**Journal of Industrial Microbiology & Biotechnology (2001) 27,** 104–110
© 2001 Nature Publishing Group 1367-5435/01 $17.00
www.nature.com/jim

# FOOD-BORNE PATHOGENS

# Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination[†]

SL Burnett and LR Beuchat

*Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797*

**The ability of public health agencies to identify, through enhanced epidemiologic and surveillance techniques, raw fruits, vegetables, and unpasteurized juices as probable sources of infectious microorganisms, has undoubtedly resulted in increased numbers of documented outbreaks. Changes in agronomic, harvesting, distribution, processing, and consumption patterns and practices have also likely contributed to this increase. The risk of illness associated with raw produce and unpasteurized produce products can be reduced by controlling or preventing contamination, or by removing or killing pathogenic microorganisms by washing or treating them with sanitizers. However, the hydrophobic cutin, diverse surface morphologies, and abrasions in the epidermis of fruits and vegetables limit the efficacy of these treatments.** *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 104–110.

**Keywords:** fruit; vegetable; pathogen; sanitizer

## Introduction

The frequency of documented outbreaks of human illness associated with consumption of raw fruits and vegetables, as well as unpasteurized juices, has increased in the United States in recent years [19]. Salmonellosis has been linked to tomatoes, seed sprouts, cantaloupe, watermelon, apple juice, and orange juice. *Escherichia coli* O157:H7 infection has been associated with lettuce, alfalfa sprouts, and apple juice, and enterotoxigenic *E. coli* has been linked to carrots. Associations of shigellosis with lettuce, scallions, and parsley, cholera with strawberries, hepatitis A virus with lettuce, raspberries, and frozen strawberries, and Norwalk/Norwalk-like virus with melon, salad, and celery have also been documented. Most recently, *Cryptosporidium* infection linked to apple cider and *Cyclospora* infection linked to raspberries, lettuce, and basil have broadened awareness that produce-associated illnesses are not confined to bacteria and viruses as causative agents.

The epidemiology of foodborne diseases has undoubtedly contributed to an increased frequency of outbreaks of infections linked to raw produce. Changes in dietary habits, methods of produce production and processing, sources of produce, and the emergence of pathogens previously not recognized for their association with raw produce have enhanced the potential for outbreaks associated with raw fruits and vegetables [8,26].

Although much is known about the ecology of pathogens in foods of animal origin, the behavior of pathogens in association with naturally occurring microflora on fruits and vegetables is less defined. Differences in surface morphology and metabolic functions of leaves, stems, florets, fruits, roots, and tubers provide a wide range of diverse ecological niches selective for specific species or groups of microorganisms. Bruised and cut surface tissues exude fluids containing nutrients and numerous phytoalexins and other antimicrobials such as organic acids that may enhance or retard the growth of naturally occurring microflora and pathogens. Colonization and biofilm development ensue, resulting in spoilage and growth of bacterial pathogens. Thus, viability of parasites and other infectious agents as affected by extrinsic and intrinsic factors unique to fruits and vegetables is largely unknown.

The microbial ecosystem on the surface of raw fruits and vegetables is diverse and complex. The presence and numbers of microorganisms differ, depending on the type of produce, agronomic practices, geographical area of production, and weather conditions before harvest [11,32,35]. Microbial ecosystems on produce after harvesting can be greatly influenced by handling and storage conditions as well as conditions of processing, packaging, distribution, and marketing. Numerous factors influence the range and populations of microorganisms associated with fruits and vegetables at any given point throughout their production and postharvest handling, thus influencing the rate and type of spoilage. The environment in which plants are grown impose extrinsic factors that affect associated surface microflora, whereas intrinsic parameters such as the nature of the epithelium and protective cuticle, tissue pH, and the presence of antimicrobials dictate which groups of produce may be more likely to harbor certain types of microorganisms in damaged tissues. The types of microorganisms recovered from raw fruits and vegetables at harvest most often reflect the microflora present in the field, orchard, grove, or vineyard at the time of harvest [19,32,48]. Climatic and agricultural determinants affecting the microbial ecosystem at harvest include geographic location, history of precipitation, wind, irrigation practices, preharvest, harvest, and postharvest practices, and the presence of insects, animals, and birds [11].

---

Correspondence: Dr LR Beuchat, Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223-1797, USA

[†] This paper was previously published in *Journal of Industrial Microbiology & Biotechnology* (2000) 25, 281–287.
Received 23 June 2000; accepted 17 October 2000

Gram-negative bacteria dominate the microflora associated with most vegetables, whereas mold and weakly fermentative yeasts often comprise the majority of microflora on raw fruits, largely due to the acidic pH of fruit tissue, which generally is less than 4.0 [41].

