Comprehensive Reviews in Food Science and Food Safety

Chapter V
Methods to Reduce/ Eliminate Pathogens from Fresh and Fresh-Cut Produce

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Scope

In response to the current public health concerns with the microbiological safety of fresh and fresh-cut produce, researchers have investigated the efficiency of numerous physical, chemical, and biological methods for reducing the microbiological load of produce. This chapter focuses on this growing area of research with a particular emphasis on human pathogenic microorganisms; however, research related to mitigation treatment effects on nonpathogenic organisms is also included. There have been several reviews that address this topic and they are pointed out throughout the chapter; therefore, the focus here is on the latest and most significant research findings. A matrix (Table V-1) summarizing the characteristics of intervention methods is also included at the end of the chapter.

1. Introduction

It is well established that pathogenic microorganisms associated with whole or fresh-cut produce can cause disease outbreaks, thereby demonstrating the need for improved mitigation efforts to reduce risks associated with these products. Issues related to outbreaks (see Chapter III), surface contamination, mild processing, and mitigation strategies for produce have been recently reviewed (Beuchat 1998, 2000; Francis and others 1999; NACMCF 1999; Seymour 1999).

There are a variety of methods used to reduce populations of microorganisms on whole and fresh-cut produce. Each method has distinct advantages and disadvantages depending upon the type of produce, 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 totally eliminate 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 due to their delicate structure and problems with mold proliferation. These and some other produce items are often packaged in the field with minimal postharvest handling or washing.

In reference to food contact surfaces, 21 CFR 110.3(o) (CFR 2000b) defines the word sanitize: "to adequately treat food-contact surfaces by a process that is effective in destroying vegetative cells of microorganisms of public health significance." An additional definition of "sanitize" is found in the FDA Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (FDA 1998): "to treat clean produce by a process that is effective in destroying or substantially reducing the numbers of microorganisms of public health concern, as well as other undesirable microorganisms, without adversely affecting the quality of the product or its safety for the consumer." This definition addresses the need to maintain produce quality while enhancing safety by reducing populations of pathogenic microorganisms of public health significance that might theoretically exist on the produce.

Traditional methods of reducing microbial populations on produce involve chemical and physical treatments. Control of contamination requires that these treatments be applied to equipment and facilities as well as to produce. Methods of cleaning and sanitizing produce surfaces usually involve the application of water, cleaning chemicals (for example, detergent), and mechanical treatment of the surface by brush or spray washers, followed by rinsing with potable water. The rinse step may include a sanitizer treatment. It is important to ensure that water used for washing and sanitizing purposes is clean so that it does not become a vehicle for contamination.

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, 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 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.

The relationship between human pathogens and the native microflora, including postharvest spoilage organisms, on produce is of interest for at least two reasons. First, it has been suggested that reducing/controlling the native microbial populations by washing and sanitizing or by controlled atmosphere storage can allow human pathogens to flourish on produce surfaces (Brackett 1992). Concern has been expressed that reductions in surface populations reduces competition for space and nutrients thereby providing growth potential for pathogenic contaminants. In theory, this scenario can result in an unspoiled product that is unsafe for consumption. Berrang and others (1989ab) showed that pathogens grow to higher levels on produce stored under controlled atmosphere for extended shelf life than traditionally stored produce. While the cut salad industry traditionally uses natural spoilage as a food safety control measure, lengthening product shelf life would not be desirable if it increases the risk that pathogens would grow before spoilage is detectable. Secondly, a proliferation of postharvest spoilage organisms may compromise peel in-

Table V-1—Matrix of methods to mitigate the presence of microorganisms on whole and cut produce

Mitigation Method	Advantages	Limitations	Comments on current use	Comments on research
Hypochlorite	• Long history of use	• Potential adverse health effects of chlorinated by-products • Corrosive to equipment • Sensitive to temperature, light, air, metals and organic materials • pH dependent • Some resistance by bacterial spores and protozoan oocysts	• Commonly used in the 50 to 200 ppm range with a 1 to 2 min contact time. • Usefulness on many produce commodities has been investigated	• Very high concentrations may not eliminate pathogens on produce • Commonly used concentrations produce a maximum 1 to 2 log reduction on many commodities.
Acidified sodium chlorite	• Greater efficacy than hypochlorite due to low pH	• Little information on production of chlorinated by-products • Limited amount of research conducted	• Studied for use on meats, seafood, poultry, produce • 500 to 1200 ppm range studied	• Usefulness for produce needs further research
Chlorine dioxide	• Less reactivity than hypochlorite with organics • Fewer chlorinated by-products • Better antimicrobial activity at neutral pH than hypochlorites	• Stability • Not permitted for cut produce	• Up to 5 ppm allowed on whole fruits and vegetables • 1 ppm maximum allowed on peeled potatoes	• Studied concentrations range from about 1 ppm to 500 ppm on commodities such as alfalfa seeds and sprouts, cucumbers, shredded lettuce, cabbage, oranges • Studies conducted with fungal spores, native microflora, *Listeria monocytogenes*, *E. coli, E. coli* O157:H7, *Salmonella*, Cryptosporidium parvum oocycts. • Reductions of a few logs reported
Bromine	• Possible synergy with chlorine compounds	• Information lacking on production of brominated by-products and their potential health effects	• Not widely used as a sanitizer	• More effective against *E. coli*, *Salmonella Typhosa* and *Staphylococcus aureus* than against *Pseudomonas aeruginosa.* • Not as effective as hypochlorite against *Bacillus cereus* spores
Iodine	• Less corrosive than chlorine at low temperature • Broad spectrum • Iodophor less volatile than iodine	• Stains commodities and equipment • Corrosive above 50 °C	• Commonly used on food contact surfaces and equipment • No direct contact use on produce	• May have significant sporocidal capacity • Possible usefulness on some whole produce deserves investigation
Trisodium phosphate	• Less corrosive than most other compounds	• Listeria relatively resistant • Has very high pH (11-12)	• Occasional use on fresh-market citrus • Authorized for use on raw poultry	• Concentrations between 1 and 15% yielded reductions in pathogen populations from 0 to 6 logs
Quaternary ammonium compounds	• Colorless, odorless • Stable at high temperature • Noncorrosive • Good penetrating ability • Relatively stable to organic compounds • Leaves residual	• Limited usefulness at low pH (<6) • Not compatible with soaps or anionic detergents • Costly	• Commonly used on food contact surfaces and equipment	* As effective as chlorine at reducing populations of Xanthomonas campestris pathovar vesicatoria. • Reduced native orange-surface microflora 95% compared to 60% reduction on control fruit.
Acids	• Economical, depending upon type of acid and use	• Low pH use only • Antimicrobial effect dependent upon type of acid and strain of microorganism	• Acidification to preserve foods commonly used • Acid sprays on meat carcasses commercially used • Phosphoric acid/anionic compounds commonly used on citrus at about 200 ppm	• Lemon juice and vinegar may be useful for limited household sanitation of produce. • Organic acids studied for use on several produce commodities for control of native populations as well as specific pathogens (*Salmonella spp, Campylobacter spp, Yersinia spp, Shigella spp., Listeria spp.*) • Peracetic acid concentrations up to 200 ppm effectively used on whole and cut produce.

