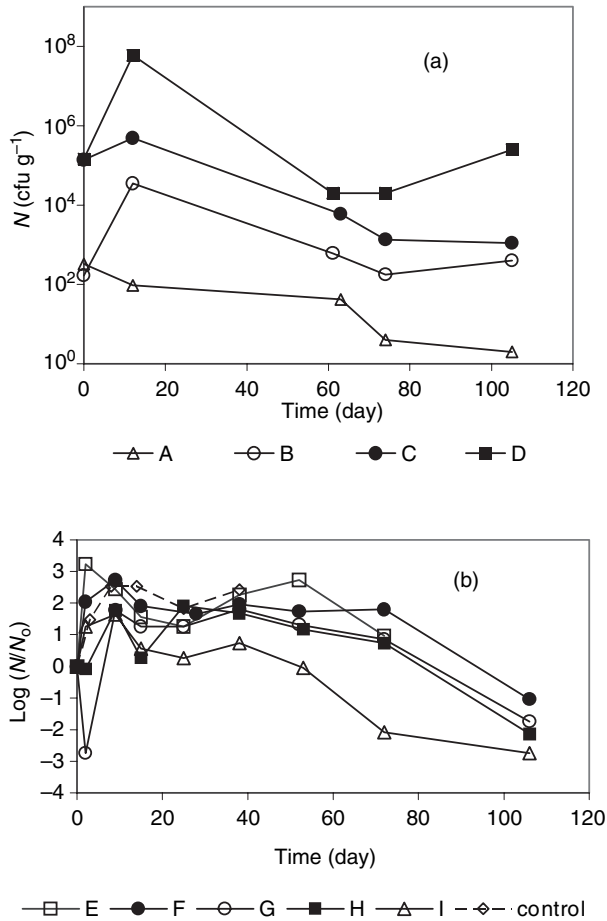

Fig. 19.1 (a) Evolution of aerobic mesophilic plate count of banana purées adjusted to selected combinations of hurdles during incubation at 25 °C (N = microbial concentration). A, thermal treatment; KS = 100 ppm; $NaHSO_4$ = 400 ppm; AA = 250 ppm; pH = 3.4; a_w = 0.97. B, thermal treatment; KS = 100 ppm; $NaHSO_4$ = 400 ppm; AA = 250 ppm; pH = 3.4. C, KS = 100 ppm; $NaHSO_4$ = 400 ppm; AA = 250 ppm; pH = 3.4. D, thermal treatment. (b) Evolution of *B. coagulans* ATCC 8038 plate count of banana purées adjusted to selected combinations of hurdles (N_0 = initial microbial concentration). E, banana purée natural. F, pH = 3.4. G, pH = 3.4; KS = 100 ppm; $NaHSO_4$ = 400 ppm; AA = 250 ppm. H, pH = 3.4; a_w = 0.97. I, pH = 3.4; KS = 100 ppm; $NaHSO_4$ = 400 ppm; AA = 250 ppm; a_w = 0.97. Control: nutrient broth. (KS is potassium sorbate; AA is ascorbic acid.)

osmotolerant and non-osmotolerant yeasts (*Zygosaccharomyces rouxii* ATCC 8383; *Z. bailii* NRRL Y-1446, *Saccharomyces cerevisiae*), various moulds (*Aspergillus niger*, *Eurotium amstelodami*, *Paecylomyces varioti*), *Bacillus coagulans* Hammer ATCC6013, *Clostridium pasteurianum* and *C.*

butyricum. Control of pH alone led to the inhibition of *C. pasteurianum* and *C. butyricum* (data not shown) but only when all hurdles were combined, banana was refractory to growth of *B. coagulans* (Fig. 19.1(b)). This latter combination was lethal to the three yeasts, but in the absence of any of these factors, the yeasts grew as well as in fresh banana. No growth of moulds was detected in banana at pH 3.4 and with additives.

Z. bailii is a recognised problem in this type of high moisture fruit product, because of its tolerance of acidic conditions, a high osmotic pressure and of preservatives such as sorbate (Warth, 1986; Tapia de Daza *et al*., 1996). The combined effects of pH (4.0, 3.5 or 3.0, adjusted with citric acid), a_w (0.99, 0.98 or 0.97, adjusted with sucrose), incubation temperature (15 or 25 °C), potassium sorbate (KS; 0, 250, 500, 750, 1000, 1250 or 1500 ppm) and/or sodium benzoate (NaB; 0, 250, 500, 750, 1000, 1250 or 1500 ppm) on *Z. bailii* inhibition were evaluated in a model broth system (Palou *et al*., 2004). Individual preservative minimal inhibitory concentrations (MICs) decreased as pH and incubation temperature decreased and were lower for KS. The smallest MICs were observed for a_w 0.97, at pH 3.0 and 15 °C, being 250 ppm for KS and 750 ppm for NaB. Figure 19.2 presents the MIC of NaB for the conditions studied. Minimal lethal concentrations (MLCs) followed the same pattern but with higher concentrations. Most of the preservative combinations assayed were additive or antagonic, depending on a_w, pH and incubation temperature, and therefore these combinations were not recommended for use in fruit systems under the same pH and a_w conditions.

One of the process aspects that has recently received consideration in order to improve these techniques was at the equilibration stage (Alzamora *et al*., 2000b). There are novel (in their application) and refined impregnation techniques for developing minimal processes. Pulsed vacuum osmotic dehydration, a new method of osmotic dehydration that takes advantage of the porous microstructure of vegetable tissues, uses vacuum impregnation (VI) to reduce process time and improve additives incorporation. In fact, the use of vacuum impregnation techniques by apple processors for firming the tissue and improving the quality of canned and frozen apple slices dates back to the 1950s.

