Factors influencing the microbial safety of fresh produce: A review

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A B S T R A C T

Increased consumption, larger scale production and more efficient distribution of fresh produce over the past two decades have contributed to an increase in the number of illness outbreaks caused by this commodity. Pathogen contamination of fresh produce may originate before or after harvest, but once contaminated produce is difficult to sanitize. The prospect that some pathogens invade the vascular system of plants and establish "sub-clinical" infection needs to be better understood to enable estimation of its influence upon risk of human illness. Conventional surface sanitation methods can reduce the microbial load, but cannot eliminate pathogens if present. Chlorine dioxide, electrolyzed water, UV light, cold atmospheric plasma, hydrogen peroxide, organic acids and acidified sodium chlorite show promise, but irradiation at 1 kGy in high oxygen atmospheres may prove to be the most effective means to assure elimination of both surface and internal contamination of produce by pathogens. Pathogens of greatest current concern are Salmonella (tomatoes, seed sprouts and spices) and Escherichia coli O157:H7 on leafy greens (spinach and lettuce). This review considers new information on illness outbreaks caused by produce, identifies factors which influence their frequency and size and examines intervention effectiveness. Research needed to increase our understanding of the factors influencing microbial safety of fresh produce is addressed.

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1. Introduction

Fresh produce is popular worldwide because it is recognized as an important source of nutrients, vitamins and fibre for humans. The global production of fruit and vegetables grew by 94% from 1980 to 2004 (Fig. 1). From 1994 to 2004 in the United States importation of fruits and vegetables doubled to $12.7 billion (Aruscavage et al., 2006), and by 2005 daily sales of cut produce in North America reached 6 million packages (Jongen, 2005). Consumption of fresh produce has increased over the past two decades for many reasons; for example, consumers are more concerned about staying healthy and eating correctly, and in response to this demand a large variety of domestic and imported produce has become available in all seasons (Warriner et al., 2009). Globally, fruit and vegetable consumption increased on average 4.5% yearly between 1990 and 2004 (EU, 2007). The annual consumption of fruits and vegetables in the United States during 1997–1999 was 25% above the levels during 1977–1979 (FDA, 2001). In Canada, from 1963 to 2010, the annual consumption of fruits and vegetables increased by 56% and 26%, respectively (Statistics Canada, 2002, 2011).

At the same time, outbreaks of foodborne illnesses associated with the consumption of fresh produce have increased (Warriner et al., 2009). This increase may be due to: changes in personal consumption, increased intensity of livestock production near areas of intense (extensive) produce production, greater availability of produce worldwide (some originating from countries with uncertain sanitary practices), and increased numbers of immunocompromised consumers (Beuchat, 2002). It is common to find scholarly reports that Salmonella and Escherichia coli O157:H7 are the major pathogens contributing to outbreaks of foodborne illness associated with fresh produce (Buck et al., 2003; FDA, 1998; Warriner et al., 2009).

Since most fresh produce receives minimal processing and is often eaten raw, pathogen contamination can represent serious risk. Further, cutting, slicing or peeling cause tissue damage which releases nutrients and facilitates growth of microorganisms (Harris et al., 2003). Microbial contamination can occur during any of the steps in the farm-to-consumer continuum (production, harvest, processing, wholesale storage, transportation or retailing and handling in the home) and this contamination can arise from environmental, animal or human sources (FDA, 2001; WHO/FAO, 2008). To reduce the risk of pathogen contamination, the FDA in 1998 released a “Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables” which underlined the major reservoirs of pathogen contamination and methods required for their control (FDA, 1998). Post-harvest washing of fresh produce, usually with chlorine, is an important method for pathogen...
reduction (Warriner et al., 2009); however, factors limiting its effectiveness include internalization of pathogens within plant tissue, biofilm formation by bacteria as well as the hydrophobicity of plant surfaces (Whipp et al., 2008). Alternative methods for pathogen control on fresh produce include: irradiation (Gomes et al., 2009; Mahmoud, 2010); ozone (Najafi and Khodaparast, 2009; Selma et al., 2007); application of bacteriophages (Abuladze et al., 2008; Kocharunchitt et al., 2009; Sharma et al., 2009b); antagonistic bacteria (Cooley et al., 2003, 2006; Scolari et al., 2008; Scharff, 2010) and a combination of antagonistic bacteria with bacteriophages (Ye et al., 2009, 2010).

2. Outbreaks related to fresh produce

Foodborne illness is a major public health concern worldwide in terms of numbers of persons affected and economic cost. An updated estimate of yearly foodborne illnesses in the United States by the Centers for Disease Control and Prevention (CDC) concluded that about 48 million persons acquire foodborne illnesses with 128,000 hospitalization and 3000 deaths each year (Scallan et al., 2011). Health Canada (2011) estimates 11–13 million cases of foodborne illnesses occur in Canada every year.

In the last two decades foodborne illness outbreaks and cases associated with fresh produce have rapidly increased (Warriner et al., 2009). Scharff (2010) estimated that produce (fresh, canned or processed) causes 20 million illnesses (24%) costing 38.6 $ billion every year in the US. Produce was infrequently recognized in the US as a vehicle of illness outbreaks/cases in the 1970s (0.7% and 1%, respectively), but as epidemiological evidence mounted, by the 1990s frequencies changed to 6% and 12%, respectively (Sivapalasingam et al., 2004). This increased further from 1990 to 2003 in the US and reached 16% of outbreaks and 30% of cases (De Jong, 2007). Similarly, DeWaal and Bhuiya (2009) concluded that produce was responsible for 13% of illness outbreaks and 21% of cases in the US from 1990 to 2005. When a more recent interval was studied (1998–2007) it was found (CSPI, 2009) that fresh produce was associated with 14.8% of illness outbreaks that accounted for 22.8% of all foodborne illnesses in the US (Fig. 2). Produce, including salads, vegetables and fruits were linked to 345, 228 and 111 illness outbreaks, respectively, with the largest number of illnesses \((\geq 11,200)\) caused by each of the former two categories. An analysis of outbreak data in the US over the period 1988 to 2008 (Bean et al., 1996; FDA, 2009a; Olsen et al., 2000) shows there were an average of 6.3–13.2 illness outbreaks each year caused by produce where, more recently, leafy greens were responsible for one third (FDA, 2009a). In Canada, Sewell and Farber (2001) reported that 15 outbreaks were associated with produce between 1991 and 2000 which caused more than 1360 cases of foodborne illness.

Microorganisms that have been frequently associated with illness outbreaks related to consumption of fresh produce include: viruses (hepatitis A virus and norovirus); protozoa such as Cyclospora cayetanensis and Cryptosporidium parvum; and bacteria such as Aeromonas (A.) hydrophila, Bacillus (B.) cereus, Clostridium (Ct.) spp., E. coli O157:H7, Listeria (L.) monocytogenes, Salmonella (S.) spp., Shigella (Sh.) spp., Vibrio (V.) cholerae, Campylobacter (C.) spp. and Yersinia (Y.) enterocolitica (Arusacavage et al., 2006; Beuchat, 2002; Buck et al., 2003; Rangel et al., 2005; Sivapalasingam et al., 2004). It is notable that Salmonella and E. coli O157:H7 consistently cause large outbreaks of foodborne illness associated with fresh produce (Buck et al., 2003; FDA, 1998; Warriner et al., 2009).