(continued on next page)

Table V-1—Matrix of methods to mitigate the presence of microorganisms on whole and cut produce *(continued from previous page)*

Mitigation Method	Advantages	Limitations	Comments on current use	Comments on research
Hydrogen peroxide	• Sporicidal • Rapid breakdown to nontoxic products	• Possible effects on product color (browning or bleaching)	• Limited industry use on food contact surfaces and packaging.	• Vapor and aqueous dips (1 to 5% range) studied on numerous produce commodities. • Variable effectiveness reported by researchers.
Ozone	• Effective at low concentrations and short contact time • Broad spectrum • Good penetration ability • Effectiveness against protozoa reported • Decomposes to nontoxic products	• Physiological injury of produce possible • Corrosive to equipment • Deterioration of produce flavor and color possible • Unstable; very highly reactive • Possible human toxic effects in processing facilities	• Commonly used for water treatment	• Effective against a variety of postharvest pathogens reported on fruits and vegetables • Reduced *Salmonella* and *E. coli* populations on ground black pepper 3 to 4 log/g. • Further research on produce is warranted
Irradiation	• No chemical treatment • Can be conducted after packaging • Shelf life extension of produce observed	• Image of irradiation by consumers • Negative sensory effects possible	• 1 to 10 kGy used to reduce pathogens in/on foods • <1 kGy used to inhibit sprouting of tubers, bulbs, roots and to eliminate insects from produce	• Variable effectiveness against postharvest pathogens reported in literature • Little information exists regarding effectiveness against human pathogens in produce
Biocontrol	• No chemical treatments	• Limited spectrum • Possible public reaction to consumption of live microorganisms	• Used on apples for control of postharvest plant pathogens • Competitive exclusion useful in poultry to prevent intestinal colonization by pathogens • Starter cultures used for fermented meat and dairy products	• Limited research on use of biocontrol measures against human pathogens on produce

tegrity and alter product pH thereby enhancing the survival and growth of human pathogens (Conway and others 2000).

These issues, along with primary methods of pathogen control for whole and fresh-cut produce are described in more detail below. Although the intent of this report is to describe methods to reduce or eliminate pathogens from produce, information regarding mitigation against nonpathogenic microorganisms is included in the text to illustrate the overall effectiveness of certain intervention technologies.

1.1. Combined methods and hurdles

This report does not specifically address the antimicrobial effects of combinations of various mitigation strategies; however, it would be expected that combinations of sanitizers and/or other intervention methods, such as heat or irradiation, would have additive, synergistic or antagonistic interactions (Parish and Davidson 1993).

The concept of using multiple intervention methods is analogous to hurdle technology where two or more preservation technologies are used to prevent growth of microorganisms in or on foods (Leistner and Gorris 1995; Leistner 2000; Howard and Gonzalez 2001)

2. Intervention methods

2.1. Temperature

Refrigerated temperatures cannot be relied upon to prevent growth of pathogenic microorganisms on produce. Populations of *Listeria monocytogenes* remained constant or grew on a variety of whole and cut produce stored at refrigerated temperatures (Farber and others 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. Austin and others (1998) reported toxin production by *Clostridium botulinum* on unspoiled onions and butternut squash stored under modified atmosphere at 15 °C (59 °F). Piagentini and others (1997) reported that *Salmonella* Hadar could survive and proliferate on chilled shredded cabbage prior to detection of spoilage. 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 and others 1997; Zhao and others 1993).

Hot water is used as a mitigation treatment of some fruits to control insects and postharvest plant pathogens that cause product spoilage. Fruits investigated for hot water treatment include apple, cherry, grapefruit, lemon, mango, melon, papaya, pear, or tomato (Breidt and others 2000; Puerta and Suslow 2001, personal communication, unreferenced). Although adverse effects on color, texture, and flavor limit the usefulness of this treatment, hot water may have application as a sanitizer of produce, especially for fresh-cut products or unpasteurized juices where inedible outer rinds, skins or peels are discarded during processing. Pao and Davis (1999) determined that immersion of oranges in hot water (70 °C [158 °F] for 2 min, or 80 °C [176 °F] for 1 min) effectively reduced *Escherichia coli* on overall fruit surfaces by 5 log CFU/cm^2, although reductions on the stem-end tissue were not as great. One disadvantage is that thermally treated produce might not be considered "fresh" by FDA based on 21 CFR part 101.95 (CFR 2000a).

The hygiene and temperature of water used during the handling of produce are of primary importance. Immersion of warm whole or fresh-cut produce in cool process solutions may induce infiltration of the solution (including contaminating microorganisms)

into the product through openings in the peel such as stem-end vascular tissue, lenticels, stomata, puncture wounds, or other physical disruptions. Research by Bartz (1982), Bartz and Showalter (1981) and Showalter (1979) showed that bacteria in a cool (20 to 22 °C) (68 to 71.6 °F) aqueous suspension penetrate into stem tissue of warm tomatoes after a 10 min exposure. A negative temperature differential of 15 °C (77 °F) allowed the infiltration of *Salmonella* Montevideo into the core of tomatoes at significantly higher rates than without a temperature differential (Zhuang and others 1995). The issue of infiltration is of special concern during hydrocooling where water is used to cool the product. It is imperative that water used for this purpose be sanitary and free of human pathogens.

Buchanan, Edelson, Miller and others (1999) determined that *E. coli* O157:H7 can penetrate into the core of warm apples placed in a cool suspension of the pathogen. Results of Burnett and others (2000) suggest that this same pathogen may infiltrate through apple floral tubes regardless of temperature differences although infiltration was greater for apples under a negative temperature differential. These studies point out the importance of maintaining adequate disinfectant levels to eliminate pathogens in water from dump tanks or other handling procedures before they have the opportunity to penetrate into the produce interior. It should be noted that temperatures in hot water dump tanks that are used to kill insect pests might aid survival of some pathogens. In a recent salmonellosis outbreak from Brazilian mangoes, the hot water treatment to kill fruit flies was at 46 to 47 °C (114.8 to 116.6 °F) for 65 to 90 min (Anonymous 2000). This was followed by a cooling procedure that could have caused internalization of the salmonellae into the fruit. Alternatively, the salmonellae could have simply attached to structures on the peel surface and not crossed the peel barrier into the fruit interior.

It is well documented that microorganisms, including some human pathogens, are capable of crossing peel barriers to enter the interior of produce (Bartz, 1982, 1988, 1991; Bartz and Showalter 1981; Buchanan, Edelson, Miller and others 1999; Burnett and others 2000; Showalter 1979; Zhuang and others 1995). This internalization phenomenon deserves closer scrutiny since it is a viable hypothesis that could explain some disease outbreaks. Studies on possible infiltration under commercial handling conditions are warranted. It is important to reproduce field, handling, packing, and storage conditions during infiltration experiments. Also, mechanisms by which microorganisms cross peel barriers to enter the interior portions of produce are an obvious focal point for research. It should be noted that the usefulness of dye penetration as a model for bacterial internalization may be limited since dyes can readily penetrate through tissue fissures too small for the passage of microbial cells (Pao and others 2001).

Upon soaking cut lettuce in suspensions (10^9 CFU/mL) of *E. coli* O157:H7 for 24 hours at various temperatures, the pathogen penetrated into cut edges to a greater degree at 4 °C (39.2 °F) than at higher temperatures (Takeuchi and Frank 2000). Cells penetrated the cut lettuce tissue to an average depth of 74 ¼m at 4 °C (39.2 °F) and were unaffected by treatment with chlorine. Penetration was lower at higher incubation temperatures. In a study on apples, Burnett and others (2000) determined that *E. coli* O157:H7 penetrated damaged tissue around puncture wounds to a depth of 70 ¼m. It has also been reported that produce affected by postharvest soft rot may harbor human pathogens at a higher frequency than healthy produce (Wells and Butterfield 1997). The mechanism by which this harborage occurs is not clearly understood, although compromised peel integrity and pH changes may each play a role.