To date, chemical treatments administered to whole and cut produce for the purpose of killing or removing pathogens have not been demonstrated to reduce populations by more than about 3 $\log_{10}$ CFU/g [7]. Reasons for their ineffectiveness stem largely from an inability of the potentially lethal chemical components to access microbial cells lodged in discontinuities and biofilms on the surface of produce. Protection against contact of cells with sanitizers results in an increased likelihood of the presence of pathogens on fruits and vegetables at the time of consumption and, therefore, an increased risk of illness. Summarized here are recent outbreaks of infections associated with raw fruits and vegetables and possible reasons for difficulties in achieving decontamination.

## Sources of contamination

Although spoilage bacteria, yeasts, and molds dominate the microflora of fruits and vegetables, the occasional presence of foodborne pathogens associated with these foods has been recognized for many years [7,22]. Any type of produce has a potential to harbor pathogens [11], but *Shigella* spp., *Salmonella*, enterotoxigenic and enterohemorrhagic *E. coli*, *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses, and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of greatest public health concern [6,7,19,36,44]. Table 1 lists examples of raw fruits and vegetables from which pathogenic bacteria have been isolated.

**Table 1** Examples of raw produce from which bacterial pathogens have been isolated[a]

| Pathogen | Produce |
|---|---|
| *B. cereus* | Cress sprouts, mustard sprouts, soybean sprouts |
| *Campylobacter jejuni* | Mushrooms |
| *C. botulinum* | Cabbage, mushrooms |
| *E. coli* O157:H7 | Alfalfa sprouts, cabbage, celery, cilantro, coriander |
| *L. monocytogenes* | Bean sprouts, cabbage, chicory, cucumbers, leafy salad greens |
| *Salmonella* | Alfalfa sprouts, artichoke, beet greens, cabbage, cauliflower, celery, eggplant, endive, fennel, lettuce, mung bean sprouts, mustard cress, parsley, peppers, salad greens, spinach |
| *Shigella* | Lettuce, parsley, salad vegetables, scallions |
| *Staphylococcus* | Lettuce, parsley, radish, salad vegetables, seed sprouts |
| *Y. enterocolitica* | Carrots, cucumbers, lettuce, tomatoes |
| *Vibrio cholerae* | Cabbage |

[a]From Beuchat [6], DeRoever [19], and NACMCF [34].

**Table 2** Examples of pathogens implicated in causing outbreaks of diseases associated with raw produce and produce products[a]

| Produce | Pathogen |
|---|---|
| *Whole and puree* | |
| Soy, cress, mustard sprouts | *B. cereus* |
| Raspberries, mesclun lettuce, basil | *C. cayetanensis* |
| Lettuce, alfalfa sprouts, radish sprouts | *E. coli* O157:H7 |
| Carrots | *E. coli* (enterotoxigenic) |
| Salad vegetables | *G. lamblia* |
| Lettuce, raspberries, frozen strawberries | Hepatitis A virus |
| Cabbage | *L. monocytogenes* |
| Sliced melon, green salad, celery | Norwalk/Norwalk-like virus |
| Tomatoes, watermelon, cantaloupe, sprouts (alfalfa, clover, mung bean), mamey, mango | *Salmonella* |
| Lettuce, scallions, parsley | *Shigella* |
| Cabbage | *Vibrio cholerae* |
| *Juice* | |
| Apple | *C. parvum* |
| Apple | *E. coli* O157:H7 |
| Apple, orange | Salmonellae |
| Coconut milk | *Vibrio cholerae* |

[a]From DeRoever [19], Kapperud *et al* [29], NACMCF [34], Nguyen-The and Carlin [35], and Taormina *et al* [44].

Pathogens, along with spoilage microorganisms, may contaminate fruits and vegetables *via* several different routes and at several points throughout the preharvest and postharvest system. Sources of contamination have been described [6,8]. Potential preharvest sources of microorganisms include soil, feces, irrigation water, water used to apply fungicides and insecticides, dusts, insects, inadequately composted manure, wild and domestic animals, and human handling. Potential postharvest sources include feces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, rinse water, ice, transport vehicles, and processing equipment [6–8,19,28]. Janisiewicz *et al* [28] demonstrated that fruit flies contaminated with a fluorescent-tagged nonpathogenic strain of *E. coli* served as a vector in colonizing apple wounds. These researchers isolated fluorescing *E. coli* from apple wounds within 48 h of exposure of apples to the flies.

Though the presence of pathogens on fruits and vegetables may be transient and secondary to spoilage microorganisms, produce have long been known to serve as vehicles for infectious agents. More recently, however, an increase in the number and frequency of outbreaks associated with produce has been documented (Table 2). According to statistics compiled by the Centers for Disease Control and Prevention (CDC), the number of reported produce-related outbreaks per year doubled between the period 1973–1987 and 1988–1992 in the United States [5].