As a result of better detection and epidemiological methods in the US, viruses have been more frequently implicated in causing illness outbreaks from produce: from 20% during 1973–1997 (Sivapalasingam et al., 2004) to \(>51\%\) during 1998–2007 (CSPI, 2009). Nonetheless, during the latter period E. coli O157:H7 and Salmonella were responsible for 7% and 17% of produce outbreaks, respectively (CSPI, 2009). Brandl (2006) reviewed food poisoning outbreaks associated with single items of fresh produce in the United States from 1990 to 2004. The most common bacterial agents were E. coli O157:H7 and S. enterica. The latter group was responsible for 76%, 60% and 30% of outbreaks caused by fruits, seed sprouts and leafy vegetables, respectively; E. coli O157:H7 were responsible for 19%, 40% and 48%, respectively. Further, results show that the majority of foodborne outbreaks associated with contamination of fresh produce were due to E. coli O157:H7 strains and Salmonella spp. (Sivapalasingam et al., 2004).

Outbreaks of illness associated with fresh produce from 2005 to 2011 are summarized in Table 1. In the US, those linked to fresh produce as the source of 21% of E. coli O157:H7 outbreaks from 1982 to 2002 in the United States. From 2000 to 2004, fresh produce was the second most commonly identified vehicle causing E. coli O157 foodborne illness outbreaks (Arusacavage et al., 2006; Rangel et al., 2005). E. coli O157 outbreaks have been linked to apple cider, lettuce, radish, alfalfa sprouts and other mixed salads (Beuchat, 2002). The largest E. coli O157:H7
outbreak occurred in 1996 in Japan which involved more than 12,000 cases with 12 deaths, and these were linked to the consumption of raw radish sprouts (Michino et al., 1999). In 2006, spinach was responsible for a serious outbreak of severe illness caused by \textit{E. coli} O157:H7 in the US and Canada; 199 \textit{E. coli} O157:H7 cases with 3 deaths were reported in 26 states. Fifty one percent of these cases were hospitalized and 16% developed acute renal failure (CDC, 2006b). In 2010 an outbreak of human \textit{E. coli} O145 infections linked to shredded romaine lettuce occurred in 5 US states where 26 confirmed and 7 probable cases (no deaths) were reported (CDC, 2010a). In May 2011, a large outbreak of illness caused by \textit{E. coli} O104:H4-contaminated fenugreek seed sprouts germinated locally from seeds believed imported from Egypt two years earlier (EFSA, 2011). A second lethal outbreak in 2011 was caused by cantaloupe contaminated with \textit{L. monocytogenes} that was distributed to 28 states in the US where 146 illnesses with 30 deaths (20.5%) were reported in addition to one miscarriage (CDC, 2011e).

Usually foodborne illness from \textit{Salmonella} is linked to consumption of poultry (Greig and Ravel, 2009); however, fresh produce has proven to be a frequent vehicle (Sivapalasingam et al., 2004). In 2008 a large outbreak of salmonellosis occurred in 43 states in the US and Canada, which involved 1442 illnesses that were linked to the consumption of hot peppers (Mody et al., 2011; CDC, 2008b). Another salmonellosis outbreak occurred during late 2010 and early 2011 where 140 illnesses were reported in 26 US states.

![Outbreaks linked to fresh produce from 2005 to 2011.](Fig. 3. Percent of outbreaks and illnesses attributable to produce (from Scharff, 2010).)
states. This outbreak was linked to the consumption of alfalfa or spicy sprouts at a multistate restaurant chain (CDC, 2011b).

Green salad, lettuce, seed sprouts, tomatoes and cantaloupes are the types of fresh produce consistently responsible for foodborne illness outbreaks. Several pathogen-produce combinations more frequently occur in outbreaks; *Salmonella* and cantaloupes, tomatoes or sprouts; *E. coli* O157:H7 and leafy green vegetables; *Cyclospora* and raspberries; and hepatitis A with green onions (DeWaal and Bhuiya, 2009; Lynch et al., 2009).

Understanding the reasons for the increasing contribution of contaminated produce to the overall burden of foodborne illness will shed light on measures likely to be most effective in reversing this trend.

### 3. Sources of pathogens contaminating fresh produce

Although spoilage bacteria, yeasts and molds predominate on raw fruits and vegetables, isolations of pathogenic bacteria, parasites and viruses are not infrequent (Beuchat, 1998). This contamination can occur either pre- or post-harvest (Beuchat and Ryu, 1997). Pre-harvest sources include soil, feces, irrigation water, reconstituted fungicides and insecticides, dust, insects, inadequately composted manure, wild or domestic animals and human handling. Human handling can contribute to post-harvest contamination along with harvesting equipment, transport containers, insects, dust, rinse water, ice, transport vehicles and processing equipment (Beuchat, 2002).

Soil is a natural environment for variety of human pathogens including *B. cereus*, *Cl. botulinum*, and *Cl. perfringens*, *L. monocytogenes* and *Aeromonas* but this profile of pathogens is broadened considerably by the addition of animal wastes to soil (Whipp et al., 2008). It has been reported that *E. coli* O157:H7 and *Salmonella* may survive in soil from 7 to 25 weeks depending on the soil type, moisture level, temperature and source of contamination (Erickson et al., 2010a; Guo et al., 2002; Lang and Smith, 2007; Zhang et al., 2009b). Nicholson et al. (2005) reported that the maximum survival of *E. coli* O157, *L. monocytogenes*, *Salmonella*, and *C. jejuni* in soil ranged from 45 to 100d. It is evident from accumulated work that these zoonotic pathogens, including *Salmonella* (Holley et al., 2006) survive longer in moist clay-based soils at lower temperatures in the presence of manure.

The conditions at the growing location are major factors that affect the microbial safety of fresh produce (Brackett, 1999). Fields that contain animal manure are more likely to be contaminated with enteric pathogens because of their ability to survive in soils for months or years (Doyle and Erickson, 2008). Feces may naturally contain between 10^2 and 10^5 CFU/g *E. coli* and between 10^4 and 10^7 CFU/g *Salmonella* spp. (Himathongkham et al., 1999); slurry between 10 and 10^4 CFU/g *E. coli* and *Yersinia* spp. (Kearney et al., 1993), and manure between 10^2 and 10^5 CFU/g *Salmonella* spp. (Pell, 1997). The manure of ruminants (cattle, sheep) and sewage are considered the main sources of *Salmonella* and *E. coli* O157:H7. Also, *C. jejuni* is a normal member of the gastrointestinal microflora of poultry, pigs and cattle (Warriner et al., 2009). Since *L. monocytogenes* is widely distributed in nature (soil, decaying vegetation), the pathogen is a common contaminant of vegetables, especially root crops (Lianou and Sofos, 2007). The prevalence of pathogens in manure or feces from domesticated food animals is summarized in Table 2.