2.2. Physical removal of microorganisms

Many hardy produce items are brush-washed with oscillating brushes to scrub surfaces for the physical removal of soil and microorganisms. This is often done in conjunction with a detergent followed by a rinse of potable water. Brushing also removes a portion of the natural waxy cuticle on the produce surface that acts as a barrier to microorganisms. Commercial waxes are occasionally added to the produce surface after washing to replace the natural waxes that are removed. It should be noted that microorganisms can become enmeshed within waxy materials on produce making their removal most difficult (Kenney and others 2001). At the same time, the addition of hot wax (50+ °C [122+ °F] for 2 min) onto orange surfaces had an antimicrobial effect (Pao and others 1999). Some produce items that may be damaged by brushes are washed in a bath, or under a spray. This may or may not include gentle agitation and/or detergents to aid in removal of soils.

Washing efficiency varies with commodity, type of washing system, type of soil, contact time, detergent, and water temperature. In one study, brush-washing of oranges in plain water reduced the surface microbial population approximately 60 to 70% compared to 90% reduction when a sanitizer was included (Winniczuk 1994). In several studies on chemical sanitizers, simple rinsing of produce in plain water reduces the surface populations although the reduction is usually well less than 1 log. A concern regarding washing system efficiency is the quality of wash water, especially if the water is recycled and not treated prior to reuse. The use of disinfectant chemicals in wash water provides a barrier to cross contamination of produce. Research on new or more efficient methods to physically remove microorganisms from produce surfaces may be warranted.

2.3. Chlorine (Hypochlorite)

Chlorine has been used for sanitation purposes in food processing for several decades and is perhaps the most widely used sanitizer in the food industry (Walker and LaGrange 1991; Cherry 1999). Chemicals that are chlorine based are often used to sanitize produce and surfaces within produce processing facilities, as well as to reduce microbial populations in water used during cleaning and packing operations. Safety concerns about the production of chlorinated organic compounds, such as trihalomethanes, and their impact on human and environmental safety have been raised in recent years, and alternatives to chlorine have been investigated. At the foodservice and household levels, chlorine remains a convenient and inexpensive sanitizer for use against many foodborne pathogens.

The most common forms of free chlorine include liquid chlorine and hypochlorites. (Chlorine dioxide and acidified sodium chlorite will be discussed in the next section.) Liquid chlorine and hypochlorites are generally used in the 50 to 200 ppm concentration range with a contact time of 1 to 2 min to sanitize produce surfaces and processing equipment. Higher concentrations have been investigated for use on seeds for sprout production. Hypochlorous acid (HOCl) is the form of free available chlorine that has the highest bactericidal activity against a broad range of microorganisms. In aqueous solutions, the equilibrium between hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-) is pH dependent with the concentration of HOCl increasing as pH decreases. Typically, pH values between 6.0 and 7.5 are used in sanitizer solutions to minimize corrosion of equipment while yielding acceptable chlorine efficacy. HOCl concentration is also significantly affected by temperature, presence of organic matter, light, air, and metals. For example, increasing levels of organic matter decreases HOCl concentration and overall antimicrobial activity. Maximum solubility in water is observed near 4 °C (39.2 °F); however, it has been suggested that the temperature of processing water should be maintained at least 10 °C (50 °F) higher than that of produce items in order to reduce the possibility of microbial infiltration caused by a temperature-generated pressure

differential. The opportunity for infiltration of microorganisms is also minimized when the sanitary condition of the water is maintained. There are readily available commercial systems for in-line monitoring and application of chlorine to maintain water cleanliness. This is particularly applicable to water used in dump tanks or for cleaning or cooling purposes.

Effects of chlorine on bacterial pathogens inoculated onto produce have been investigated with mixed results. Studies indicate those chlorine concentrations traditionally used with produce (<200 ppm) are not particularly effective at reducing microbial populations on lettuce. Survival of *E. coli* O157:H7 on cut lettuce pieces after submersion for 90 seconds in a solution of 20 ppm chlorine at 20 or 50 °C (68 or 122 °F) was not significantly different from the non chlorine treatment (Li and others 2001). Spray treatment of lettuce with 200 ppm chlorine was no more effective at removing *E. coli* O157:H7 than treatment with deionized water (Beuchat 1999). Increasing the exposure time from 1 to 5 min did not result in an increased kill. Likewise, Adams and others (1989) indicated that a standardized washing procedure for lettuce leaves was only slightly improved with inclusion of 100 ppm chlorine over tap water alone. Although a reduction of pH of the chlorine solution to between 4.5 and 5.0 increased lethality up to 4-fold, longer wash times (from 5 to 30 min) did not result in increased removal of microorganisms.

Research reported by Nguyen-the and Carlin (1994) suggests that inactivation of *L. monocytogenes* on vegetables by chlorine is limited. Zhang and Farber (1996) showed that treatment of shredded lettuce and cabbage with 200 ppm chlorine for 10 min reduced the population of *L. monocytogenes* by 1.7 and 1.2 log CFU/g, respectively. Reductions were only marginally greater when exposure time was increased from 1 to 10 min. Similarly, 10-minute exposures of *Yersinia enterocolitica* on shredded lettuce to 100 and 300 ppm chlorine resulted in population reductions of roughly 2 to 3 log (Escudero and others 1999). Results at 4 °C (39.2 °F) and 22 °C (71.6 °F) were not significantly different ($P < 0.05$). In this same study, a combination of 100 ppm chlorine and 0.5% lactic acid inactivated *Y. enterocolitica* by greater than 6 log. These results suggest that *Y. enterocolitica* may be more sensitive to chlorine than some other pathogens. Brackett (1987) reported that the reduction in numbers of *L. monocytogenes* on Brussels sprouts changed from 90% (dipped 10 seconds in sterile water without chlorine) to 99% with the addition of 200 ppm chlorine. When inoculated into cracks of mature green tomatoes, *Salmonella* Montevideo survived treatment with 100 ppm chlorine (Wei and others 1995).

Treatment of produce with higher concentrations of chlorine (>500 ppm) has been studied. For example, sprouts have unique attributes and microbiological issues that have required investigations of nontraditional sanitation regimens. Treatment of alfalfa seeds and sprouts with chlorine to control salmonellae and *E. coli* O157:H7 has been studied (Jaquette and others 1996; Beuchat and Ryu 1997; Taormina and Beuchat 1999a, 1999b). Chlorine concentrations up to 100 ppm reduced populations of pathogens on alfalfa seeds; however, concentrations between 100 and 1000 ppm were not more effective (Jaquette and others 1996). Treatment of alfalfa sprouts for 2 min with a 500 ppm chlorine dip reduced salmonellae populations by 3.4 log per gram, and, after treatment with 2000 ppm chlorine, salmonellae populations were undetectable (<1 CFU/g) (Beuchat and Ryu 1997). The effect of chlorine treatment on sensory aspects of the sprouts was not reported. *Escherichia coli* O157:H7 populations were reduced significantly after exposure to $Ca(OCl)_2$ at 500 and 1000 ppm; however, treatment with 20,000 ppm $Ca(OCl)_2$ did not eliminate this microorganism from seeds (Taormina and Beuchat 1999a). Application of 2000 ppm sodium or calcium hypochlorite significantly reduced the population of *E. coli* O157:H7 on germinated alfalfa seeds but did not control growth of the pathogen on sprouts during the sprouting process (Taormina and Beuchat 1999b).