Foods exhibiting a porous microstructure can be impregnated, that is, their pores can be filled with a suitable solution, introducing solvents and solutes of choice into the porous spaces that are occupied by a certain amount of occluded gas. The volume of this gas can be modified, substituting it for the impregnation solution as a result of capillary action or by the combined effect of capillary action and pressure gradients, which are imposed on the system (Fito and Pastor, 1994; Fito and Chiralt, 1995; Salvatori *et al*., 1998). The impregnation produced by pressure gradients, which act as driving forces, can be controlled by the expansion or compression of the occluded gas. A way to accomplish this is to apply a vacuum to the product for short periods (i.e. 5–10 min) while it is immersed in the liquid and then re-establish the atmospheric pressure. These alternating

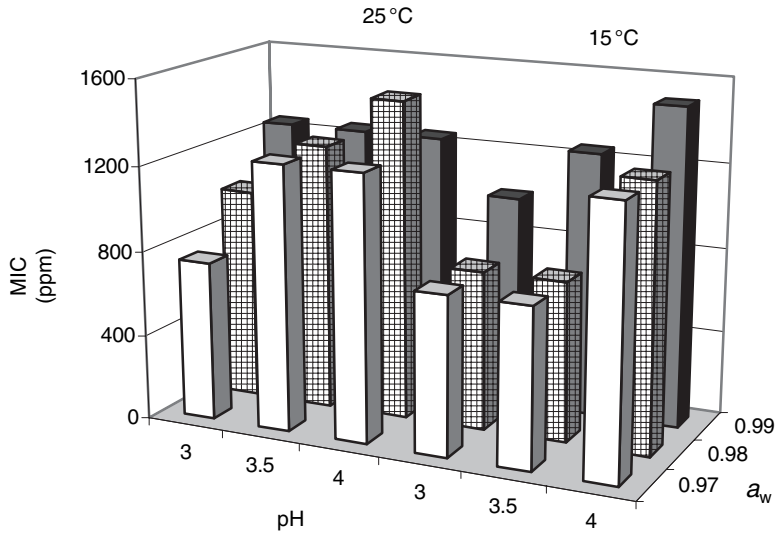

Fig. 19.2 Sodium benzoate minimal inhibitory concentrations (MIC) to inhibit *Zygosaccharomyces bailii* during 30 days growth in laboratory media at selected pH, water activity and incubation temperatures.

pressures cause the gas to be expelled from the pores and to be replaced by the entering liquid. Penetrating aqueous solutions may contain sugar, calcium salts, organic acids, pigments, flavours, sulphurous salts and a combination of them. A proper formulation of the impregnation solution allows expeditious compositional modifications of the solid matrix, which may result in quality and stability enhancement of final products, without submitting the food structure to the eventual stress caused by long exposure to gradient solute concentration. The final products generally exhibit structural, physical and chemical properties very different from those of atmospheric infused fruits.

An important advantage of using low pressures (approximately 50 mbar) in minimal preservation of fruit is that equilibration times are shorter than at atmospheric pressure (e.g. 15 min under vacuum compared with a few hours in forced convection under atmospheric conditions, or a few days in media without agitation to reduce a_w to 0.97 in apple slices or halves) (Alzamora *et al.*, 2000b). This process could be very appropriate in the development of new minimally processed products at high a_w (i.e. a_w 0.97) in the case of fruits with high porosity, or combined with osmotic dehydration at atmospheric pressure if a greater reduction of a_w is desired or for fruits of low porosity. Papaya, melon, orange segments, kiwi fruit and papaya impregnated with passion fruit juice have been successfully preserved at a_w 0.97–0.98 using vacuum impregnation. The high moisture fruits obtained exhibited different shelf-lives depending on pH, storage

temperature, antimicrobial level and type of packaging (Tapia *et al*., 1999; López Malo, 1999, Vergara-Balderas *et al*., 1998; Welti-Chanes *et al*., 1998, Leúnda *et al*., 2000).

The use of antimicrobials of natural origin as replacements (total or partial) for sorbates, benzoates and other synthetic additives, in order to meet consumers' concerns about chemicals, was another aspect considered to improve combined techniques (Alzamora and López-Malo, 2002). Cerrutti *et al*. (1997) evaluated the potential utilisation of vanillin (3000 pm) as an antimicrobial (instead of potassium sorbate and sodium bisulphite) in the formulation of a combined technique for obtaining minimally processed strawberry purée. Mild heat treatment, addition of 500 ppm ascorbic acid, reduction of a_w to 0.95 with sucrose and control of pH (≅3.0) were the other inherent hurdles that were combined to reach the desired microbiological stability of the fruit. The microbial stability of the purée was addressed by studying the evolution of native flora (aerobic and anaerobic mesophilic bacteria, yeasts and moulds) and by challenge testing with the microorganisms of concern (*S. cerevisiae*, *Z. rouxii*, *Schizosaccharomyces pombe*, *Pichia membranaefaciens*, *Botrytis* sp., *Byssochlamys fulva*, *Bacillus coagulans* and *Lactobacillus delbrueckii*).