## Increased consumption

Increases in numbers of produce-related outbreaks on an international scale have been attributed to a higher *per capita* consumption of fresh or minimally processed fruits and vegetables [8,22]. Data from the National Agricultural Statistics Service of the U.S. Department of Agriculture reveal a rise in the *per capita* consumption of fresh fruits and vegetables in the United States by almost 20 pounds from 1988 to 1996 [45]. This increase can be attributed, in part, to the consumer's desire to maintain a diet that promotes better health [2,26]. Also, advances in agronomic and harvesting practices, processing, packaging, distribution, and marketing have enabled year-round importation of high-quality raw produce from Central and South America, as well as from countries in other parts of the world, to the United States [7].

Hedberg *et al* [26] described some factors that contribute to the epidemiology of foodborne disease associated with fresh produce and other foods. These include changes in diet, increased consumption of food in commercial food service establishments, increased handling, and the development of large and complex international networks of distribution. Brakett [10] stated that improper refrigeration is probably the single greatest hazard associated with safety of chilled foods. Because of the complex nature of distribution networks that supply fresh produce to wholesale and retail markets, especially in the case of imported fruits and vegetables that may require longer delivery time, temperature abuse may result in an increased risk of microbiological spoilage and/or growth of pathogenic bacteria. At least two major produce-associated outbreaks can be attributed to globalization of the food supply. Outbreaks of shigellosis in Norway, Sweden, and the United Kingdom in 1994 were linked to contaminated iceberg lettuce imported from Spain [30] and an outbreak of cyclosporiasis in the United States was linked to consumption of raspberries imported from Guatemala [27].

*E. coli* O157:H7, *Salmonella*, and *Cryptosporidium* infections associated in recent years with the consumption of unpasteurized apple and orange juices [15–18] have increased awareness of the presence of pathogens associated with fruits previously considered too acidic to serve as vehicles for pathogenic bacteria. Outbreaks associated with fruit juices have caused the U.S. Food and Drug Administration to alter labeling laws, requiring manufacturers of unpasteurized juices to include warnings on product labels describing the risk of exposure to pathogenic microorganisms. In addition, these outbreaks have raised interest in developing efficacious methods to kill, reduce, or remove pathogens that may be present on fruits intended for juicing.

## Surface morphology influences ease of removing pathogens

Washing and rinsing some types of fruits and vegetables prolong shelf life by reducing the number of microorganisms on the surface. However, only a portion of the microflora is removed with this simple treatment, only delaying the growth of spoilage and pathogenic microorganisms. With the addition of a disinfectant to wash water, the efficacy of decontamination can be enhance by up to 100-fold [7]. Sanitizers vary greatly in their ability to disinfect raw produce. The mechanism of bactericidal action, the nature and the location of the microorganisms, and the type of produce all influence the efficacy of decontamination treatments. The inability of sanitizers to remove all microorganisms on the surface of raw produce suggests that they are ineffective in removing cells more intimately associated with morphological structures. Microorganisms, including pathogens, may reside in protected sites on the epidermis of fruits and vegetables [7,23,40]. Although the protective mechanism of these sites is not well understood, the concept that hydrophobicity of microbial cells aids in their protection by inhibiting penetration of the disinfectant has been proposed.

The epidermis of fruits and vegetables is covered with a multilayered hydrophobic cuticle (1–15 $\mu$m thick) that provides the primary barrier against fungal invasion, insect and physical damage, and desiccation. The cuticle is composed of cutin, which is composed of high-molecular-weight lipid polyesters of long-chain substituted aliphatic acids. Imbedded within the cutin are crystalline and amorphous wax molecules that are responsible for the highly water-repellent nature of plant surfaces. Gas exchange takes place through pores in the epidermis called stomata. Stomata are protected by guard cells that open and close in response to changes in internal turgor pressure caused by environmental stimuli.

Lenticels are formed from stomata in maturing pome fruits and first appear to the unaided eye as small white or cream areas on the surface of the intact fruit. Glenn *et al* [24] observed that as apples mature, stomata open and become distorted. Following this transformation, 1000–1200 lenticels are formed on each apple. The invasion of fungi through lenticels of apples was studied as early as 1925 [31]. Surfaces of several vegetables and fruits are covered with tubular protuberances called trichomes that aid in inhibiting the invasion of insects and molds. As is the case with lenticels, the number of trichomes does not change with age, but are simply more widely dispersed over the surface of the vegetable or fruit as they increase in size.