Foodborne pathogens can survive for long periods in animal manure at cool temperatures. *E. coli* O157:H7 survived in bovine manure for over 70d at 5 °C (Semenov et al., 2007), but only 48d at 22 or 30 °C (Semenov et al., 2007; Wang et al., 1996). Hutchison et al. (2005) studied the survival of *Salmonella*, *E. coli* O157:H7, Campylobacter and *Listeria* in 35,000L tanks of fresh livestock waste (pig and cattle slurries with non-potable water). Survival was found

#### Table 2

Prevalence of pathogens in different kinds of manure or feces.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Manure or feces</th>
<th>Country</th>
<th>Prevalence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157</td>
<td>Cattle</td>
<td>Great Britain</td>
<td>120/2553 (4.7)</td>
<td>Milnes et al., 2008</td>
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<tr>
<td></td>
<td>Great Britain</td>
<td>107/810 (13.2)</td>
<td>Hutchison et al., 2004</td>
<td></td>
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<tr>
<td></td>
<td>Norway</td>
<td>3/1541 (0.2)</td>
<td>Johnsen et al., 2001</td>
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<tr>
<td></td>
<td>Nigeria</td>
<td>42/407 (10.3)</td>
<td>Ojo et al., 2010</td>
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<td></td>
<td>Great Britain</td>
<td>21/2825 (0.7)</td>
<td>Milnes et al., 2008</td>
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<tr>
<td></td>
<td>Nigeria</td>
<td>5/24 (20.8)</td>
<td>Hutchison et al., 2004</td>
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<td></td>
<td>Great Britain</td>
<td>9/168 (5.4)</td>
<td>Ojo et al., 2010</td>
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<td></td>
<td>Great Britain</td>
<td>6/2114 (0.3)</td>
<td>Milnes et al., 2008</td>
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<td></td>
<td>Norway</td>
<td>2/1976 (0.1)</td>
<td>Johnsen et al., 2001</td>
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<tr>
<td></td>
<td>Nigeria</td>
<td>20/409 (4.9)</td>
<td>Ojo et al., 2010</td>
<td></td>
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<tr>
<td></td>
<td>Canada</td>
<td>12/359 (3.3)</td>
<td>Farzan et al., 2010</td>
<td></td>
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<tr>
<td><em>Salmonella</em></td>
<td>Cattle</td>
<td>Great Britain</td>
<td>36/2553 (1.4)</td>
<td>Milnes et al., 2008</td>
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<tr>
<td></td>
<td>Great Britain</td>
<td>62/810 (7.7)</td>
<td>Hutchison et al., 2004</td>
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<td></td>
<td>USA</td>
<td>273/4977 (5.5)</td>
<td>Fedorka-Cray et al., 1998</td>
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<td></td>
<td>Ireland</td>
<td>6/200 (3.0)</td>
<td>Madden et al., 2007</td>
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<td></td>
<td>Great Britain</td>
<td>30/2825 (1.1)</td>
<td>Milnes et al., 2008</td>
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<td>Great Britain</td>
<td>2/24 (8.3)</td>
<td>Hutchison et al., 2004</td>
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<td>Great Britain</td>
<td>17/287 (5.9)</td>
<td>Pao et al., 2005</td>
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<td></td>
<td>Great Britain</td>
<td>124/529 (23.4)</td>
<td>Milnes et al., 2008</td>
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<td></td>
<td>USA</td>
<td>10/126 (7.9)</td>
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<td>Orji et al., 2005</td>
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<td>India</td>
<td>34/231 (14.7)</td>
<td>Murukugar et al., 2005</td>
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<tr>
<td><em>Campylobacter</em></td>
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<td>Great Britain</td>
<td>364/667 (54.6)</td>
<td>Milnes et al., 2008</td>
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<td></td>
<td>Great Britain</td>
<td>104/810 (12.8)</td>
<td>Hutchison et al., 2004</td>
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<td></td>
<td>UK</td>
<td>(16.5)</td>
<td>Chatre et al., 2010</td>
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<td></td>
<td>France</td>
<td>52/220 (24.8)</td>
<td>Madden et al., 2007</td>
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<td></td>
<td>Ireland</td>
<td>30/32 (94)</td>
<td>Klein et al., 2010</td>
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<td></td>
<td>Australia</td>
<td>312/713 (43.8)</td>
<td>Milnes et al., 2008</td>
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<td>Hutchison et al., 2004</td>
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<td>93/518 (18.0)</td>
<td>Salihu et al., 2009</td>
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<td>Hutchison et al., 2004</td>
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<td>Workman et al., 2005</td>
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<td>Barbados</td>
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<td>Farzan et al., 2010</td>
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<td>Brazil</td>
<td>13/67 (19.4)</td>
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<td>Brazil</td>
<td>65/89 (94.2)</td>
<td>Workman et al., 2005</td>
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<td>Barbados</td>
<td>18/24 (75.0)</td>
<td>Franchin et al., 2005</td>
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<td><em>Listeria</em></td>
<td>Cattle</td>
<td>Great Britain</td>
<td>241/810 (29.8)</td>
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<td>Nigeria</td>
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<td>2277/6180 (36.8)</td>
<td>Esteban et al., 2009</td>
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<td></td>
<td>Turkey</td>
<td>6/130 (4.6)</td>
<td>Kalender, 2003</td>
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<td></td>
<td>Great Britain</td>
<td>7/24 (29.2)</td>
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to be in the order *Campylobacter* < *Listeria* < *Salmonella* < *E. coli* O157:H7. Nicholson et al. (2005) examined the survival of *E. coli* O157:H7, *Listeria*, *Campylobacter*, and *Salmonella* in solid and liquid manures (dairy and pig solids, broiler litter, and dairy slurry at 7% and 2% dry matter and in non-potable water). They found that survival in waste solids varied from 2 to 32d and was in the order *Campylobacter* < *Listeria* < *Salmonella* < *E. coli* O157:H7 in solid manure, but was in the order *Salmonella* < *Listeria* < *E. coli* O157:H7 < *Campylobacter* in liquid manure and non-potable water.

While it is likely that *L. monocytogenes* is ubiquitous in natural environments, it is evident that *Campylobacter*, *Salmonella*, and *E. coli* are less likely to survive outside host animals. *Salmonella* spp. in general are better survivors outside animal hosts and in insect vectors than *E. coli* O157:H7, and are more resistant to desiccation and exposure to brackish aquatic environments than *E. coli* (Winfield and Groisman, 2003).

The quality of irrigation water and type of irrigation system influence the microbial safety of fresh produce (Artucavage et al., 2006; Brackett, 1999; Warriner et al., 2009). Flood and spray irrigation represent the greatest risk because contaminated water can be directly deposited onto the edible leaves of produce (FDA, 1998). Solomon et al. (2002) studied the effect of irrigation types on the presence of *E. coli* O157 and found that 90% of lettuce plants which had been spray-irrigated with water containing 7 log CFU/ml of *E. coli* O157 were contaminated, while only 19% were contaminated when surface irrigation was used with the same concentration of *E. coli* O157.

The cost and inconsistent availability of potable water in some regions can lead to the reuse of non-potable (grey or waste) water of uncertain quality for irrigation, which may exacerbate produce contamination. The WHO/FAO (2006) recommended that the fecal coliform level of wastewater used for irrigation of fresh produce should not exceed 1000 CFU or MPN/100 ml. A number of studies have reported that poor quality water has been used for agricultural purposes. Gemmell and Schmidt (2011) assessed the microbiological quality of river water used for fresh produce irrigation in South Africa and found that the number of total coliforms and *E. coli* reached 6 log and 5.5 MPN/100 ml, respectively. Ahmed et al. (2009) reported that 25%, 3% and 28% of pond and tidal creek water samples in Australia were positive for *C. jejuni*, *Salmonella*, and *E. coli* O157, respectively. Chigor et al. (2010) isolated *E. coli* O157 from 2.1% of river waters used in Nigeria for fresh produce irrigation. Haley et al. (2009) isolated *Salmonella* from 79.2% of surface water samples with the highest concentrations occurring in the summer in the United States. In Western Canada, Gannon et al. (2004) isolated *E. coli* O157:H7 and *Salmonella* from 1.7% and 10.3% of river and irrigation canal water, respectively.