The combination assayed prevented the growth of native and inoculated flora for at least 60 days storage at room temperature. Control of only pH and a_w inhibited growth of *B. coagulans* and *L. delbrueckii*, but vanillin addition exerted a bactericidal effect. Vanillin in the presence of the other hurdles affected the behaviour of the yeasts, although spice sensitivity was dependent on the species inoculated. *S. cerevisiae* was the most sensitive and counts decreased abruptly in the presence of vanillin. *P. membranaefaciens* was the most resistant, although growth was practically inhibited in the presence of the spice. The number of cells of the other yeasts gradually decreased during storage. It is noteworthy that vanillin appeared to be effective against *Z. bailii* and *P. membranaefaciens*, both well-known preservative-resistant yeasts (Pitt and Richardson, 1973). In addition, vanillin appeared to control the growth of *Z. rouxii*, the most common spoilage organism of osmotolerant yeasts.

Z. rouxii can spoil foods with high acidity, low redox potential and low to intermediate a_w. A combination of 100 ppm SO_2, 500 ppm potassium sorbate and pH < 4.0 has been suggested to prevent its growth in this type of minimally processed fruits owing to the high sensitivity of the yeast to SO_2 (Tapia de Daza *et al*., 1995). Vanillin could be a natural alternative for eliminating the use of sulphites in the control of this yeast. *B. fulva* (a species most commonly involved in strawberry disintegration) and *Botrytis* sp. showed no growth in the preserved purée during 60 days of storage. Although microbial behaviour was similar at room temperature or under refrigeration, colour was better preserved at temperatures ≤10 °C.

A combined procedure proposed by Castañón *et al*. (1999) to prepare minimally processed banana using vanillin as an antimicrobial agent is

described next. Bananas are washed, peeled, cut transversally into slices (approximately 1 cm thick) and blanched in saturated water vapour for 5 min. Immediately, the slices are sprinkled with a 1% w/v ascorbic acid solution (approximately 0.2 ml g^{-1} banana) and processed to obtain the purée. Sucrose and phosphoric acid (or citric acid) solution are added to reduce the initial a_w from 0.986 to 0.97 and to adjust the pH to 3.3, respectively. Then, 3000 ppm vanillin are added. The purée is placed in sterile polyethylene bags, sealed and stored. Native flora (standard plate, yeast and moulds) changes during storage at different temperatures (15, 25 and 35 °C) were evaluated by Castañón *et al.* (1999) in banana purée preserved using the technique just described above and also in purées preserved in the same way but with the addition of 1000 ppm vanillin or 1000 ppm potassium sorbate instead of 3000 ppm vanillin. The addition of 1000 ppm vanillin only increased the lag phase up to 16 days at 15 °C, and the time to detect the microbial spoilage was extended to around 21 days. In the presence of 3000 ppm vanillin or 1000 ppm potassium sorbate, after three days and up to 60 days of storage at any of the three temperatures studied, microbial growth (<10 cfu g^{-1}) was not detected. However, browning of the fruit was the factor that determined the shelf-life.

These findings show that addition of vanillin in combination with a slight reduction of a_w and regulation of pH may be a promising technique for natural fruit preservation. It is to be noted that although these experiments were made using purées to facilitate microbiological studies, these techniques can also be applied to whole or sliced fruits.

19.4 Strengths and weaknesses: future trends

Consumer tastes are changing and high quality foods with fresh-like attributes are being demanded. Processing at higher temperatures and/or reducing water activity in combination with additives may markedly enhance microbial and enzyme inactivation, but with resulting decreases in fresh quality and 'freshness' as compared with the raw or non-thermal processed fruits and vegetables.

Some of these limitations may be challenged by focusing on adequate preservation system design, product formulation and/or process conditions (i.e. new decontamination techniques for raw materials; employment of additional, emerging and traditional stress factors in combination to reduce the levels of humectants and antimicrobials; vacuum procedures to adjust a_w, etc.).

Combinations of preservative factors mentioned above were selected to assure the microbial stability and sensory quality of the products but, at the same time, minimal facilities, services and equipment available were also considered. The selected hurdles and their intensities, as well as the way in which the hurdles are applied, should not be regarded as inflexible, since

other combinations can be equally or more suitable for reaching microbial stability, or enhancing sensory acceptability, and/or increasing the shelf-life. However, the modification, elimination and/or reduction of any hurdle or the level of its presence must be carefully evaluated as well as the sequence of hurdle application (Argaiz *et al*., 1995; Alzamora *et al*., 1993). Some issues of concern in the design and optimisation of these combined techniques are summarised below.

19.4.1 Adaptation to stresses

Any food processing, decontamination or storage method may cause shock, adaptation, or shock/adaptation, and induce cross-protection against environmental-related stresses (Samelis and Sofos, 2003). Bacterial stress responses to a stressful food environment and during processing may have serious consequences in foods. The expression of induced tolerance depends on stress intensity and on the way stresses are applied (simultaneously, in series gradually over longer periods of time or in series over very short periods of time). Leitsner (2000) indicated that simultaneous exposure of microorganisms to different stress factors requires increased energy consumption and leads microorganisms to cellular death through metabolic exhaustion and disturbed homeostasis. On the other hand, stresses applied gradually permit microorganisms to adapt and may result in pathogenic survivors of increased resistance under unfavourable conditions. Samelis and Sofos (2003) also stated that stresses applied successively, depending on type, order of application, magnitude and duration, may allow survival and resistance.