Beuchat and others (1998) showed that the maximum reduction in human pathogen populations on apples, tomatoes, and lettuce was 2.3 log CFU/cm^2 after dipping in solutions of 2000 ppm chlorine for 1 min. On fresh-cut cantaloupe cubes, 2000 ppm chlorine resulted in less than a 90% reduction in viable cells of several strains of salmonellae (Beuchat and Ryu 1997). Populations of salmonellae or *E. coli* O157:H7 inoculated onto the surfaces of cantaloupes and honeydew melons were reduced between 2.6 and 3.8 log CFU (as compared to a water wash control) when treated for 3 min with 2000 ppm sodium hypochlorite or 1200 ppm acidified sodium chlorite (Park and Beuchat 1999). These treatments were less effective when applied to asparagus spears, thereby indicating that it may be necessary to customize sanitation treatments for different types of produce. Populations of *Shigella sonnei* inoculated onto whole parsley leaves were reduced more than 7 log CFU/g after treatment for 5 min with 250 ppm free chlorine (Wu and others 2000).

Reduction in populations of microflora on whole and fresh-cut produce is dependent upon the type of produce and the type of natural microflora present. Senter and others (1985) determined that total plate counts and Enterobacteriaceae populations on tomato surfaces decreased when chlorine levels of process water were raised from about 115 to 225 ppm. Pao and Davis (1999) showed that populations of *E. coli* inoculated onto orange surfaces were reduced more than 2 log CFU/cm^2 after immersion in 200 ppm chlorine at 30 °C (86 °F) for 8 min. This reduction was only slightly higher than that resulting from immersion in deionized water alone. Murdock and Brokaw (1958) used water containing 20 to 50 ppm free chlorine to reduce total microbial populations on the surface of oranges by 92 to 99%, as compared to 79% for oranges washed in water. Winniczuk (1994) determined that dipping washed oranges in 1000 ppm HOCl for 15 seconds reduced the microbial population on the surface by about 90%, as compared to 60% for control oranges dipped in plain water. Populations of *E. coli* inoculated onto lettuce leaves and broccoli florets were generally reduced <1 log CFU/g after a 5 min dip in 100 ppm free chlorine compared to a plain water dip (Behrsing and others 2000).

Results of Mazollier (1988) indicated that microbial reductions on leafy salad greens were essentially the same when treated with 50 or 200 ppm chlorine. Total microbial populations were reduced about 1000-fold when lettuce was dipped in water containing 300 ppm total chlorine, but no effect was seen against microbial populations on red cabbage or carrots (Garg and others 1990). Coliform bacteria were reduced by 81% on parsley, 93% on lettuce, 98% on strawberries, and 85% on coriander after a 10-minute contact time in a solution of 300 ppm chlorine (Lopez and others 1988). Microbial populations of cut potato strips were not effectively controlled by dips in 300 ppm hypochlorite (Gunes and others 1997). Treatment of honeydew melons and cantaloupes with 200 ppm hypochlorite significantly ($P < 0.05$) reduced surface microbial populations compared to water-washed controls (Ayhan and others 1998).

Since chlorine reacts with organic matter, components leaching from tissues of cut produce surfaces may neutralize some of the chlorine before it reaches microbial cells, thereby reducing its effectiveness. Additionally, crevices, cracks, and small fissures in produce, along with the hydrophobic nature of the waxy cuticle on the surface of many fruit and vegetables, may prevent chlorine and other sanitizers from reaching the microorganisms. Surfactants, detergents, and solvents, alone or coupled with physical manipulation such as brushing, may be used to reduce hydrophobicity or remove part of the wax to increase exposure of microorganisms to sanitizers. However, such treatments may cause deterioration of sensory quality, thereby limiting their usefulness

to applications just prior to consumption (Adams and others 1989; Zhang and Farber 1996).

2.4. Chlorine dioxide and acidified sodium chlorite

The major advantages of chlorine dioxide (ClO_2) over HOCl include reduced reactivity with organic matter and greater activity at neutral pH; however, stability of chlorine dioxide may be a problem. ClO_2 forms fewer organohalogens than HOCl, although its oxidizing power is reported as 2.5 times that of chlorine (Benarde and others 1967). A maximum of 200 ppm ClO_2 is allowed for sanitizing of processing equipment and 3 ppm maximum is allowable for contact with whole produce. Only 1 ppm maximum is permitted for peeled potatoes. Treatment of produce with chlorine dioxide must be followed by a potable water rinse or blanching, cooking, or canning (CFR 2000c).

There is less information about the effectiveness of ClO_2 than HOCl as a sanitizer for produce. As with HOCl, microbial susceptibility to ClO_2 differs with strain and environmental conditions of application. A population of *L. monocytogenes* inoculated onto shredded lettuce and cabbage leaves was reduced an additional 1.1 and 0.8 log at 4 and 22 °C (39.2 and 71.6 °F), respectively, after treatment with 5 ppm ClO_2 for 10 min when compared to washing in tap water (Zhang and Farber 1996). Use of ClO_2 gas reduced the numbers of *E. coli* O157:H7 on injured green pepper surfaces (Han and others 2000). Treatment of surface-injured green peppers with 0.6 and 1.2 ppm ClO_2 gas reduced populations of *E. coli* O157:H7 by 3.0 and 6.4 log cycles, respectively. These researchers noted that no significant growth of *E. coli* O157:H7 was observed on uninjured pepper surfaces, but significant growth occurred on injured pepper surfaces within 24 hours at 37 °C (98.6 °F). The use of ClO_2 in a gaseous state, as opposed to an aqueous solution, warrants further study.

Roberts and Reymond (1994) demonstrated mortality of postharvest spoilage fungi to ClO_2. Greater than 99% kill of conidia or sporangiophores was observed after 1 min in water containing 3 or 5 ppm ClO_2. Fungal populations on conveying equipment were reduced upon treatment with foam containing 14 to 18 ppm ClO_2. Costilow and others (1984) reported that 2.5 ppm ClO_2 was effective against microorganisms in wash water, but concentrations as high as 105 ppm did not reduce the microflora in or on cucumbers. Similar results were reported by Reina and others (1995). Immersion of oranges in 100 ppm chlorine dioxide at 30 °C (86 °F) for 8 min produced a 3-log reduction of nonpathogenic *E. coli* compared to about a 2-log reduction when immersed in deionized water only (Pao and Davis 1999).

Acidified sodium chlorite has been approved for use on certain meats, seafood, poultry, and raw fruits and vegetables as either a spray or dip in the range of 500 to 1200 ppm (CFR 2000d). Reactive intermediates of this compound are highly oxidative with broad spectrum germicidal activity. Applications of 500 ppm acidified ClO_2 significantly reduced populations of *E. coli* O157:H7 (>1 log) on germinated alfalfa seeds, but did not control the growth of the pathogen during the sprouting process (Taormina and Beuchat 1999b). Park and Beuchat (1999) showed that acidified sodium chlorite has a substantial antimicrobial effect against *E. coli* O157:H7 and salmonellae inoculated onto cantaloupes, honeydew melons and asparagus spears. Pathogen reductions were in the range of 3 log. There is a need for more published information on the general usefulness of acidified sodium chlorite for produce.

2.5. Bromine

Little is known about the usefulness of bromine as a sanitizer for produce. Kristofferson (1958) and Shere and others (1962) observed a synergistic antimicrobial relationship when bromine was added to chlorine solutions. Within 15 min at 24 °C (75.2 °F), free bromine (200 ppm) was shown to kill *E. coli*, *Salmonella* Typhosa, and *Staphylococcus aureus*, but not *Pseudomonas aeruginosa* (Gershenfeld and Witlin 1949). Dibromodimethyl hydrantoin was as effective as chlorine against *Streptococcus faecalis* (Ortenzio and Stuart 1964), but was less effective against *Bacillus cereus* spores (Cousins and Allan 1967). As with free chlorine, there are safety concerns about the production of brominated organic compounds and their impact on human and environmental safety.