Pathogens have the ability to survive for long periods in treated waters of different origin. *E. coli* O157:H7 survived in filtered and autoclaved municipal water for up to 91d at 8 °C or 49d at 25 °C (Wang and Doyle, 1998) or in filtered and autoclaved farm water for up to 65d at 15 °C (Artiz and Killham, 2002). Santo Domingo et al. (2000) found that *Salmonella* survived in sterile municipal or river water for more than 45d at 23 °C. It should be noted that bacterial predation by protozoa can reduce the length of time bacteria remain viable in raw waters (Artiz and Killham, 2002).

Several studies have shown that reconstituted pesticides may serve as potential sources of *Salmonella*, *Shigella*, *E. coli* O157:H7 and *L. monocytogenes* (Guan et al., 2005; Ng et al., 2005). Ng et al. (2005) tested 10 commercial pesticides (insecticides, herbicides and fungicides) and after reconstitution in sterile water to their recommended concentration, two of the pesticides supported the survival and growth of inoculated *Pseudomonas*, *Salmonella* and *E. coli* spp. Pesticides were also reconstituted in different types of agricultural water (bore, dam and river) and examined for survival and growth of microorganisms naturally present. During storage at 30 °C for 48 h, 9 pesticides supported the growth of bacterial species present naturally (Ng et al., 2005). Guan et al. (2005) studied the ability of several pathogens to survive or grow in commercial horticultural pesticide solutions and found that both *Salmonella* and *E. coli* O157:H7 survived exposures used. After 24 h, average *Salmonella* Heidelberg and *E. coli* O157:H7 numbers in 7 solutions decreased 0.2 and 1.7 log CFU/ml, respectively. They also found that *E. coli* and *Salmonella* survived for > 45 and < 15d, respectively, on tomato leaves when sprayed in fungicide contaminated with these organisms. *Salmonella* survived better than *E. coli* O157:H7, *Shigella flexneri* or *L. monocytogenes* in the pesticides used during this study.

Harvesting and processing influence the microbiological safety of fresh produce. These activities include human and mechanical contact, immersion in water, and cutting or slicing, which not only have the potential to contaminate produce with pathogens, but also can enhance bacterial growth (Brackett, 1999; Doyle and Erickson, 2008). Personal hygiene of farm workers is considered an important factor that influences transfer of pathogenic bacteria to fresh produce. Coliforms have been isolated from fresh produce at different stages of production and processing, and while they are often considered an indicator of animal and human fecal contamination, their presence on produce can be ambiguous (Johnston et al., 2005). In contrast, infected workers are considered the primary source of viruses that cause foodborne illness (Berger et al., 2010) and *Shigella* (Warriner et al., 2009).

The conditions of fresh produce distribution can affect bacterial safety by facilitating or preventing cross-contamination of fresh produce and then by preventing opportunity for bacterial multiplication by use of appropriate storage temperatures. Rosset et al. (2004) showed that foodborne illness events develop following temperature abuse during food distribution. Cold storage reduces the growth rate of most human pathogens including *L. monocytogenes*, but the latter can still grow on a wide variety of vegetables at temperatures used to maintain produce quality (Brackett, 1999). Normally, fresh produce should be maintained below 5 °C to reduce the proliferation of spoilage and pathogenic organisms (Rediers et al., 2009).

It is evident that pathogens of concern can survive for extended periods in environments where plants are grown for food use. Produce contamination is more likely to occur when crops are grown in soil containing pathogens, when soils are fertilized with untreated liquid or solid manures, when non-potable water is used for irrigation or formulating pesticides for use on crops, and when worker habits are less hygienic.

4. Incidence of pathogenic organisms on fresh produce

Fresh produce following cutting has higher water activity and more readily available nutrients at cut surfaces than when intact, which support the growth of a variety of foodborne pathogens.
Fresh produce can be contaminated with foodborne pathogens (Table 3) at any point from the farm to consumer, including retailing and handling at home (WHO/FAO, 2008). This contamination occurs most frequently in the field, during initial processing and during the final preparation in the commercial or domestic kitchen (Lynch et al., 2009).

Johnston et al. (2005) studied the quality of fresh produce at different stages of production and processing in the Southern United States and found that coliforms were between 1.0 log and 3.5 log CFU/g at harvest, washing, and packaging. Allende et al. (2004) studied the microbial quality of commercial fresh processed red lettuce in Spain and found the number of coliforms to be 3.67 log CFU/g. Valentin-Bon et al. (2008) isolated coliforms (55%) and E. coli (16%) from 100 bagged lettuce and spinach samples collected in Washington. Recently, Seow et al. (2012) analyzed 125 fresh vegetable and fruit samples (intact and cut) collected from local markets in Singapore. The aerobic mesophilic counts ranged from 1.6 to 9.1 log CFU/g, with the lowest and the highest numbers recorded for orange and bean sprouts, respectively. The highest level of coliforms was found in bean sprouts and fresh-cut salad, with 50% of samples containing more than 5 log CFU/g. Eni et al. (2010) examined the microbial quality and safety of fruits (sliced and intact) and vegetables (intact L. monocytogenes were obtained from Norwegian markets to assess their microbial safety. Salleh et al. (2003) examined different L. monocytogenes were isolated from 56 (8.2%) of 673 intact samples including lettuce, spinach, carrots and green onions. Bacterial numbers ranged from 0.48 to 3.04 log MPN/g. E. coli was not isolated from tomatoes or strawberries. The frequencies of positive samples ranged from 4.4% (9 of 206) for carrots to 27.1% (16 of 59) for spinach. Arthur et al. (2007) evaluated the microbial contamination of 1183 Ontario-grown fresh fruits and vegetable samples during the summer of 2004. One sample each of Roma tomato and organic leaf lettuce were positive for Salmonella. E. coli prevalence was highest in parsley (13.4%) followed by organic leaf lettuce (11.6%), leaf lettuce (6.5%), scallops (6.4%) and cantaloupe (4.9%). Park and Sanders (1992) studied the occurrence of thermotolerant Campylobacter spp. in 1564 fresh samples of 10 types of vegetables sold at 553 outdoor farmers markets and 1031 supermarkets. In samples from the outdoor markets, Campylobacter spp. were detected on 6 types of vegetables; the detection rates were spinach, 2 of 60 (3.3%); lettuce, 2 of 67 (3.1%); radish, 2 of 74 (2.7%); green onions, 1 of 40 (2.5%); parsley, 1 of 42 (2.4%); and potatoes, 1 of 63 (1.6%). C. jejuni was the predominant species (22 of 25; 88%), with the remainder being C. lari (2 of 25; 8%) and C. coli (1 of 25; 4%). Of the samples from supermarkets, all were negative for Campylobacter spp., whether purchased in summer or winter. Although numbers of organisms present are rarely reported, widespread contamination of fresh produce by pathogens capable of causing foodborne illness illustrates the risk these crops may represent when consumed uncooked, since the infective dose of some of the pathogens is very low. Further, cross-contamination during washing, residual surface moisture or condensate, and temperature abuse during storage can enhance the level of risk represented by low levels of pathogen contamination (Danylyuk and Schaffner, 2011).