Although pathogen challenge and validation studies have not been performed in the development of these combined techniques, their long-term effects on natural and inoculated flora addressed during long-term storage indicated that survivors after exposure to the hurdles assayed became energetically exhausted during storage to be able to survive. Counts of native and inoculated flora after stabilisation of pineapple, papaya, strawberry and mango have been reported to be very low or below the limit of detection (Alzamora *et al*., 1989, 1995; Tapia de Daza *et al*., 1995). Microbial load has also been found to decrease further during storage of stabilised product. This fact would indicate that, as blanching was followed by the other stresses (low a_w and pH, and preservatives) immediately afterwards, and these last were applied simultaneously, heat injury survivors may not have time or enough energy to survive and develop resistance. However, the potential exhausting effect of the combined techniques on bacterial pathogens requires experimental verification.

19.4.2 Interactions between stress factors

When two antimicrobials are combined, a greater antimicrobial action against microorganisms is *a priori* expected. But antimicrobial interactions

can be additive (the antimicrobial effectiveness is not reduced or enhanced in the presence of the second compound), synergistic (the antimicrobial activity for a compound is enhanced by the presence of a second one) or antagonistic (the antimicrobial effectiveness is reduced in the presence of the second compound) (Parish and Davidson, 1993). To analyse antimicrobial binary combinations, MIC data are usually transformed to fractional inhibitory concentrations (FIC), as defined by Davidson and Parish (1989):

FIC_A = MIC of A in presence of B/MIC of A

FIC_B = MIC of B in presence of A/MIC of B

The FIC Index was calculated as follows, using the FICs for individual antimicrobials: $FIC_{Index} = FIC_A + FIC_B$.

The characteristics of the interaction of synthetic and natural preservatives against various fungi were examined in model systems resembling the environmental factors typical of high-moisture fruit products. As an example, FIC isobolograms for combinations of vanillin and ethylenediamine tetra-acetic acid (EDTA), sodium bisulphite and potassium sorbate at a_w 0.99 or 0.95 (reduced with sucrose) and pH 4.5 or 3.5 (adjusted with hydrochloric acid) against *Z. bailii* are shown in Fig. 19.3 (Rivera Carriles, 2002). Data on the straight line connecting unity (FIC = 1) on the *x* and *y* axes indicate an additive effect; curves deviating to the left of the additive line indicate synergistic interactions and curves deviating to the right of the additive line indicate antagonistic interactions. In the same way, a FIC index near 1 implies additivity; <1 implies synergy; and >1 implies antagonism (Davidson and Parish, 1989). The effect of the binary combinations of these antimicrobials depended on a_w and pH. Vanillin acting in the presence of EDTA, potassium sorbate or sodium bisulphite exhibited antagonistic, synergistic or additive effects dependent not only on a_w and pH but also on the relative amount of each antimicrobial in the binary mixture.

In similar studies, combinations of vanillin and potassium sorbate (pH 3.5 or 4.5), citral and potassium sorbate (pH 3.5), sodium benzoate and vanillin (pH 3.5) and sodium benzoate and eugenol (pH 3.5) against *Aspergillus flavus* for different incubation times and a_w 0.99 were analysed (López-Malo, 2000). At pH 3.5, several combinations of vanillin and potassium sorbate were synergistic, while at pH 4.5 most of the combinations were antagonistic. Moreover, results indicate that, as incubation time increased, the synergistic characteristic of the interaction vanillin–potassium sorbate evolved towards an additive one. The same trend was observed for the initially synergistic potassium sorbate–citral interaction. Additive effects were found practically when eugenol and sodium benzoate were used in combination, while the interaction vanillin–sodium benzoate was antagonistic for most of the combinations assayed.

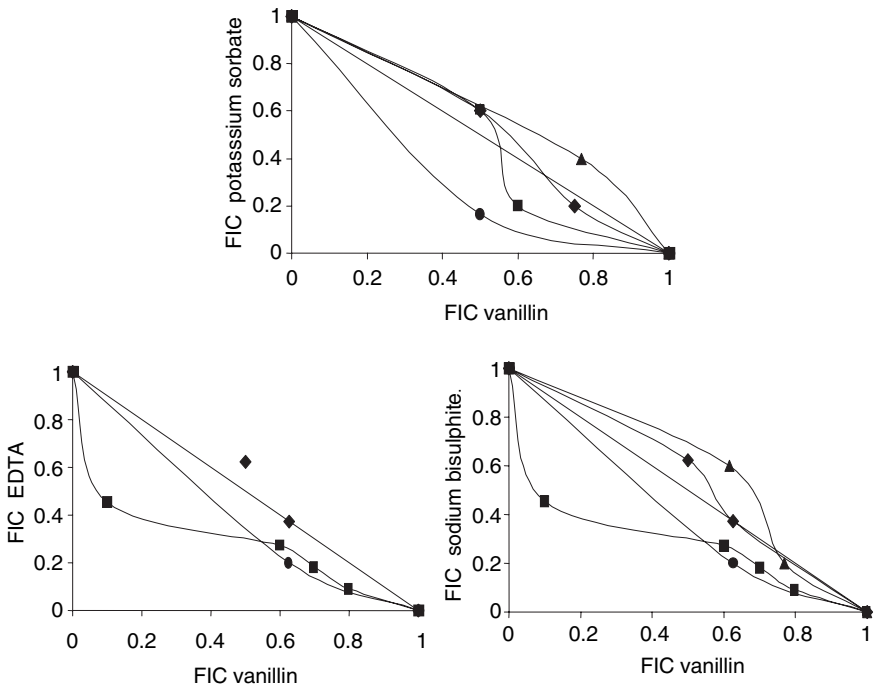

Fig. 19.3 FIC isobolograms for combinations of vanillin and EDTA, sodium bisulphite or potassium sorbate against *Zygosaccharomyces bailii* in potato dextrose agar. (-◆- a_w 0.99, pH = 3.5; -■- a_w 0.99, pH = 4.5; -●- a_w 0.95, pH = 4.5; -▲- a_w 0.95, pH = 3.5).