## Attachment and infiltration

Bacterial attachment on the surface of sound produce is limited in contrast to attachment on processed meat tissues. However, attachment and infiltration of microbial cells do occur and are facilitated by the stomata, lenticels, broken trichomes, and bruises and cracks in the skin surface of fruits and vegetables [20,47]. Decontamination treatments are less effective in killing bacteria attached to or located within these protective structures. Using confocal scanning laser microscopy, Seo and Frank [40] observed metabolically active *E. coli* O157:H7 cells within stomata of lettuce leaves after treatment with a 20 ppm chlorine solution. They also observed that *E. coli* O157:H7 preferentially attached to cut edges as opposed to the intact leaf surface. Cells penetrated the interior of the cut tissue, resulting in protection against exposure to free chlorine in the treatment solution. Infiltration of *E. coli* O157:H7 into cut tissue of lettuce is enhanced at 4°C compared to 37°C [43]. Compromises in plant surfaces may also occur as a result of insect damage or mechanical injury during postharvest handling [37], or may form naturally [33,46]. The formation of cracks in the cuticle of apples is attributed primarily to a tendency toward marked restriction of growth in the epidermal layer late in the growing season while the fleshy portions of the fruit enlarge rapidly [46]. Cracks tend to occur at weak areas on the surface, e.g., around lenticels and trichomes. Furthermore, cavities within the epidermis may develop from cuticular cracks as the fruit develops [33], entrapping microorganisms and providing even more protection from decontamination treatments. Growth of microorganisms in protected areas on produce can result in the formation of biofilms [14] that are difficult for sanitizers to penetrate. The mechanisms of microbial attachment or lodging on the surface and internal tissues of fruits and vegetables are not well understood. It has been hypothesized, however, that hydrophobic interactions between the epidermal layer and microbial cells play a major