5. Survival or growth of pathogens on fresh produce

Survival of microorganisms on fresh produce is affected by nutrient availability, UV radiation, toxic compounds released by the plant, competition from other microorganisms and desiccation (Whipp et al., 2008). E. coli O157:H7 and Salmonella can survive on produce for long periods of time, with maximum survival on parsley in the field reported to be 177 and 231d, respectively (Islam et al., 2004a,b). Zhang et al. (2009b) studied the survival of inoculated E. coli O157:H7 on intact lettuce and found that the organism survived for at least 25d on leaf surfaces, with greater survival on the abaxial (under) side of the leaves than on the adaxial side. The interaction of enteric pathogens like E. coli O157:H7 and Salmonella with plant surfaces can lead to development of biofilms or internalization within plant tissue (Aruscavage et al., 2006), and bacterial fimbiae or flagella can facilitate plant infection. Biofilms on fresh produce occur as groups of bacterial cells aggregate in exopoly saccharide materials that serve to protect the cells from environmental stresses, including desiccation and bactericidal agents (Morris and Monier, 2003). Formation of biofilms on surfaces of spinach, lettuce, Chinese cabbage, celery, leek, basil, parsley and
Table 3
Fresh produce from which bacterial pathogens have been isolated.

<table>
<thead>
<tr>
<th>Produce</th>
<th>Country</th>
<th>Pathogen</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
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<td></td>
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<td></td>
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<td>Mouzin et al., 1997</td>
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<td></td>
<td>USA</td>
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<td>Portnoy et al., 1976</td>
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<tr>
<td>Alfalfa seeds</td>
<td>USA</td>
<td>S. Havana</td>
<td>Taormina et al., 1999</td>
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<td></td>
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<td>S. Cubana</td>
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<td></td>
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<td>S. Tennesssee</td>
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<td>Heisick et al., 1989a</td>
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(continued on next page)
endive leaves has been demonstrated (Morris and Monier, 2003). Morris et al. (1998) estimated that 10—40% of bacteria on the surface of intact parsley and endive leaves were associated in biofilms. Olmez and Temur (2010) observed the initiation of biofilm formation by E. coli and L. monocytogenes on intact lettuce surfaces after 24 h of incubation at 10 °C. Niemira and Cooke (2010) reported that E. coli O157:H7 had the ability to form biofilms on intact spinach and lettuce after 24 h of storage at 4 °C. Intergeneric microbial interactions facilitate biofilm development and enhance environmental protection. For example, Salmonella Thompson formed biofilms with Pantoea agglomerans along the veins of cilantro leaves (Brandl and Mandrell, 2002) and E. coli O157:H7 in biofilms were observed associated with Pseudomonas fluorescens (See and Frank, 1999). The most common areas of bacterial aggregations on plants were at the base of trichomes, around the stomata and along veins in the leaves (Aruascavage et al., 2006). These regions have high wettability which promotes water availability and nutrient leaching that in turn support microorganism growth (Brandl and Mandrell, 2002). Cooley et al. (2003) found E. coli O157:H7 and S. enterica grew and their numbers increased to 7 log CFU/g on intact leaf tissue of Arabidopsis thaliana (thale cress) at 100% humidity. Berrada et al. (2006) demonstrated that intact vegetables like lettuce, tomatoes, endive, carrots, cabbage, asparagus, broccoli and cauliflower supported the growth of L. monocytogenes.

The ability of pathogens to attach to fresh produce depends on intrinsic and extrinsic factors including motility of the pathogen, their interaction with other organisms and leaching of nutrients from the plant (Aruascavage et al., 2006; Frank, 2001). Motility facilitates pathogen entry into wounds, stomata or other openings (Cooley et al., 2003; Kroupitski et al., 2009). Motility is an important factor which contributes to plant infiltration by E. coli O157:H7. Shiga-toxigenic E. coli O157:H7 possess mechanisms (e.g., through chemotaxis, motility, and quorum sensing) that direct them toward stomata and induce guard cells to open these gateways (Saldana et al., 2011). Intact salad leaves (Eruca vesicaria (rocket), lettuce, basil and spinach) were colonized by enterotoxigenic E. coli (ETEC) using flagella (Shaw et al., 2011) or by enteropathogenic E. coli (EAEC) using aggregative adherence fimbriae, which are usually used for adhesion of AECC to human intestinal mucosa (Berger et al., 2009b). In another study, S. Senftenberg also attached to salad leaves (basil, lettuce, rocket and spinach) and flagella played a major role in these interactions (Berger et al., 2009a). Kroupitski et al. (2009) found that mutations in Salmonella that reduced motility and chemotaxis inhibited bacterial internalization. E. coli O157:H7 and S. enterica can move around on the external surfaces of plants and this may contribute to the successful colonization of damaged regions at the surface (Cooley et al., 2003), where enzyme action and nutrient leakage may provide opportunity for pathogen growth (Aruascavage et al., 2006).

Pathogens may persist in cracks of the produce skin and contaminate edible parts during cutting, slicing and peeling, or pathogens may enter and spread inside produce through the stem scar (FDA, 2009b). It was reported that the number of E. coli O157:H7 in damaged apples was greater than in noninjured apples by 1—3 fold after 48 h (Dingman, 2000; Janisiewicz et al., 1999). In damaged honeydew melon slices, Salmonella Enteriditis was able to increase 5 log CFU/g over 168 h at 20 °C (Leverentz et al., 2001). Generally, the pH of most vegetables is suitable for growth of pathogenic bacteria (Beuchat, 2002) and even though damaged produce can have a higher pH compared to noninjured produce (Dingman, 2000) these changes are not inhibitory. In addition, pathogens are afforded protection within damaged tissue where spoilage bacteria or fungi have been active (Brandl, 2008). Seo and Frank (1999) reported that E. coli O157:H7 adhered better to cut lettuce leaf surfaces, broken trichomes and stomata than intact leaf surfaces. This attachment affords pathogens protection from post-harvest treatments, making them difficult to remove or inactivate (Aruascavage et al., 2006; Frank, 2001).