An examination of these developments makes one realise that, when considering binary mixtures of antimicrobials, it is not easy to anticipate the effects or to explain the observed activity. Moreover, there is an increasing awareness that many combinations may be antagonistic. However, many synergistic combinations previously mentioned could be useful to reduce the amounts of antimicrobial needed to inhibit growth, diminishing concerns about the use of chemical preservatives.

19.4.3 Stress factor stability and/or availability

Hurdles in foods may change during processing and storage. Sorbic acid and sulphur dioxide are depleted in stored fruits, decreasing their effectiveness as hurdles to microbial growth and/or enzymatic browning. Sorbic acid is a key factor in achieving microbial stability of fruits preserved as above. Being diunsaturated, it degrades appreciably as a function of time, temperature and pH (Gerschenson *et al.*, 1986a,b). After 4 months storage at 30 °C, losses of sorbic acid in these fruit systems of low pH and a_w have

been reported to be nearly 40–55% (Alzamora *et al*., 1995; Corte *et al*., 2004).

The total sulphite content in papaya of high moisture after 150 days was around 54% of the initial content. Alzamora *et al*. (1989) reported a loss of this compound (probably due to volatilisation, oxidation and/or irreversible combination with fruit components) of approximately 50% in pineapple immersed in glucose syrup during 4 months of storage at 27 °C. Argaiz *et al*. (1991, 1993) reported a rapid loss of approximately 60% of total sulphite in papaya, pineapple and peaches immersed in sucrose syrups and stored in glass jars at 35 °C. They also reported a total sulphite loss in peaches stored without syrup in plastics bags after 60 days at 35 °C. Corte *et al*. (2004) found that the growth of *Z. bailii* was retarded for 3 months in minimally processed papaya and then it was able to grow; this fact was coincident with 50% losses in potassium sorbate and bisulphite fruit contents. Colour changes of preserved banana (a_w 0.97; pH 3.4, 100 ppm potassium sorbate; 250 ppm ascorbic acid) as they were influenced by $NaHSO_4$ concentration (200, 400 or 600 ppm), storage temperature (15, 25 or 35 °C) and thermal treatment time after packaging (0, 1 or 2 min) were analysed by Guerrero *et al*. (1996). Their findings showed that storage temperature had a highly significant effect on colour changes because of its influence on the availability of free bisulphite ion, the rate of browning and the chilling injury effect. As an example, Figure 19.4 shows the changes in total reversible combined and free sodium bisulphite concentration together with the corresponding Brown Index (BI) for banana with different treatments and storage temperatures. $NaHSO_4$ concentration ranging from 400–440 ppm, storage temperature ranging from 19–36 °C and thermal treatment time after packaging equal to 1 min were the optimum levels of the variables needed to ensure minimum browning development in preserved banana during 4 months storage.

On the other hand, it is well known that the antimicrobial properties of some additives, combined or not, in systems like water, buffers or broths are, in general, a poor indication of their performance in complex food systems. This issue gains relatively high importance in the case of naturally occurring antimicrobials, where the extrapolation is more difficult. In most cases, the actions responsible for reducing the antimicrobial activity are interactions with food components (like proteins, lipids, aldehydes and many macromolecules) and other preservation factors (Sofos *et al*., 1998). Thus, concentrations required for inhibitory or inactivation effects on microorganisms in real foods are considerable higher in comparison with laboratory media and frequently above tolerable taste thresholds. For instance, a lesser effect of vanillin was found in banana and in mango compared with that in apple, strawberry, papaya and pineapple. It was attributed to the greater content of fat and/or protein in the flesh, which are known to bind and/or solubilise phenolic compounds (Cerrutti and Alzamora, 1996).