2.6. Iodine

Iodophors have a broad spectrum of antimicrobial activity, are less corrosive than chlorine at low temperatures, and are less volatile and irritating to skin than other types of iodine solutions (Lawrence and others 1957). However, iodine-containing sanitizer solutions may be corrosive (upon vaporization above 50 °C [122 °F]), have reduced efficacy at low temperature, and may stain equipment, clothes, and skin. The use of iodine-containing solutions as direct contact sanitizers for produce is further limited due to a reaction between iodine and starch that results in a blue-purple color. Despite these limitations, iodine solutions such as iodophors (combinations of elemental iodine and nonionic surfactants or carriers) are commonly used as sanitizers for food contact surfaces and equipment in the food processing industry (Bartlett and Schmidt 1957; Hays and others 1967; Mosley and others 1976; Lacey 1979; Jilbert 1988). Although iodine solutions are not used for direct food contact, a peroxidase-catalyzed chemical solution that included sodium iodide as an antimicrobial constituent was active against salmonellae inoculated onto chicken breast skin (Bianchi and others 1994) and may warrant investigation for some produce items.

As with most sanitizers, iodophors are more active against vegetative cells than bacterial spores. Decimal reduction values for vegetative bacterial cells are between 3 and 15 seconds at 6 to 13 ppm available iodine at neutral pH (Hays and others 1967; Mosley and others 1976; Gray and Hsu 1979). D values for spores of *Bacillus cereus, Bacillus subtilis*, and *C. botulinum* Type A treated with 10 to 100 ppm of iodophor are 10- to 1000-fold greater than for vegetative cells (Odlaug 1981). Although iodophors are not approved for direct food contact, they might have some usefulness for treatment of produce items that are peeled before consumption. This type of use would require regulatory approval and a demonstration that produce treated by these compounds are safe for consumption.

2.7. Quaternary ammonium compounds

Commonly called "quats," quaternary ammonium compounds are cationic surfactants that are odorless, colorless, stable at high temperatures, noncorrosive to equipment, nonirritating to skin, and able to penetrate food contact surfaces more readily than other sanitizers (Walker and LaGrange 1991). The antimicrobial activity of quats is greater against the fungi and gram-positive bacteria than gram-negative bacteria. Thus, *L. monocytogenes* is more sensitive to quats than coliforms, *Salmonella* spp., pathogenic *E. coli*, or pseudomonads. Due to their high surface-active capability, the mechanism of activity for quats possibly involves a breakdown of the cell membrane/wall complex (Marriott 1999). Some concern has been expressed about the potential for development of resistance to quats due to the common spread of Class 1 integrons among bacteria. The practical impact of possible quat resistance has not been demonstrated.

Quat sanitizers form a residual antimicrobial film when applied to most hard surfaces and are relatively stable to organic compounds. They are most effective when used at pH 6 to 10, and are not compatible with acidic environments, soaps or anionic detergents. Although they are not approved for direct food contact,

quats may have some limited usefulness with whole produce that must be peeled prior to consumption. As with iodine compounds, direct food contact would require regulatory approval and a demonstration that produce treated by quats is safe for consumption.

Brown and Schubert (1987) determined that a 30-second exposure of oranges to a 500 ppm quat solution reduced *Xanthomonas campestris* pv. *vesicatoria* as effectively as 150 to 250 ppm chlorine for 2 min. The surface microflora of oranges brush-washed in water and dipped in 200 ppm quat for 15 seconds was reduced about 95% compared to 60% for washed oranges dipped in plain water (Winniczuk 1994).

2.8. Acidic compounds with or without fatty acid surfactants

Organic acids are commonly used as antimicrobial acidulants to preserve foods either by direct addition or through microbiological fermentation (Foegeding and Busta 1991). Since many pathogens generally cannot grow at pH values much below 4.5, acidification may act to prevent microbial proliferation. Organic acids may also possess bactericidal capabilities. The antimicrobial action of organic acids is due to pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid. Many types of produce, especially fruit, naturally possess significant concentrations of organic acids such as acetic, benzoic, citric, malic, sorbic, succinic, and tartaric acids, which negatively affect the viability of contaminating bacteria. Fruits such as melons and papayas contain lower concentrations of organic acids than other fruits and therefore are at pH values above 5.0, which does not suppress growth of pathogenic bacterial contaminants.

In contrast to their use as preservatives, organic acids, primarily lactic acid, are also successfully used as sanitizers on food animal carcasses and may have potential for application to produce surfaces for the purpose of reducing populations of microorganisms. Treatment with citric acid in the form of lemon juice has been shown to reduce populations of *Salmonella* Typhi inoculated onto cubes of papaya and jicama (Fernandez Escartin and others 1989). Castillo and Escartin (1994) investigated survival of *C. jejuni* on cubes of watermelon and papaya treated at room temperature with lemon juice. Six hours after treatment, populations of *Campylobacter jejuni* ranged from 0 to 14.3% of the original inoculum on cubes treated with lemon juice, and from 7.7 to 61.8% on cubes not treated with lemon juice. The antimicrobial activity was more pronounced on papaya than watermelon.

Use of acetic acid to inactivate pathogenic bacteria on fresh parsley was studied by Karapinar and Gonul (1992). Populations of *Y. enterocolitica* inoculated onto parsley leaves were reduced > 7 log cycles after washing for 15 min in solutions of 2% acetic acid or 40% vinegar. Treatment in 5% acetic acid for 30 min did not result in any recovery of aerobic bacteria, while treatment with vinegar gave a 3 to 6 log decrease in aerobic counts, depending upon vinegar concentration and exposure time. Treatment of whole parsley leaves for 5 min at 21 °C (69.8 °F) with vinegar (7.6% acetic acid) reduced populations of *S. sonnei* more than 7 log per gram (Wu and others 2000). Vinegar and lemon juice have potential as inexpensive, simple household sanitizers; however, possible negative sensory effects when used on produce would be a disadvantage.

Various combinations of acetic acid, lactic acid and chlorine were observed to reduce populations of *L. monocytogenes* on shredded lettuce (Zhang and Farber 1996). Lactic or acetic acids in combination with 100 ppm chlorine were slightly more antagonistic toward *L. monocytogenes* than either acid or chlorine alone; however, the increased antagonism might be due to an additive effect of the combined compounds or due to an increase in hypochlorous acid at the reduced pH levels of the acid combinations. A 2 min dip in 5% acetic acid at room temperature was the most effective treatment of several investigated for reducing populations of *E. coli* O157:H7 inoculated onto apple surfaces (Wright and others 2000). The 5% acetic acid treatment reduced the population more than 3 log CFU/cm^2 as compared to less than a 3 log reduction by a commercial preparation with 80 ppm peroxyacetic acid. It was noteworthy that the 2 min dip treatment with a commercial 0.3% phosphoric acid-based fruit wash caused sublethal injury to *E. coli* O157:H7 as measured by a comparison of counts on selective and nonselective media.

Antimicrobial activity varies among the organic acids. Citric acid was much less effective than tartaric acid in preventing growth of microorganisms on salad vegetables (Shapiro and Holder 1960). A concentration of 1500 ppm citric acid did not affect bacterial growth, while treatment with 1500 ppm tartaric acid resulted in a 10-fold reduction in counts after 4 days at 10 °C (50 °F). Priepke and others (1976) reported that microbial populations of cut lettuce, endive, carrots, celery, radishes, and green onions treated with 2000 ppm sorbate and/or 10,000 ppm ascorbate, then stored 10 days at 4.4 °C (40 °F), were not effectively controlled. Coliforms and fecal coliforms were reduced about 2 and 1 log/g, respectively, on mixed salad vegetables treated with 1% lactic acid (Torriani and others 1997). In the same study, treatment of the mixed vegetables with a 3% sterile permeate from a culture of *Lactobacillus casei* reduced the total mesophilic count about 5 log/g and prevented growth of coliforms, enterococci, and *Aeromonas hydrophila* after 6 days at 8 °C (46.4 °F).