Pathogens such as E. coli O157:H7 and Salmonella have the ability to internalize or infect the vascular system of growing plants. Itoh et al. (1998) observed that E. coli O157:H7 adhered to the outer surfaces of radish sprouts produced from contaminated seeds and became internalized during sprout growth. Several studies showed that human pathogens can enter stomata (Fig. 4) and cut edges of
fresh produce (Gomes et al., 2009; Seo and Frank, 1999). E. coli O157:H7 internalized at depths of 20 μm–100 μm below the external surfaces of produce (Beuchat and Ryu, 1997; Solomon et al., 2002). Internalization of Salmonella or L. monocytogenes was observed within core tissue of tomatoes or at the stomata of lettuce and spinach, respectively (Olmez and Temur, 2010; Niemira and Cooke, 2010; Zhuang et al., 1995). Saldaña et al. (2011) reported that at low inoculation levels (10–50 organisms) E. coli O157:H7 was able to reach the internal cavity of stomata, intercellular spaces of the spongy mesophyll and vascular tissue (xylem and phloem) of cut baby spinach leaves using its pilus, flagella and the type 3 secretion system. Li et al. (2008) reported that vacuum cooling increased the internalization of E. coli O157:H7 within intact lettuce tissue (6.4 log CFU/g) compared to the non-vacuum-treated control (5.3 log CFU/g). Kroupiński et al. (2009) studied the effects of light (0, 3 and 100 μE m⁻² s⁻¹) and temperature (4, 25 and 37 °C) on internalization of S. enterica within intact iceberg lettuce leaves during storage, and they found elevated internalization rates at high light intensity and higher temperatures (25 and 37 °C). Franz et al. (2007) reported that E. coli O157:H7 and two strains of S. Typhimurium internalized within intact lettuce tissue that was contaminated via soil. Microbes were recovered in large numbers (3.95 and 2.57 log CFU/g, respectively) and with high frequency (29% and 23%, respectively).

In contrast, several studies found that internalization of human pathogens in fresh produce was rare and was affected by numerous factors including the type of fresh produce, the pathogen strain, contamination level and plant maturity (Dong et al., 2003; Golberg et al., 2011; Jablonske et al., 2005; Mitra et al., 2009; Pu et al., 2009). Golberg et al. (2011) examined the frequency of S. Typhimurium internalization in different types of intact vegetables and fresh herb leaves during a 2 year study. The frequency varied significantly among the different inoculated plants. The highest frequencies were observed in arugula leaves (88%) and iceberg lettuce (81%) followed by basil (46%), red lettuce (20%), romaine lettuce (16%), parsley (1.9%) and tomato (0.5%). Moreover, internalization of Salmonella in iceberg lettuce varied widely (0–100%), with a higher incidence occurring mainly in the summer. Dong et al. (2003) studied the abilities of different strains of E. coli, S. enterica and K. pneumoniae to internalize from the rhizosphere to the interior of alfalfa sprouts and found that these strains differed substantially in their invasiveness. K. pneumoniae and E. coli were the best and poorest colonizers, respectively. In addition, there were differences in colonization success between strains of each of S. enterica and E. coli. Pu et al. (2009) found internalization of E. coli O157:H7 occurred only in 1 of 120 intact spinach samples inoculated at 3 or 7 log CFU/ml after seed germination. Also with spinach, Hora et al. (2005) found that mechanical or biological disruption of roots did not enhance internalization of E. coli O157:H7 into the leaves. Warriner et al. (2003) found infection of E. coli in spinach sprouts was limited when 20-day-old sprouts were transferred to contaminated soil. Jablonske et al. (2005) showed that internalization of E. coli O157:H7, S. Typhimurium and L. monocytogenes depended mainly on plant type, stage of cultivation as well as the pathogen. Microbes were inoculated on cress, lettuce, radish and spinach seeds. After 9 days of germination, E. coli O157:H7 was internalized in all plants while S. Typhimurium was internalized in lettuce and radish. However, L. monocytogenes did not become internalized. It was significant that the pathogens were not recovered from mature plants (49 days post-germination). Mootian et al. (2009) studied the transfer of E. coli O157:H7 at low numbers from soil, manure-amended soil and water to growing young (12d) or mature (30d) lettuce plants. E. coli O157:H7 was detected by enrichment in 21% (113 of 552) of young lettuce plants harvested at 1, 10, 20, and 30d and 30% (36 of 120) of mature plants that were harvested at 15d. Since surface tissue was sterilized before analysis, it was concluded that E. coli O157:H7 was located in protected sites of the lettuce tissue. Bernstein et al. (2007) found that S. enterica serovar Newport became internalized within the aerial parts of romaine lettuce via the root at 33 day-old plants but not when 17 or 20 day-old plants were tested.

There is also a body of literature showing the failure of foodborne pathogens to consistently become internalized within edible plant tissue. Zhang et al. (2009b) reported E. coli O157:H7 was not internalized within intact lettuce leaves and roots, regardless of the type of lettuce, age of plants, or strain of E. coli O157:H7. Only 0.3% of surface-sanitized leaf and root samples from plants grown in inoculated soil were positive for E. coli O157:H7. Mitra et al. (2009) found that there was no evidence for internalization of E. coli O157:H7 within intact spinach leaves. Zhang et al. (2009a) studied the effect of heat stress on internalization of E. coli O157:H7 in lettuce that was planted in contaminated soil and exposed to 36 °C during the day and 15 °C at night for 2 days or 32 °C during the day and 15 °C at night for 3d, while control plants were held at 23 °C during the day and 7 °C at night for 3d. They detected E. coli O157:H7 by enrichment in all inoculated soil and rhizosphere samples. However, heat stress during growth of lettuce did not enhance internalization of E. coli O157:H7 in lettuce. Sharma et al. (2009a) studied the internalization of E. coli O157:H7 within spinach through roots when planted in inoculated soils and were unable to recover E. coli O157:H7 by spiral plating from internal tissues of spinach plants after 28d. However, E. coli cells were microscopically observed in root tissues in 23 (21%) of 108 spinach plants, while no internalized cells were observed in shoot tissue. Erickson et al. (2010a) did not detect E. coli O157:H7 within any roots or leaves of spinach, lettuce, and parsley during use of contaminated irrigation water and compost. Erickson et al. (2010b) used E. coli O157:H7 in spray irrigation water and detected the organism on the surface and within tissues of spinach plants after irrigation; however, 7d after spraying, all spinach leaves tested were negative for surface or internal contamination. They also found that E. coli O157:H7 internalization of lettuce leaves was more persistent when contaminated water was sprayed on the abaxial side (up to 14d) than on the adaxial side (2d).

Some bacterial strains are better able to colonize produce surfaces than others, and biofilm formation, tissue damage, plant species as well as level of host maturity influence pathogen persistence. It is evident that plant infection by zoonotic bacteria can occur; however, it is also clear that mere contact between pathogens and potential plant hosts does not mean that a successful infection will be established. Further, pathogen internalization may be transient and affected by plant maturity and maturation.
rate. It is less frequently observed in field experiments where daily variations in humidity, temperature, and UV light intensity vary. The potential infection of plants by human pathogens is a food safety concern because these pathogens are less likely to be removed or killed during washing and sanitizing after harvest than are surface contaminants.

6. Pathogen control

In 1998, the FDA developed guidelines to minimize the microbial hazards associated with fresh produce based on the following principles: (1) prevention of fresh produce contamination by bacteria is better than depending on corrective actions; (2) use of good agricultural and management practices is necessary; (3) fresh produce can be contaminated at any point along the farm-to-table food chain; (4) potential contamination from water used with fresh produce must be minimized; (5) there must be proper management of animal manure when used as fertilizers and (6) worker hygiene and sanitation practices play a critical role in fresh produce safety. Also, in 2004, the same agency published an action plan to reduce foodborne disease associated with fresh produce. The plan describes strategies for improving safe produce production from the farm to the retail store with four objectives: preventing fresh produce contamination by pathogens; minimizing the public health impact when contamination of fresh produce occurs; improving communication about fresh produce safety with producers, packers, processors, transporters, distributors, preparers, consumers, and other government entities; and facilitating and supporting research relevant to the contamination of fresh produce (FDA, 2004).