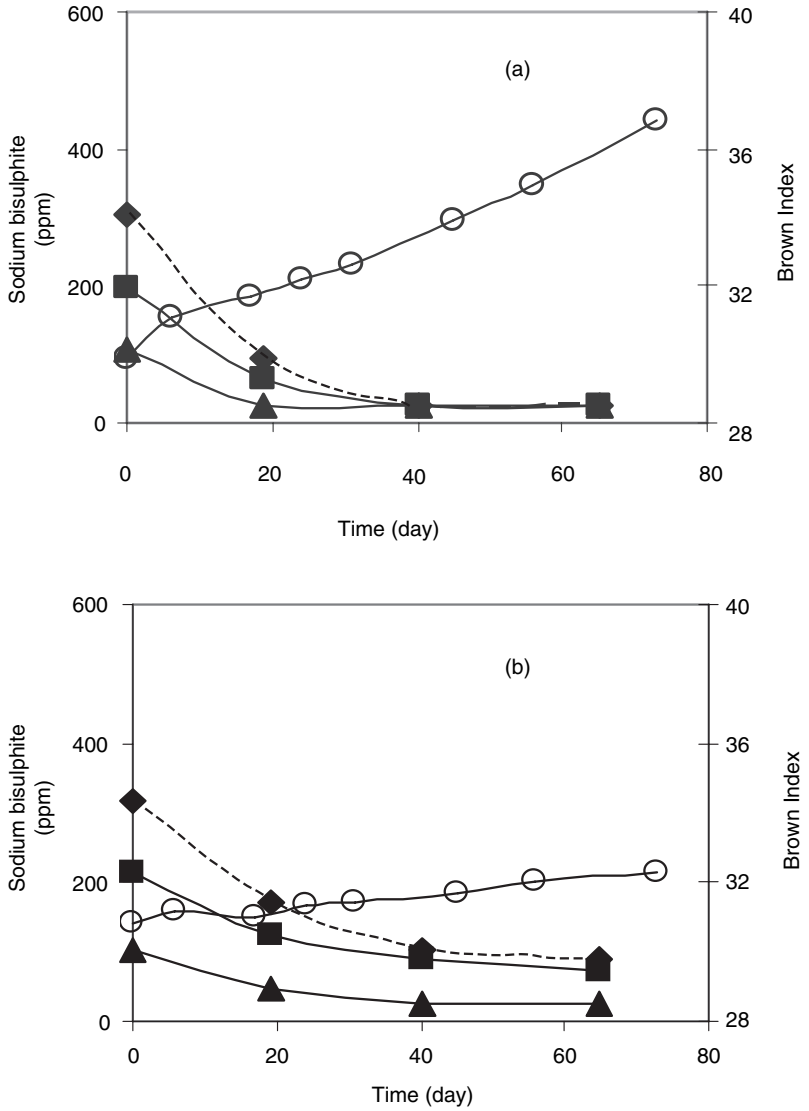

Fig. 19.4 Changes in Brown Index and sodium bisulphite content values in banana purée. (a) 400 ppm $NaHSO_4$; 35 °C; 2 min TT. (b) 400 ppm $NaHSO_4$; 25 °C; 1 min TT. (c) 600 ppm $NaHSO_4$; 15 °C; 1 min TT. (d) 600 ppm $NaHSO_4$; 35 °C; 1 min TT. TT = thermal treatment after packaging. -◆-, total; -▲-, combined; -■-, free; -○-, Brown Index.

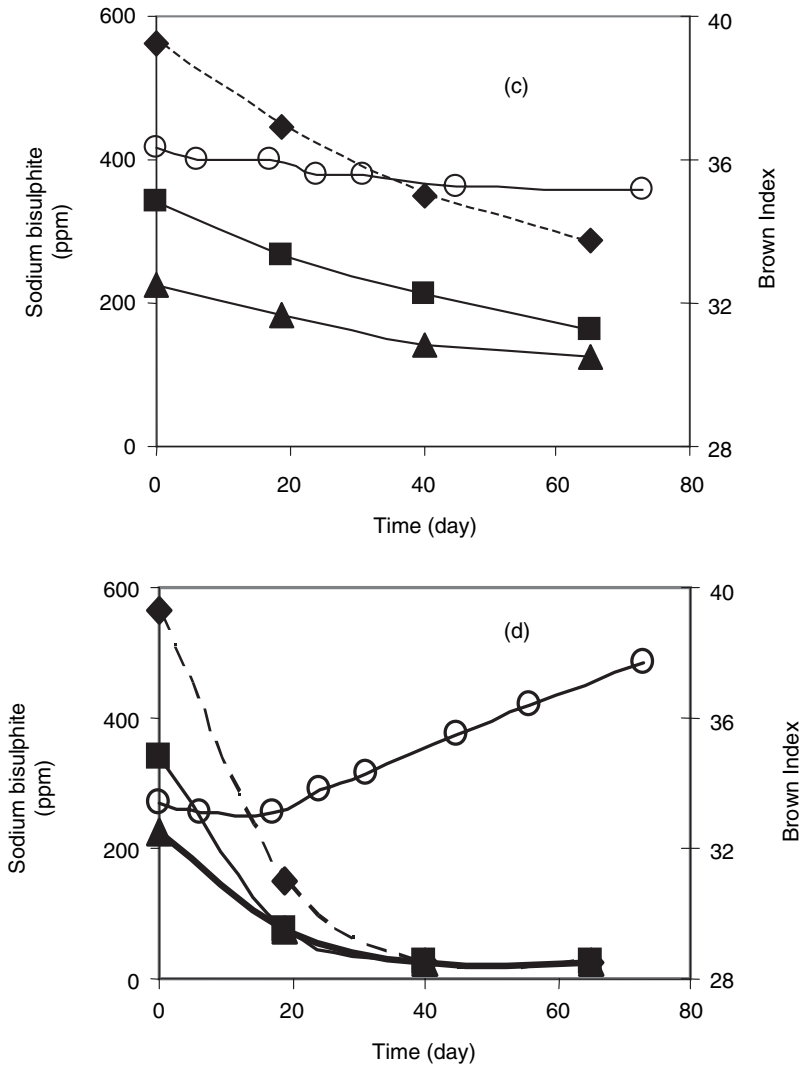

Fig. 19.4 *Continued*

19.4.4 Quantification of microbial response to factors in combination

Although since the mid-1990s there has been a rapid progress in the development, validation and application of microbial mathematical models, little of this approach appears to have found application in the estimation of microbial behaviour when designing shelf-stable or refrigerated high-moisture fruits. On the contrary, microbial response has been generally obtained using the traditional approach via challenge tests with pathogens

or spoilage microorganisms of concern, without a rigorous kinetic analysis of the data. This approach is expensive, slow and does not provide a sound basis for a rational development of the processes.