Orthophosphoric acid with added surfactants is commonly used in the citrus processing industry for both cleaning and sanitizing purposes. Pao and Davis (1999) demonstrated that immersion of oranges in a 200 ppm phosphoric acid/surfactant solution decreased *E. coli* populations only slightly better than immersion in deionized water alone. Winniczuk (1994) determined that dipping oranges for 15 seconds in 500 ppm of a commercial phosphoric acid surfactant solution after brush-washing in water reduced surface populations approximately 85%, as compared to 60% for brush-washing alone.

2.9. Alkaline compounds

In a laboratory study of suspended and attached cells of various foodborne pathogens on nonfood surfaces, *E. coli* O157:H7 populations were reduced 5 and 6 log after a 30-second treatment with 1% trisodium phosphate (TSP) at 10 °C (50 °F) and room temperature, respectively (Somers and others 1994). *Campylobacter jejuni* was almost as sensitive as *E. coli* O157:H7 to TSP. Treatment with 8% TSP decreased populations of *L. monocytogenes* only 1 log cycle. Resistance of *L. monocytogenes* to TSP was also reported by Zhang and Farber (1996). A 5-minute treatment with 2% TSP produced a 1 log reduction of *Salmonella* Chester attached to the surface of apple disks (Liao and Sapers 2000). *Salmonella* Montevideo populations on the surface of tomatoes were reduced from 5.2 log CFU/cm^2 to nondetectable levels after 15 seconds in 15% TSP (Zhuang and Beuchat 1996). A significant reduction in population was observed after 15 seconds in 1% TSP. Populations of *S.* Montevideo within the core tissue of tomatoes were less affected by TSP, although significant reductions were observed. A 30-second treatment of 4% TSP reduced the numbers of *E. coli* O157:H7 on alfalfa seeds from 2.5 log CFU/g to nondetectable levels (<0.30 log CFU/g) (Taormina and Beuchat 1999a). Reductions of populations of *E. coli* inoculated onto orange surfaces were not significantly different after immersion in 2% TSP for 8 min as compared to immersion in deionized water (Pao and Davis 1999). Various high pH cleaners containing sodium hydroxide, potassium hydroxide, sodium bicarbonate,

and/or sodium orthophenylphenate (with or without surfactants) reduced populations of *E. coli* on orange surfaces (Pao and others 2000). These same researchers determined that high pH waxes used on fresh market citrus provided substantial inactivation of *E. coli* on orange fruit surfaces (Pao and others 1999). The high pH of typical alkaline wash solutions (11 to 12) and concerns about environmental discharge of phosphates may be limiting factors for use of certain alkaline compounds on produce.

2.10. Peracetic acid alone and in combination with fatty acids

The efficacy of peracetic acid against microorganisms on produce has not been extensively reported. On stainless steel chips in the presence of organic matter, peracetic acid, and peroctanoic acid inactivated mixed-culture biofilms of *L. monocytogenes* and *Pseudomonas* sp. more effectively than chlorine (Fatemi and Frank 1999). When used at 40 and 80 ppm, a sanitizer that contains peracetic acid (Tsunami™ Ecolab, Mendota Heights, MN) significantly (*P*d"0.05) reduced salmonellae and *E. coli* O157:H7 populations on cantaloupe and honeydew melon surfaces (Park and Beuchat 1999). These treatments were less effective on asparagus spears. The brand of sanitizer used in this study is reported by the manufacturer to maintain its efficacy over a broader pH range and organic demand than hypochlorite, although it is more expensive.

Nearly 100-fold reductions in total counts and fecal coliforms on cut-salad mixtures were observed after treatment with 90 ppm peroxyacetic (peracetic) acid or with 100 ppm chlorine (Masson 1990). The subsequent inhibition of microbial growth during storage of salads was attributed to residual peracetic activity. Microbial populations on the surface of oranges were reduced about 85% after brush-washing in water followed with a 15-second dip in 200 ppm peracetic acid, compared to a 60% reduction on oranges that were brush-washed and dipped in plain water (Winniczuk 1994).

Confidential research results from one company indicated that a static 2-minute treatment of inoculated tomatoes with a sanitizer formulation containing 60 ppm peracetic acid in combination with surfactants reduced populations of *Salmonella* Javiana, *L. monocytogenes*, and *E. coli* O157:H7 by 96%, 99.96% and 99.5%, respectively, compared with treatment in sterile water. Similar results were obtained with a second sanitizer formulation containing 40 ppm peracetic and surfactants.

2.11. Hydrogen peroxide

Juven and Pierson (1996) reviewed research reports on the antimicrobial activity of H_2O_2 and its use in the food industry. H_2O_2 possesses bactericidal and inhibitory activity due to its properties as an oxidant, and due to its capacity to generate other cytotoxic oxidizing species such as hydroxyl radicals. The sporicidal activity of H_2O_2 coupled with rapid breakdown makes it a desirable sterilant for use on some food contact surfaces, and packaging materials in aseptic filling operations. Residual H_2O_2 level may vary dependent on the presence or absence of peroxidase in the produce item.

Use of H_2O_2 on whole and fresh-cut produce has been investigated in recent years. *Salmonella* populations on alfalfa sprouts were reduced approximately 2 log CFU/g after treatment for 2 min with 2% H_2O_2 or 200 ppm chlorine (Beuchat and Ryu 1997). Less than 1 log CFU/g reduction was observed on cantaloupe cubes under similar test conditions. Treatment with 5% H_2O_2 bleached sprouts and cantaloupe cubes. Treatment of whole cantaloupes, honeydew melons, and asparagus spears with 1% H_2O_2 was less effective at reducing levels of inoculated salmonellae and *E. coli* O157:H7 than hypochlorite, acidified sodium chlorite or a peracetic acid-containing sanitizer (Park and Beuchat 1999). Use of a 1% H_2O_2 spray on alfalfa seeds and sprouts did not control growth of *E. coli* O157:H7 (Taormina and Beuchat 1999b). H_2O_2 (3%), alone or in combination with 2 or 5% acetic acid sprayed onto green peppers, reduced *Shigella* populations approximately 5 log cycles, compared to less than a 1-log reduction by water alone (Peters 1995). In the same study, *Shigella* inoculated onto lettuce was reduced approximately 4 log after dipping in H_2O_2 combined with either 2 or 5% acetic acid; however, obvious visual defects were noted on the treated lettuce. The same treatment gave similar results for *E. coli* O157:H7 inoculated onto broccoli florets or tomatoes with minimal visual defects.

Microbial populations on whole cantaloupes, grapes, prunes, raisins, walnuts, and pistachios were significantly reduced upon treatment with H_2O_2 vapor (Sapers and Simmons 1998). Treatment by dipping in H_2O_2 solution reduced microbial populations on fresh-cut bell peppers, cucumber, zucchini, cantaloupe, and honeydew melon, but did not alter sensory characteristics. Treatment of other produce was not as successful. H_2O_2 vapor concentrations necessary to control *Pseudomonas tolaasii* caused mushrooms to turn brown, while anthocyanin-bleaching occurred in strawberries and raspberries. Shredded lettuce was severely browned upon dipping in a solution of H_2O_2. Combinations of 5% H_2O_2 with acidic surfactants at 50 °C (122 °F) produced a 3 to 4 log reduction of nonpathogenic *E. coli* inoculated onto the surfaces of unwaxed Golden Delicious apples (Sapers and others 1999). Further research is necessary to determine the usefulness of H_2O_2 treatment on other fruits and vegetables.