In 2008 the WHO/FAO developed a broad range of recommendations to control foodborne pathogens and increase the safety of fresh produce at pre- or post-harvest. These recommendations included: the use of effective methods to minimize pathogen contamination by wildlife in crops; the conduct of topographical and climate risk assessments prior to farm establishment and planting; and prevention of contamination from flooding. Wells, septic systems and water and sewage treatment systems should be developed and operated safely and effectively during periods of excessive rainfall. Crop growth areas should be protected from faecal contamination; composting should be done properly to inactivate pathogens in manure; surface water and groundwater resources should be protected from any potential contamination source including wildlife, animal waste, agricultural run-off, human activity, sewage or industrial effluent; there should be application of good agricultural and manufacturing practices, especially acceptable personnel health habits; access to adequate sanitary facilities and proper sanitation of equipment associated with growing and harvesting; appropriate training and education of farm workers and consumers; specific attention should be afforded to cooling processes and the quality of water used for cooling and packaging; implementation of good manufacturing and hygiene practices with standard operating procedures (SOPs), as part of an HACCP-based approach at all stages of production and processing; grading and selection of produce prior to primary packaging should include discarding damaged plants to reduce the likelihood of attached and internalized pathogens; particular attention should be given to processing areas including knives, blades, utensils, conveyors and other food contact surfaces where biofilms may form; packaging design and materials should provide adequate protection to minimize contamination of produce and prevent damage; and packaging materials or gases used must be non-toxic and not pose a threat to the safety and suitability of food for consumption under specified conditions of storage and use.

Krtinic et al. (2010) asserted that stringent regulations on irrigation water quality and fertilizer control are important for produce safety. To guide some of these actions, regulatory agencies specify the required minimum time delay between manure application and fresh produce harvest. Canadian regulations identify 3 months for tree fruits and grapes, 15 months for small fruits and 12 months for vegetables (Warriner et al., 2009). The USDA identifies 4 months for fresh produce when the edible portion has direct contact with the soil surface or 3 months if there is no direct contact. In addition, composting temperatures should reach between 55 and 77 °C for 3–15d, depending on composting system (USDA, 2011). Post-harvest washing of fresh produce can be an important measure to reduce pathogen contamination. However, not all washing methods and washing solutions are effective.

When sanitizers are ineffective, produce washing may redistribute pathogens and cause cross-contamination. Zhang et al. (2009c) found that a 10% organic load (lettuce slurry) reduced the anti- E. coli O157:H7 effectiveness of 30–50 ppm chlorine to almost that of water. A peracid mix or peroxyacetic acid at 30 ppm were less affected by the organic matter. The importance of this observation was underlined by results of the modeling work by Danylik and Schaffner (2011), where it was estimated that when E. coli O157:H7 is present on 0.1% of produce samples at harvest, cross-contamination at washing could be responsible for up to 95% of illnesses caused by the produce product.

Although some studies have shown that chlorine is ineffective in elimination of pathogens, it is the most widely used sanitizing agent to prevent potential cross-contamination during washing. The recommended concentrations range from 50 to 200 ppm with a contact time of 1–2 min (Beuchat, 1998). Washing using solutions containing approximately 20–200 ppm free chlorine cannot eliminate pathogens completely, but reductions of 1–3 log CFU/g are common (Arusacavage et al., 2006). Therefore, alternatives for fresh produce sanitation such as chlorine dioxide, ozone, lactic acid, peroxyacetic acid and acidified sodium chlorite have been studied (Beuchat, 1998; Ruiz-Cruz et al., 2007).

Use of peroxyacetic acid for sanitizing specific food products including fresh produce at concentrations that do not exceed 80ppm in wash water has been approved by the FDA (USDA, 2000b). It has been confirmed that peroxyacetic acid at 80 ppm is effective for reduction of pathogens on several types of fresh produce (Rodgers et al., 2004). Park and Beuchat (1999) reported that peroxyacetic acid at 40–80 ppm reduced Salmonella and E. coli O157:H7 populations on intact melon surfaces by 2.6–3.8 log CFU/g. Hellstrom et al. (2006) reported that peroxyacetic acid achieved 1.7 log CFU/g reductions of L. monocytogenes on lettuce. Abadias et al. (2011) found that washing of fresh-cut apples using peroxyacetic acid at 20 ppm for 1 min reduced numbers of E. coli O157:H7 and L. innocua by >4 log CFU/g. Vandekinderen et al. (2009) tested the efficacy of different concentrations (25, 80, 150 and 250 ppm) of peroxyacetic acid for removal of the native microflora in 4 types of fresh-cut vegetables. Its efficacy was highly dependent on the type of fresh-cut produce: the highest microbial reductions were obtained with carrots and white cabbage (0.5–3.5 log CFU/g) followed by iceberg lettuce (0.4–2.4 log CFU/g), while the lowest efficacy was for fresh-cut leek (0.4–1.4 log CFU/g).

The FDA also approved acidified sodium chlorite as a spray or dip in the range of 500–1200 ppm (USDA, 2000a). Subsequently, it was found that acidified sodium chlorite was bactericidal during washing different types of fresh produce (González et al., 2004; Inatsu et al., 2005; Ruiz-Cruz et al., 2007). González et al. (2004) found that acidified sodium chlorite was better able to reduce pathogen numbers on shredded carrots than chlorine, citric acid or water. Acidified sodium chlorite achieved a 5.25 log CFU/g reduction at 1000 ppm. Inatsu et al. (2005) reported that exposure of intact lettuce to acidified sodium chlorite caused a 3.1 log CFU/g
reduction in Salmonella numbers. Ruiz-Cruz et al. (2007) studied the efficacy of acidified sodium chlorite in reducing E. coli O157:H7, Salmonella spp. and L. monocytogenes populations on fresh-cut carrots. They found that bacterial numbers were significantly reduced by 1, 1.5 and 2.5 log CFU/g after washing with acidified sodium chlorite at 100, 250 and 500 ppm, respectively.

Hydrogen peroxide washes have been effective in reducing pathogen viability on whole grapes, prunes, apples, oranges, mushrooms, melons, tomatoes, red bell peppers, lettuce, cucumbers, zucchini, bell peppers, and melons (Artés et al., 2007). Sapers and Sites (2003) reported that the use of hot (60–80 °C) 5% hydrogen peroxide for 2 min reduced E. coli and Salmonella numbers by more than 2 log CFU/g on intact cantaloupes without tissue damage and benefits were noted up to 26d storage at 4 °C. However, treatment of cantaloupes with 1% hydrogen peroxide at 20 °C for 15 min was ineffective against E. coli and Salmonella. In the same study, 1% hydrogen peroxide at 20 or 40 °C for 15 min reduced E. coli O157:H7 numbers by 1.8–3.5 log CFU/g on intact apples. Ukuku (2006) found that treatment of fresh cut cantaloupes with 2.5% hydrogen peroxide for 2 min reduced E. coli and Salmonella numbers by more than 2 log CFU/g on intact cantaloupes without tissue damage and benefits were noted up to 26d storage at 4 °C. However, treatment of cantaloupes with 1% hydrogen peroxide at 20 °C for 15 min was ineffective against E. coli and Salmonella.