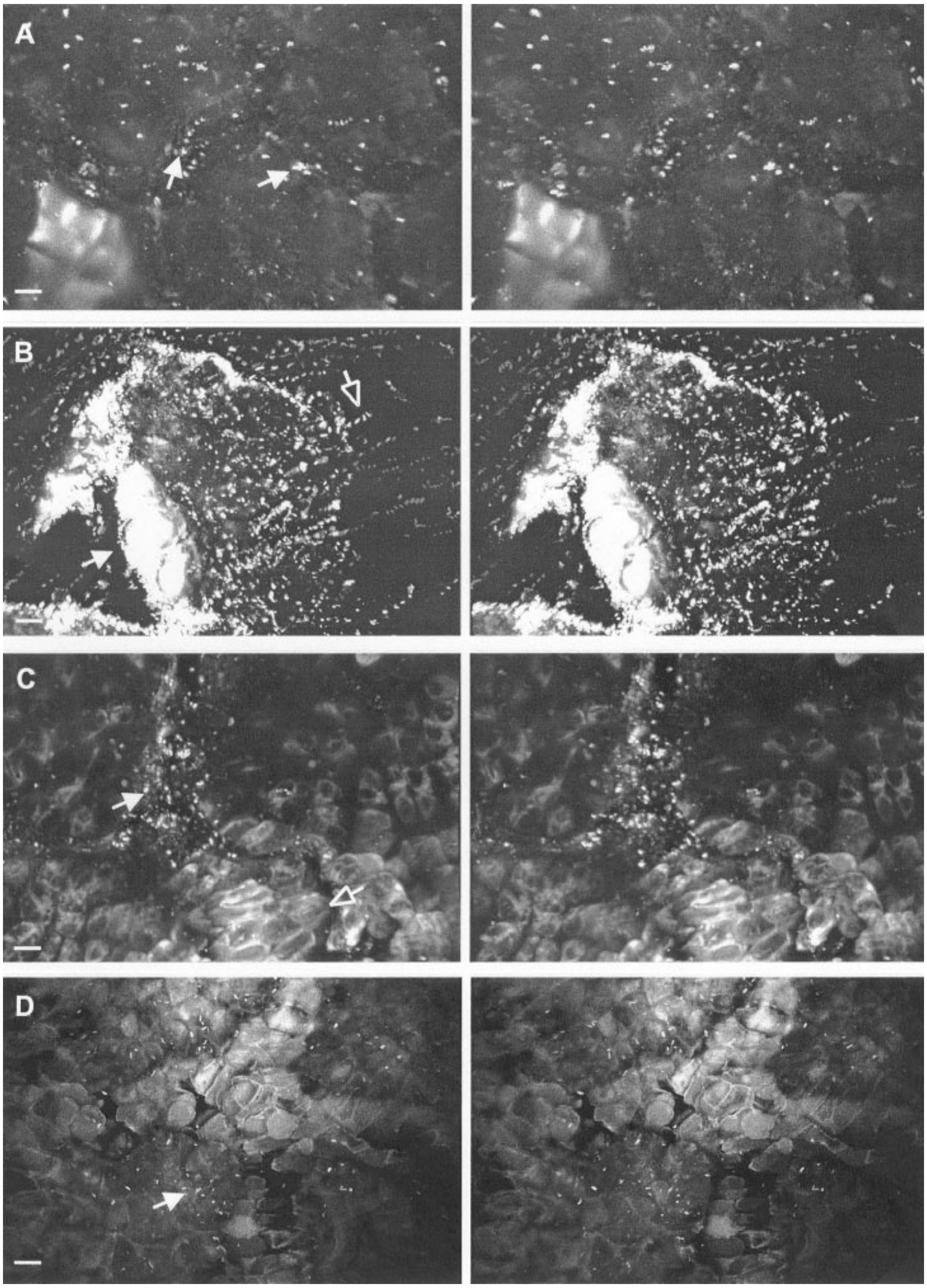

**Figure 1** Confocal scanning laser microscopy stereo images showing attachment of *E. coli* O157:H7 to apple surface structures. (A) Crevice (10 $\mu$m) in waxy cuticle of skin harboring attached cells (closed arrows). (B) Heavy colonization of attached (closed arrow) and unattached (open arrow) cells associated with damaged skin surrounding puncture (48 $\mu$m). (C) Cells (closed arrow) cells within a fissure emanating from loosely packed waxed platelets of lenticel. (D) Russet area showing cells (closed arrow) attached to cuticular ridge surrounding a groove containing wax platelets (20 $\mu$m). Bar = 10 $\mu$m. For additional information on methods used to inoculate and microscopically examine apples, see Burnett *et al* [13].

role in facilitating attachment to cuticular cracks, stomata, lenticels, and trichomes [23].

Several studies have demonstrated bacterial infiltration within produce tissues. *E. coli* O157:H7 in liquid suspension has been shown to infiltrate lenticels, russet areas, and punctures on the surface of apples (Figure 1) and suffuses into core structures *via* the floral tube and attaches to seeds, seed locules, and the cartilaginous pericarp of the core (Figure 2) [13]. Bacterial infiltration into apples is enhanced by a negative temperature differential, i.e., the temperature of the apple is higher than the temperature of the suspension in which it is immersed [12,13]. The U.S. Food and Drug Administration has recommended that packers consider the effects of water temperature when attempting to remove field heat from produce [21]. Other researchers demonstrated that washing tomatoes in water containing *Salmonella* at a temperature cooler than that of the tomatoes results in infiltration of the pathogen into the stem scar tissue [49]. Gases in the intercellular spaces in fruits or vegetables exert reduced pressure