The concepts of predictive microbiology and protocols of model development, validation and application should be integrated from the very beginning of the design of a product. Designed experiments have the advantage that they produce results that can be reliably used for prediction. Improvements in the databases of these decontamination techniques will dramatically help their design and use of predictive models would provide a basis for the rational implementation of a HACCP programme simultaneously with the product development and pilot plant prototype production. However, a literature search reveals that, for these combined techniques, not only are there many fewer data but the accuracy of the available data is poor. Many reported literature experiments cannot be reproduced because information about the fruit or the medium (pH, a_w, size and shape of the piece fruit, etc.), process parameters (temperature, specific variables of the operation), and strains and initial physiological state is lacking. Therefore, modelling concepts should be incorporated as a routine tool for data collection and analysis to obtain reliable experimental kinetic parameters of the response of microbial populations exposed to combined techniques, improving the efficacy of data collection and precision of results. Moreover, modelling will allow a unified approach to the evaluation of the efficacy of the processes, facilitate the use of consistent and reliable data by different research and potentially permit much greater collaboration throughout the world.

The quantification of the influence of various hurdles on microbial behaviour allows interaction effects between them – antagonistic, synergistic or additive – to be precisely discerned and the impact of different factors on reduction in the microbial population to the compared. The selected hurdles can be kept at their minimum levels. Moreover, to maintain freshness, a sensory-based selection of hurdles/levels may be performed between several 'safe' equivalent combinations of interactive effects determined by the models. In fact, the integral prediction of the conduct of microbial populations exposed to these combined techniques, which involve a previous inactivation step followed by other growth inhibiting factors, should encompass models for microbial inactivation for those microorganisms that are sensitive to the inactivation factor, and models for growth/survival for those refractory ones. Variables that affect death in the first case or growth and survival in the second one are different. But an important point to take into account in the prediction of the behavior of microorganisms that were not inactivated is the influence of the prior history of the cell, including the stresses and the potential injury caused by the previous inactivation process. Injury cells can be easily underestimated when enumerating surviving organisms, resulting in misleading conclusions about the efficacy of the

method. According to the specific microorganism, growth/survival and death may both be modelled separately or in combination. In this sense, the division of the total cell population into sub-populations responding differently to the treatments or hurdles suggested by Pruitt and Kamau (1993) appears to be a useful approach.

In particular, one of the most powerful tools to be applied in the design and optimisation of minimally processed fruits is the modelling of the probability of a growth/no-growth interface. Growth/no-growth interface models quantify the effects of various hurdles on the probability of growth and define a set of combinations at which the growth rate is zero or the lag phase infinite (McMeekin *et al.*, 2000; Ratkowsky and Ross, 1995). As an example, probabilistic modelling using logistic regression was used to predict the boundary between growth and no-growth (probability level, *P*: 0.05) of *S. cerevisiae* after a 10-day incubation in the presence of the following growth-controlling factors: a_w (0.99, 0.97, 0.95), pH (3.5, 4.5), and citral (0–1100 ppm) or vanillin (0–1400 ppm) concentration (Fig. 19.5). Two interesting conclusions can be obtained as follows:

1. For both antimicrobials, an increase of pH from 3.5 to 4.5 increased the probability of yeast growth, decreasing the number of combinations of citral or vanillin concentrations with likelihoods of inhibiting yeast growth. However, significant differences in the probability of growth were observed between antimicrobials at both pH values, with citral being more effective in terms of delaying growth than vanillin.
2. Lowering a_w diminished the probability of yeast growth only when combined with antimicrobials. Selecting a probability of growth of 0.05, as a_w decreased the concentration of the antimicrobials (especially citral) decreased.

This example demonstrates that very useful information about stability and factor combination adequacy can be obtained from the knowledge of the growth/no-growth interface. This quantitative approach allows improvement and/or scientific design of combined factors technologies that were developed originally through observation of cardinal parameters for growth as well as by trial and error.

19.5 Calculations involved in the adjustment of a_w of high-moisture fruits

Techniques used to control a_w (i.e. removal of water, addition of solutes by moist or dry infusion, vacuum infusion or a combination of these processes) and their principles, advantages and disadvantages as well as models for a_w prediction in non-electrolytes and electrolyte binary solutions, mixtures and