2.12. Ozone

The use of ozone as an antimicrobial agent in food processing was reviewed by Kim and others (1999b) and Xu (1999); however, little has been reported about the inactivation of pathogens on produce. Salmonellae and *E. coli* populations were reduced 3 to 4 log/g in ground black pepper after 60 min treatment with ozonated air (6.7 mg/L at a flow rate of 6 L/min); however, significant changes in the volatile oil profiles were also noted (Zhao and Cranston 1995). Volatile oils in whole black peppercorns treated in ozonated water were not significantly affected.

Ozone is an effective treatment for drinking water and will inactivate bacteria, fungi, viruses, and protozoa (Peeters and others 1989; Korich and others 1990; Finch and Fairbairn 1991; Restaino and others 1995). According to Restaino and others (1995), bacterial pathogens such as *Salmonella* Typhimurium, *Y. enterocolitica, S. aureus*, and *L. monocytogenes* are sensitive to treatment with 20 ppm ozone in water. Finch and Fairbairn (1991) investigated the sensitivity of enteric viruses to ozone, while Korich and others (1990) reported on the ozone inactivation of protozoa such as *Cryptosporidium parvum*. Treatment of *C. parvum* oocysts with 1 ppm ozone for 5 min resulted in < 1 log inactivation. In the same study, *Giardia* spp. cysts were more sensitive than *C. parvum* to ozone treatment. Peeters and others (1989) reported that 2.27 ppm ozone treatment for 8 min eliminated the infectivity of 5×10^5 *C. parvum* oocysts in water.

Treatment with ozonated water can extend the shelf life of apples, grapes, oranges, pears, raspberries, and strawberries by reducing microbial populations and by oxidation of ethylene to retard ripening (Beuchat 1998). Microbial populations on berries and oranges were reduced by treatment with 2-3 ppm and 40 ppm, respectively. Kim and others (1999a) reported a 2 log/g reduction in total counts for shredded lettuce suspended in water ozonated with 1.3 mM ozone at a flow rate of 0.5 L/min.

In contrast to the use of ozone as an initial treatment to reduce microbial populations on produce surfaces, ozone gas has also been investigated for use during storage of various foods, including fish (Haraguchi and others 1969), poultry (Sheldon and Brown 1986), peanuts and cottonseed meal (Dwankanath and others 1968), pork, beef, dairy products, eggs, mushrooms, potatoes, and fruits (Kaess and Weidemann 1968; Gammon and Ker-

elak 1973). Apples stored in an atmosphere containing ozone had reduced incidents of spoilage (Bazarova 1982). Fungal growth during storage of blackberries was inhibited by 0.1 to 0.3 ppm ozone (Barth and others 1995). Treatment of grapes by ozone increased shelf life and reduced fungal growth (Sarig and others 1996). Spoilage of vegetables such as onions, potatoes, and sugar beets was reduced upon storage in an ozone containing atmosphere (Kim and others 1999b).

Due to its strong oxidizing activity, ozone may cause physiological injury of produce (Horvath and others 1985). Bananas treated with ozone developed black spots after 8 days of exposure to 25 to 30 ppm gaseous ozone. Carrots exposed to ozone gas during storage had a lighter, less intense color than untreated carrots (Liew and Prange 1994). Ozone can also cause corrosion of metals and other materials in processing equipment. It is capital intensive and may be difficult to monitor and control in situations where highly variable organic loads are likely to occur. As with other sanitizers, employee safety and health issues must be addressed and appropriate safeguards must be in place when using ozone as a sanitizing agent. Since ozone produces toxic vapors, adequate ventilation is necessary for employee safety. However, since it has excellent ability to penetrate and does not leave a residue, ozone may have usefulness for treatment of process water, food contact surfaces, or whole produce. Industry representatives indicate that the postharvest use of ozone for treatment of produce is increasing.

2.13. Irradiation

Ionizing radiation from ^{60}Co, ^{137}Cs, or machine generated electron beams, alone or in combination with other treatments such as hot water, is used as a means of extending shelf life of produce (Diehl 1995; Thayer and others 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, fruits, and nuts, and kill parasites in meats. Medium dose treatments (1 to 10 kGy) reduce microbial populations, including pathogens, on or in foods. Elimination of pathogens on meat, seafood, and poultry by medium dose irradiation has been studied. It should be noted that produce treated by doses above the level of 1 kGy cannot use the term "fresh" (21CFR101.95). High doses of irradiation (10 to 45+ kGy) produce shelf-stable packaged meats and specialized hospital meals.

In a review on irradiation and produce, Thayer and Rajkowski (1999) state, "To date, relatively little effort has been applied to the control of foodborne pathogens on fresh foods. However, ionizing irradiation has recently been used to eliminate *Escherichia coli* O157:H7 from apple juice, *Toxoplasma gondii* and/or *Cyclospora cayetanensis* from raspberries, and *E. coli* O157:H7 and salmonellae from seed and sprouts." Research on the effectiveness of irradiation against human pathogens has been conducted mostly on food products of animal origin (Mossel and Stegeman 1985; Farkas 1989; Monk and others 1995); however, Rajkowski and Thayer (2000) reported that salmonellae were not recovered from alfalfa sprouts irradiated with 0.5 kGy even though the seeds used to produce the sprouts contained detectable levels of the pathogen. These researchers concluded that ionizing radiation can be used to reduce pathogen populations on sprouts. Buchanan and others (1998) determined that 1.8 kGy will produce a 5-log reduction of *E. coli* O157:H7 in apple juice. These same researchers reported that acid-resistant stationary phase cells of enterohemorrhagic *E. coli* are more resistant to irradiation than non-acid-resistant cells (Buchanan, Edelson, and Boyd 1999).

Doses in the range of <1 to 3 kGy have been shown to reduce or eliminate populations of foodborne pathogens, postharvest spoilage organisms, and other microorganisms on produce (Moy 1983; Urbain 1986; Farkas 1997). Most medium and high level doses are not appropriate for produce because they can cause sensory defects (visual, texture, and flavor) and/or accelerated senescence due to irreparable damage to DNA and proteins (Thomas 1986; Barkai-Golan 1992). Treatment of unpasteurized orange juice with 3 kGy electron-beam irradiation reduced *E. coli* populations inoculated into the juice by at least 5 log, but had unacceptable sensory consequences (Parish and Goodrich 2000; personal communication; unreferenced). Strawberry shelf life can be extended with treatments in the range of 2 to 3 kGy (Sommer and Maxie 1966; Zegota 1988; Marcotte 1992; Diehl 1995). Maxie and others (1971) asserted that strawberry is the only domestic fruit or vegetable with adequate potential to utilize irradiation for shelf life extension, since other commodities do not tolerate dosage levels needed to control spoilage. Research conducted since that time suggests that irradiation can be an important treatment to enhance safety of other types of produce. Postharvest disease incidence in apples and Bosc pears was reduced after 0.3 to 0.9 kGy irradiation treatment (Drake and others 1999). Disease incidence of Anjou pears was not reduced.

Use of ionizing radiation to eliminate insect pests, and to control postharvest spoilage organisms on fresh produce has been reviewed (Clarke 1959; Willison 1963; Staden 1973; Moy 1983; CAST 1986, 1989; Barkai-Golan 1992; Wilkinson and Gould 1996) and guidelines for treatment have been issued (Anonymous 1991a, 1991b, 1993). Combinations of ionizing radiation with other treatments have been studied. A combination of 0.75 kGy irradiation with a 10 min dip in 50 °C (122 °F) water provided much better control of postharvest spoilage organisms of papayas and mangoes than either treatment alone (Brodrick and van der Linde 1981). Neither irradiation (0.3 to 0.6 kGy), hot fungicide treatment, nor a combination of the two, satisfactorily prevented postharvest spoilage of mangoes (Johnson and others 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 and others 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 1998a).