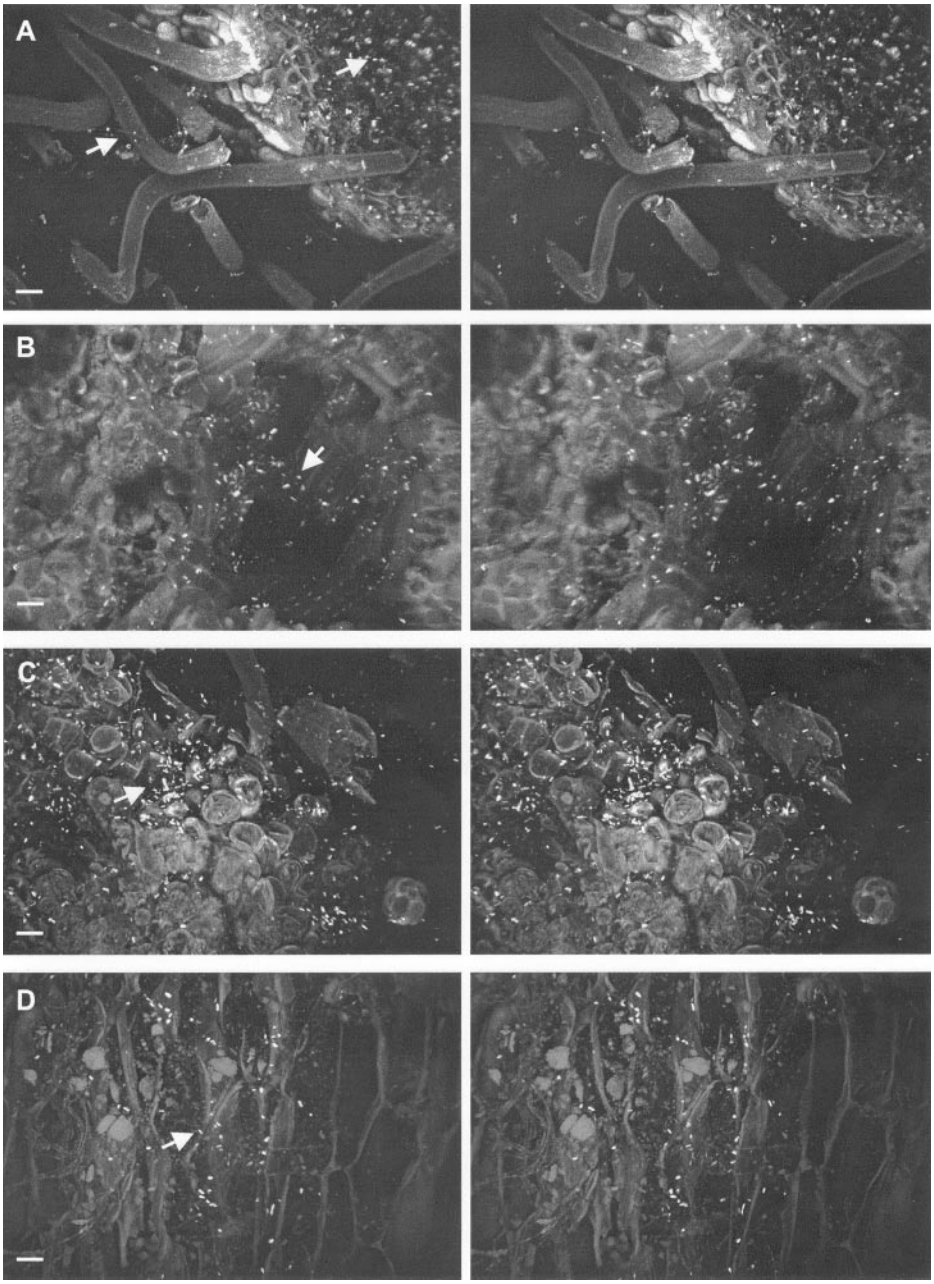

**Figure 2** Confocal scanning laser microscopy stereo images showing attachment of *E. coli* O157:H7 to internal apple structures. (A) Wall of floral tube (50 $\mu$m) showing cells (closed arrows) attached to irregular tissue and internal trichomes. (B) Tissue of ventral cavity harboring cells (closed arrow) at depths up to 40 $\mu$m. (C) Cells (closed arrow) attached to seed locule wall (28 $\mu$m). (D) Striated epidermal cells of seed integument (26 $\mu$m) harboring several bacterial cells (closed arrow). Bar = 10 $\mu$m. For additional information on methods used to inoculate and microscopically examine apples, see Burnett *et al* [13].

during cooling, which allows the combined atmospheric and hydrostatic pressure on the immersed produce to force some of the external environment, for example, contaminated water, into its apertures [3]. Bartz and Showalter [4] demonstrated that tomatoes submerged in a suspension of *Serratia marcesens* exposed to a negative temperature differential not only contained the bacterium more frequently, but also gained more mass than tomatoes exposed to a positive temperature differential. Buchanan *et al* [12] reported that immersing apples in a suspension of *E. coli* O157:H7 resulted in higher numbers of cells associated with the outer core region, which afforded them protection against a chlorine rinse. It was concluded that the potential for aspirating the pathogen into the internal structures of the fruit is increased by a negative temperature differential.

## Sanitizers have limited efficacy

Pathogens vary in their sensitivity to chlorine, for example, *L. monocytogenes* is generally more resistant than *Salmonella* and

*E. coli* O157:H7. Although chlorine and other sanitizers reduce populations of microbial cells exposed on the surface of produce by up to 2 or 3 $\log_{10}$ units, little is known about the efficacy of sanitizers in killing cells located in protected sites on the epidermis and within tissues. The influence of waxes and oils commercially applied to produce on the efficacy of sanitizers has likewise not been assessed. Several sanitizing agents have been evaluated for their effectiveness in reducing populations of microorganisms on produce. Adams *et al* [1] observed that treatment of salad greens with 100 ppm free chlorine reduced aerobic plate counts by 92% to 98%. Increasing concentrations of hypochlorite resulted in limited improvement of disinfection. This small improvement was attributed to an increased concentration of chlorine gas formed within hydrophobic structures. The addition of a surfactant, Tween 80, to hypochlorite reduced microbial numbers by 99.6% but caused unacceptable changes in sensory quality.

The use of 5 ppm chlorine dioxide ($ClO_2$) to wash whole fresh fruits and vegetables is allowed by the U.S. Food and Drug Administration. Its efficacy is less affected than hypochlorite by pH and organic matter [7]. With regard to the ability of $ClO_2$ to penetrate into hydrophobic sites on produce surfaces, Reina *et al* [38] observed that although 2.8 ppm $ClO_2$ was effective in killing planktonic bacteria in cooling water used to treat pickling cucumbers, the disinfectant had little effect on microorganisms on or in the fruit, suggesting that $ClO_2$ penetrated the protective sites on the cucumber epidermis poorly.

Ozone has been applied in the food industry in Europe for decades [25] and its use in food processing has recently been approved in the United Stated. Ozone is a more powerful sanitizer than chlorine, and can be applied to foods without concern about potentially hazardous residual compounds remaining after treatment. However, Spotts and Cervantes [42] reported that ozonated water did not control decay in wound-inoculated pears, nor did ozonated water effectively reduce fungal infection in inoculated wounds in apples.