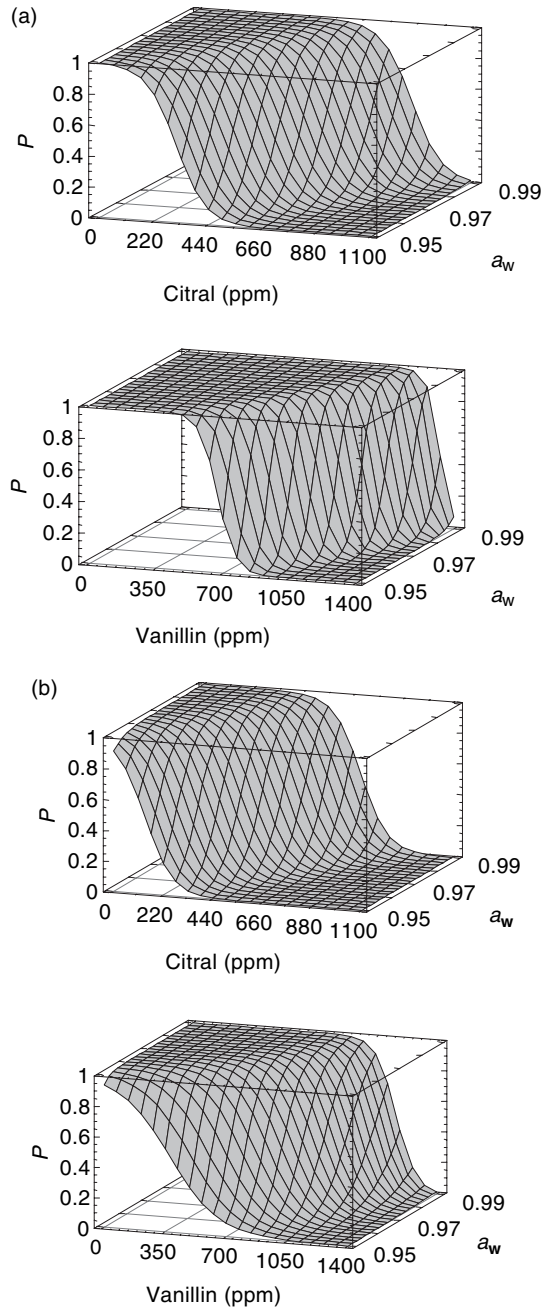

Fig. 19.5 *Saccharomyces cerevisiae* probabilities of growth after 10 days of incubation at 25 °C in model systems formulated with a_w 0.99, 0.97 or 0.95, vanillin or citral. (a) pH 4.5; (b) pH 3.5.

foods have been treated in many reviews and books (Troller and Christian, 1978; Rockland and Stewart, 1981; Rockland and Beuchat, 1987; Chirife, 1995; Fito and Chiralt, 1995). Here, calculations for adjusting a_w of high moisture fruits by osmotic dehydration at atmospheric pressure will be briefly described.

The amounts of sugar or other humectants (glucose, sucrose or other sugars or polyoles) is determined according to the weight of the fruit and to the final concentration required for the stabilisation of the product (Alzamora *et al*., 1989, 1995, 2003; Guerrero *et al*., 1994; Tapia de Daza *et al*., 1995; Welti-Chanes *et al*., 2000). To reduce a_w to a desired value, a sufficient amount of sugar or other solute is dissolved in water (moist infusion) or is directly aggregated to the fruit (dry infusion). The amount of sugar is calculated by using Ross's equation, which predicts a_w values of complex aqueous systems (in this case, the preserved fruit product) with various components when they are in equilibrium (Ross, 1975):

$$a_{w,\text{fruit product}} = a^0_{w,\text{fruit}} \cdot a^0_{w,\text{sugar}} \ldots a^0_{w,n\text{ component}} \quad [19.1]$$

where the water activity of the preserved fruit, $a_{w_{\text{fruit product}}}$, is the product of a_w values of the aqueous solutions of each n component (fruit, sugar, . . . , n component) when measured at the same molality as in the preserved fruit or complex system (i.e. as in the water of the fruit plus the water of the solution for moist infusion, or only as in the water of the fruit for dry infusion).

The value of $a^0_{w_{\text{fruit}}}$ is supposed to be equal to one (owing to the low content of soluble solids in the fresh fruit), and the value of a_w of the aqueous solutions of sugars ($a^0_{w_{\text{sugar}}}$), polyols or other organic molecules may be accurately predicted using Norrish's equation (Chirife *et al*., 1980):

$$a_{w,\text{sugar}} = x_w \cdot \exp(-Kx_s^2) \quad [19.2]$$

where x_w is the molar fraction of water, x_s the molar fraction of solute and K is a correlating constant (2.25 for glucose and fructose; 6.47 for sucrose; 1.16 for glycerol; 1.65 for sorbitol).

For moist infusion, the mass balances are the following:

Water mass balance:

$$W_T = W_F + W_{SO} \quad [19.3]$$

$$W_F = MC_F \cdot M_F \quad [19.4]$$

where W_T = grams of total water; W_F = grams of water in fresh fruit; W_{SO} = grams of water in the solution or syrup; MC_F = moisture content of the fruit; M_F = grams of fruit.

Sugar mass balance on water:

$$C_E \cdot W_T = C_F \cdot W_F + C_S \cdot W_{SO} \quad [19.5]$$

where C_E = g sugar/g total water, C_S = g sugar/g water in the solution, C_F = g sugar/g water in fresh fruit and $C_S W_{SO}$ = mass of sugar needed to prepare the solution (= M_S). C_E is the concentration of the aqueous sugar solution needed to obtain the a_w required in the equilibrium for the preserved fruit (i.e. $a_{w_{\text{fruit product}}}$) estimated by applying Norrish's equation. C_F is assumed to be equal to zero, since the amount of sugar in the fresh fruit is negligible (a_w for the fresh fruit is approximately 1).

For dry infusion, as water is only supplied by the fruit, the balances are simplified to:

Water mass balance:

$$W_F = W_F \quad [19.6]$$

$$W_F = MC_F \cdot M_F \quad [19.7]$$

Sugar mass balance on water:

$$M_S = C_E \cdot W_F \quad [19.8]$$

where C_E = g sugar/g total water = g sugar/g water of the fresh fruit. Again, C_E is the concentration of the sugar solution needed to obtain the a_w required for the preserved fruit after equilibrium (i.e. $a_{w_{\text{fruit product}}}$), estimated by applying Norrish's equation.

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