The shelf life of packaged leaf vegetables stored at 10 °C (50 °F) was extended by treatment with 1 kGy (Langerak 1978). In this study, *Enterobacteriaceae* were eliminated on endive and the shelf life was extended from 1 (for nonirradiated) to 5 days. Chervin and Boisseau (1994) concluded that irradiation of shredded carrots was superior to chlorination and spin-drying. Microbial populations (measured as total plate counts) of shredded carrots treated with 0.5 kGy or chlorine and stored 9 days under refrigeration were 1300 and 87,000 CFU/g, respectively (Hagenmaier and Baker 1998b). The same authors reported a similar reduction of microbial populations on cut iceberg lettuce treated with 0.19 kGy (Hagenmaier and Baker 1997). A combination of hot water dips and 1.0 kGy irradiation doubled the shelf life of mangoes from 25 to 50 days (El-Samahy and others 2000).

As discussed in the recent FDA report, "Kinetics of microbial inactivation for alternative food processing technologies" (FDA 2000), high intensity pulsed X-rays have been shown to reduce *E. coli* O157:H7 populations in ground beef by 3 log cycles, and to decrease *Salmonella* Senftenberg on turkey carcasses. Studies on the use of X-rays to inactivate pathogens on/in produce may be warranted.

Consumer acceptance of irradiated food remains questionable. A publication by USDA-ERS suggests that the number of consumers likely to purchase irradiated food has decreased in recent years from about 70% in 1996 to 50% in 2000 (Frenzen and oth-

ers 2000). Additionally, there is a need to ensure that research on irradiation addresses sensory aspects, such as taste, appearance and texture, of produce.

2.14. Biocontrol

There are few published reports on the use of biocontrol agents to prevent growth of human pathogens on produce. Janisiewicz and others (1999) reported that *Pseudomonas syringiae* prevented growth of *E. coli* O157:H7 in wounds of apples. Populations of the pathogen increased 2 log in wounds that were not treated with the antagonist but did not increase in wounds treated with *P. syringiae*. *Enterococcus mundtii* did not prevent growth of *L. monocytogenes* on fresh produce but did inhibit growth of the pathogen on vegetable agar (Bennik and others 1999). Mundticin, a bacteriocin produced by *E. mundtii*, was reported to have potential as a biopreservative on modified atmosphere-stored mungbean sprouts. Populations of *L. monocytogenes* inoculated onto endive leaves were inhibited by treatment with a mixed population of microorganisms originally isolated from endive (Carlin and others 1996). Strains of lactic acid bacteria were reported to inhibit *A. hydrophila, L. monocytogenes, Salmonella* Typhimurium, and *S. aureus* on vegetable salads (Vescovo and others 1996).

The application of microorganisms to prevent proliferation of postharvest spoilage organisms has been studied to a greater extent than for control of human pathogens on produce surfaces (Liao 1989; Smilanick and Denis-Arrue 1992; Stanley 1994; Janisiewicz and Bors (1995); Korsten and others 1995; Leibinger and others 1997; Calvente and others 1999; El-Ghaouth and others 2000; Usall and others 2000). Studies suggest that nonpathogenic microorganisms applied to produce surfaces might out-compete pathogens for physical space and nutrients, and/or may produce antagonistic compounds that negatively affect viability of pathogens. Research on biocontrol of human pathogens on produce is warranted.

The use of bacteriophage to reduce populations of *Salmonella* on fresh-cut fruit was recently reported (Leverentz and others 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 (41 or 50 °F). Salmonellae were not reduced on apple slices possibly due to the fruit's lower pH (4.2). Use of phage for pathogen control deserves further investigation.

The concept of "induced resistance" of plants to microorganisms that cause pathologies in plant systems is worth noting (Hammerschmidt 1999). In recent years groups of 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.

2.15. Miscellaneous

Numerous plant-derived compounds with antimicrobial properties have been studied for use in food systems (Cherry 1999). Although their usefulness may be limited due to undesirable sensory effects, naturally derived food compounds and essences have shown antimicrobial activity against human pathogens in laboratory studies. Compounds such as various bacteriocins, cinnamaldehyde, diacetyl, benzaldehyde, pyruvic aldehyde, piperonal, basil methyl charvicol, vanillin, psoralens, jasmonates, allyl-isothiocyanate, lactoferricin, hop resins, and essences of garlic, clove, cinnamon, coriander, and mint have been studied for antimicrobial activity in various food systems (Isshiki and others 1992; Tokuoka and Isshiki 1994; Bowles and others 1995; Delaquis and Mazza 1995; Lis-Balchin and others 1996; Cerrutti and others 1997; Ulate-Rodriguez and others 1997; Bowles and Juneja 1998; Buta and Moline 1998; Wan and others 1998; Chantaysakorn and Richter 2000; Fukao and others 2000). Further information is needed regarding the effects of specific plant derivatives, and other naturally occurring compounds, on human pathogens and produce.

2.16. Alternative technologies

Although nonthermal and other alternative technologies, such as high pressure, pulsed electric field, pulsed light, oscillating magnetic fields, ultrasound and UV treatments, have been investigated to reduce or eliminate microorganisms in foods, there is little published research directly related to the impact of these technologies on the safety of fresh whole or cut produce (FDA 2000). Limited data regarding the use of these technologies for unpasteurized juices has been published. Although a recent study showed 4 to 8 log reductions of *Salmonella* spp. or *E. coli* O157:H7 after high pressure processing at 615 MPa, there was no indication if death rates of the nonacid resistant inocula were influenced by the acidic nature of the fruit juices (Teo and others 2001). There is a regulatory question whether produce treated by these technologies may be labeled as "fresh"; however, further research on the effects of alternative treatments on produce is warranted.

3. Summary

The primary method to eliminate, or significantly reduce, pathogens on produce is strict adherence to Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs), Hazard Analysis Critical Control Points (HACCP), and other relevant strategies that prevent contamination from occurring. This includes the concept of "good management practices" as described in the Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (FDA 1998). Although the frequency of produce contamination by pathogens is thought to be very small, there are no known mitigation strategies that will completely remove pathogens after contamination has occurred while maintaining produce freshness. A variety of mitigation regimens and sanitizers are available to reduce microbial populations depending upon the type of produce involved. Washing and sanitizing efficiencies depend on several factors, including characteristics of the produce surface, water quality, cleaner/sanitizer used, contact time, and presence and type of scrubbing action. Based on reported data, it is likely that different sanitation mitigation strategies are needed for different produce items.

4. Research Needs

In order to adequately address safety issues associated with fresh produce, it is necessary to enhance the quantity and quality of research on mitigation strategies. A few of the research needs include:

- Investigate traditional and nontraditional sanitizers on specific pathogen/produce combinations.
- Survey extensively domestic and imported products to determine the frequency of public health microorganisms on specific produce items.
- Survey comprehensively to determine pathogen concentrations on/in various types of produce.
- Determine additive, antagonistic, or synergistic effects of sanitation treatments when used in combination.
- Evaluate the enhancement of physical washing methods by various techniques.
- Investigate the likelihood of pre- or postharvest microbial infiltration into produce interiors and the significance for produce safety.
- Assess interactions between human pathogens and posthar-

vest spoilage organisms that may cause pathogen infiltration into produce tissues.

• Investigate biocontrol and competitive exclusion as mitigation strategies.

• Develop new sanitizers and innovative technologies for sanitation treatment of produce.

• Develop treatments to eliminate pathogens in animal wastes used during production of produce.

• Identify treatments to eliminate pathogens in irrigation water.

• Investigate the use of alternative technologies on the safety of whole and cut produce.

• Investigate sanitizer effects on pathogens other than bacteria.

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