The use of $H_2O_2$ to disinfect minimally processed fruits and vegetables has been reviewed [39]. Its use may be limited to produce that contain endogenous catalase activity to remove residual $H_2O_2$ as mandated by the U.S. Food and Drug Administration. The antimicrobial behavior of $H_2O_2$, applied either as a vapor or in solution, within specific hydrophobic locations on the surface of fresh produce has not been described.

Little is known about the survival of pathogens located at protective sites on the surface of produce after the application of wax or oil coatings. However, protection against sanitizers is likely to be enhanced by the additional hydrophobic layer. In the fresh produce industry, waxes such as carnauba and shellac are sprayed onto tumbling fruits to prevent migration of water and to slow respiration rates [30]. Produce undergoes partial decontamination treatment before waxing. Wash and rinse treatments performed by the consumer may not be sufficient to remove pathogens lodged beneath and within the wax layer.

The efficacy of sanitizers in killing pathogens within protective sites on raw fruits and vegetables has received little research attention. The fact that a standard method for evaluating the efficacy of produce sanitizers has not been established [7,9] confounds validation of the performance of sanitizers. There is a need for the development of a method that can be used to evaluate the effectiveness of a range of sanitizers applied to several types of unwaxed commercially waxed produce, giving particular attention to specific morphological features on the produce surface to elucidate the nature of hydrophobic protection of pathogens against contact with sanitizers.

## References

1 Adams MR, AD Hartley and LJ Cox. 1989. Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiol* 6: 69–77.
2 Alketruse SF and DL Swerdlow. 1996. The changing epidemiology of foodborne diseases. *Am J Med Sci* 311: 23–29.
3 Bartz JA. 1982. Infiltration of tomatoes immersed at different temperatures to different depths in suspensions of *Erwinia carotovora* subsp. *carotovora*. *Plant Dis* 66: 302–305.
4 Bartz JA and RK Showalter. 1981. Infiltration of tomatoes by aqueous bacterial suspensions. *Phytopathology* 71: 515–518.
5 Bean NH, JS Goulding, MT Daniels and FJ Angulo. 1997. Surveillance for foodborne disease outbreaks — United States, 1988–1992. *J Food Prot* 60: 1265–1286.
6 Beuchat LR. 1996. Pathogenic microorganisms associated with fresh produce. *J Food Prot* 59: 204–216.
7 Beuchat LR. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/98.2. 42 pp.
8 Beuchat LR and JH Ryu. 1997. Produce handling and processing practices. *Emerg Infect Dis* 3: 459–465.
9 Beuchat LR, BV Nail, BB Adler, and MRS Clavero. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *J Food Prot* 61: 1305–1311.
10 Brackett RE. 1992. Shelf stability and safety of fresh produce as influenced by sanitation and disinfection. *J Food Prot* 55: 808–814.
11 Brackett RE. 1999. Incidence, contributing factors, and control of bacterial pathogens on produce. *Postharvest Biol Technol* 15: 305–311.
12 Buchanan RL, SG Edelson, RL Miller, and GM Sapers. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *J Food Prot* 62: 444–450.
13 Burnett SL, J Chen and LR Beuchat. 2000. Attachment of *Escherichia coli* O157:H7 to the surface and internal structures of apples as demonstrated by confocal scanning laser microscopy. *Appl Environ Microbiol* 66: 4679–4687.
14 Carmichael I, IS Harper, MJ Coventry, PWJ Taylor, J Wan, and MW Hickey. 1999. Bacterial colonization and biofilm-development on minimally processed vegetables. *J Appl Microbiol* 85 (Symp Suppl): 45S–51S.
15 Centers for Disease Control and Prevention. 1996. Outbreaks of *Escherichia coli* O157:H7 infection associated with drinking unpasteurized commercial apple juice — British Colombia, California, Colorado, and Washington, December. *Morb Mortal Wkly Rep* 276: 1865–1866.
16 Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider — Connecticut and New York, October 1996. *Morb Mortal Wkly Rep* 46: 4–8.
17 Centers for Disease Control and Prevention. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice — United States and Canada, June 1999. *Morb Mortal Wkly Rep* 48: 582–585.
18 Cody SH, MK Glynn, JA Farrar, KL Cairns, PM Griffin, J Kobayashi, M Fyfe, R Hoffman, AS King, JH Lewis, B Swaminathan, RG Bryant, and DJ Vugia. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. *Ann Intern Med* 130: 202–209.
19 DeRoever C. 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9: 321–347.
20 Dingman DW. 2000. Growth of *Escherichia coli* O157:H7 in bruised apple (*Malus domestica*) tissue as influenced by cultivar, date of harvest, and source. *Appl Environ Microbiol* 66: 1077–1083.
21 Food Safety Initiative Staff, HFS-32. 1999. Guidance to industry: guide to minimize microbial food safety hazards for fresh fruits and

vegetables. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, DC.

22 Francis GA, C Thomas and D O'Beirne. 1999. The microbiological safety of minimally processed vegetables. *Int J Food Sci Technol* 34: 1–22.

23 Frank JF. 2001. Microbial attachment to food and food contact surfaces. *Adv Food Nutr Res* 43: 319–370.

24 Glenn GM, BW Pooviah, and HP Rasmussen. 1985. Pathways of calcium penetration through isolated cuticles of 'Golden Delicious' apple fruit. *J Am Soc Hortic Sci* 110: 166–171.

25 Graham DM. 1997. Use of ozone for food processing. *Food Technol* 51: 72–75.

26 Hedberg CW, KL MacDonald and MT Osterholm. 1994. Changing epidemiology of food-borne diseases: a Minnesota perspective. *Clin Infect Dis* 18: 671–682.

27 Herwaldt BL and ML Ackers. 1997. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *N Engl J Med* 336: 1548–1556.

28 Janisiewicz WJ, WS Conway, MW Brown, GM Sapers, P Fratamico and RL Buchanan. 1999. Fate of *Escherichia coli* O157:H7 on fresh cut apple tissue and its potential for transmission by fruit flies. *Appl Environ Microbiol* 65: 1–5.

29 Kapperud G, LM Rorvik, V Hasseltvedt, EA Hoiby, BG Iverson, K Staveland, G Johnson, J Leitao, H Herikstad, Y Andersson, G Langeland, B Gondrosen and J Lassen. 1995. Outbreak of *Shigella sonnei* infection traced to imported iceberg lettuce. *J Clin Microbiol* 33: 609–614.

30 Kester JJ and OR Fennema. 1986. Edible films and coatings: a review. *Food Technol* 12: 47–59.

31 Kidd MN and A Beaumont. 1925. An experimental study of the fungal invasion of apples in storage with particular reference to invasion through the lenticels. *Ann Appl Biol* 12: 14–33.

32 Lund BM. 1992. Ecosystems in vegetable foods. *J Appl Bacteriol* 13 (Symp Suppl): 115S–126S.

33 Meyer A. 1944. A study of the skin structure of golden delicious apples. *Proc Am Soc Hortic Sci* 45: 105–110.

34 National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *Int J Food Microbiol* 52: 123–153.

35 Nguyen-The C and F Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit Rev Food Sci Nutr* 34: 371–401.

36 Ortega YR, C Roxas, R Gilman, NJ Miller, L Cabrera, C Taquira and CR Sterling. 1997. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region Peru. *Am J Trop Med Hyg* 57: 683–636.

37 Quintana MEG and RE Paull. 1993. Mechanical injury during postharvest handling of 'solo' papaya fruit. *J Am Soc Hortic Sci* 118: 618–622.

38 Reina LD, HP Fleming and EG Humphries. 1995. Microbiological control of cucumber hydrocooling water with chlorine dioxide. *J Food Prot* 58: 541–546.

39 Sapers GM and GF Simmons. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. *Food Technol* 52: 48–52.

40 Seo KH and JF Frank. 1998. Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. *J Food Prot* 62: 3–9.

41 Splittstoesser DF. 1987. Fruits and fruit products. In: Beuchat LR (Ed), Food and Beverage Mycology. Van Nostrand Reinhold, New York, pp. 101–128.

42 Spotts RA and LA Cervantes. 1992. Effect of ozonated water on postharvest pathogens of pear in laboratory and packinghouse tests. *Plant Dis* 76: 256–259.

43 Takeuchi K and JF Frank. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the affect by inculum size and temperature and the effect of chlorine treatment on cell viability. *J Food Prot* 63: 434–440.

44 Taormina PJ, LR Beuchat and L Slutsker. 1999. Infections associated with eating seed sprouts: an international concern. *Emerg Infect Dis* 5: 626–634.

45 USDA. 1998. Agricultural statistics 1998. National Agricultural Statistics Service. Washington, DC, pp. XIII–5.

46 Verner L. 1938. Histology of apple fruit tissue in relation to cracking. *J Agric Res* 57: 813–824.

47 Wells JM and JE Butterfield. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. *Plant Dis* 81: 867–872.

48 Zagory D. 1999. Effects of post-processing handling and packaging on microbial populations. *Postharvest Biol Technol* 15: 313–321.

49 Zhuang R-Y, LR Beuchat and FJ Angulo. 1995. Fate of *Salmonella montevideo* on in raw tomatoes as affected by temperature and treatment with chlorine. *Appl Environ Microbiol* 61: 2127–2131.