New chemical technologies show promise for fresh produce sanitation to ensure safety. Harris et al. (1999) tested an alkaline solution (water, oleic acid, glycerol, ethanol, potassium hydroxide, sodium bicarbonate, citric acid and distilled grapefruit oil) to remove Salmonella from intact tomato surfaces and they found that this solution reduced the population by 2–4 log CFU/tomato. Sy et al. (2005) tested the efficacy of gaseous chlorine dioxide at 4.1 ppm to reduce Salmonella on different types of fresh produce. Reductions resulting from this treatment were 3.13–4.42 log CFU/g for fresh-cut cabbage, 5.15–5.88 log CFU/g for fresh-cut carrots, 1.53–1.58 log CFU/g for fresh-cut leafy vegetables, 4.21 log CFU/apple, 4.33 log CFU/tomato, 1.94 log CFU/onion, and 3.23 log CFU/peach. Keeratipibul et al. (2011) investigated the efficacy of hypochlorous and peracetic acids in reducing E. coli levels on intact tomatoes and lettuce. The hypochlorous acid at 75 ppm reduced the level of E. coli by 3.1 and 1.3 log CFU/g on the tomato fruits and lettuce, respectively, while the peracetic acid at 50 ppm reduced the numbers by 4.5 and 2.5 log CFU/g on tomatoes and lettuce, respectively. Abadias et al. (2011) found that 3 min washing of fresh-cut apples using sodium carbonate (100 g/L), potassium carbonate (10 g/L), carvacrol (875 mg/L), hydrogen peroxide (20 mL/L), N-acetyl-L-cysteine (10 g/L) or Citrox 14WP (5 mL/L) reduced E. coli O157:H7 numbers by >4 log CFU/g. Velázquez et al. (2009) studied the efficacy of 0.1 mg/ml benzalkonium chloride and 0.2% lactic acid against E. coli O157:H7 and Y. enterocolitica on lettuce and tomatoes. On tomatoes, the benzalkonium chloride reduced E. coli O157:H7 and Y. enterocolitica by 2.1 and 4.2 log CFU/tomato, respectively, while lactic acid reduced E. coli O157:H7 and Y. enterocolitica by 2.2 and 5.1 log CFU/tomato, respectively. On lettuce, the benzalkonium chloride reduced E. coli O157:H7 and Y. enterocolitica by 2.1 and 2.2 and 1.5 log CFU/g, respectively, while lactic acid reduced E. coli O157:H7 and Y. enterocolitica by 0.4 and 2.4 log CFU/g, respectively. Use of a commercial solution containing 20 ppm of lactic acid as the active compound to wash fresh-cut escarole or lettuce reduced coliform numbers by 2.25 and 1.6 log CFU/g, respectively (Allende et al., 2008). Neal et al. (2011) found that washing of spinach leaves with 2% lactic acid at 55 °C reduced E. coli O157:H7 and Salmonella by 2.7 and 2.3 log CFU/g, respectively. The combination of 2% organic acids (malic acid, lactic acid and citric acid) with ultrasound (40 kHz) reduced numbers of E. coli O157:H7, S. typhimurium and L. monocytogenes on intact lettuce leaves by 2.3–3.2 log CFU/g (Sagong et al., 2011).

Recently, acidic, neutral or alkaline electrolyzed water has been studied as a sanitizer. Wang et al. (2004) reported that washing of fresh-cut cilantro by acidic electrolyzed water reduced the total aerobic count and coliforms by 1–1.3 log CFU/g after 4d storage at 0 °C. Issa-Zacharia et al. (2011) found that slightly acidic electrolyzed water (pH 5.6) reduced E. coli and Salmonella spp. by 2.7–2.9 log CFU/g on fresh-cut Chinese celery and lettuce or daikon sprouts after soaking for 5 min Guentzel et al. (2008) evaluated soaking of spinach and lettuce contaminated with E. coli, S. Typhimurium, S. aureus, L. monocytogenes and E. faecalis in electrolyzed oxidizing water containing 100 and 120 ppm total residual chlorine at 25 °C for 10 min. All organisms in spinach were reduced by 4.0–5.0 log CFU/ml, while in lettuce, E. coli numbers were reduced by 0.24–0.25 log CFU/ml and the other organisms were reduced by 2.43–3.81 log CFU/ml. Park et al. (2008) used a combination of acidic electrolyzed oxidizing water and chlorine for sanitizing. Washing of fresh-cut onions inoculated with different pathogens using acidic electrolyzed oxidizing water at pH 2.06 and 3.75 ± 2.5 ppm free chlorine for 3 min reduced the numbers of E. coli O157:H7, L. monocytogenes and S. Typhimurium >5.8, >5.6 and >5.2 log CFU/g, respectively. Abadias et al. (2008a) reported that neutral electrolyzed water containing 50 ppm of free chlorine (pH 8.6) caused a 1–2 log CFU/g reduction in the numbers of E. coli O157:H7, Salmonella, L. innocua and C. carotovora on lettuce. In another study, the combination of alkaline electrolyzed water and 1% citric acid at 50 °C caused a 4 log CFU/g reduction in L. monocytogenes and E. coli O157:H7 numbers on shredded carrots (Rahman et al., 2011).

The limited activity of these washing methods is believed due in part to the presence of pathogens in protected sites (within stomata, at cut surfaces or the presence of biofilms or bacterial aggregates). In addition, the hydrophobicity of the leaf surface may prevent access by decontaminating solutions (Whippo et al., 2008). Olmez and Temur (2010) reported that biofilm formation and internalization of E. coli and L. monocytogenes on intact green leaf lettuce reduced the efficacy of ozonated water (2 mg/L), chlorine (100 mg/L) and organic acid (0.25 g/100 g citric acid plus 0.50 g/100 g ascorbic acid) treatments (10 °C for 2 min). More than a 3 log CFU/g reduction was achieved with both pathogens when treatments were extended to 6 h but at 48 h there was only a further ≤1.5 log CFU/g reduction. The decreased efficacy of sanitizing treatments with increasing incubation time was believed possibly due to bacterial internalization and biofilm formation on lettuce leaves. There have been further attempts to address these issues.

Ozone is efficient in reducing pathogens on fresh produce because of its strong oxidizing capacity. Rodgers et al. (2004) reported that application of ozone to shredded lettuce reduced Salmonella numbers by 5 log CFU/g. Zhang et al. (2005) reported that intact celery sticks dipped in 0.18 ppm ozone water for 9d at 4 °C reduced bacterial viability by 1.69 log CFU/g compared to control water. Selma et al. (2007) reported that treatment of shredded lettuce with 5 ppm ozone water for 5 min reduced Shigella sonnei numbers by 1.8 log CFU/g. Najafi and Khodaparast (2009) successfully used gaseous ozone on date fruits and reduced the numbers of E. coli and Staphylococcus aureus to undetectable levels after treatment with 5 ppm for 60 min. Application of ozone to produce at the post-harvest stage is valuable because it inactivates bacteria, prevents fungal decay, can cause destruction of pesticides and chemical residues and controls storage pests (Najafi and Khodaparast, 2009). On the other hand, using ozone as a disinfectant has disadvantages including its instability and reactivity with organic materials, and thus, the effective elimination of microorganisms may require high concentrations which may cause sensory
defects in fresh produce. In addition, some nutritional components (vitamins, amino acids, enzymes, essential fatty acids) may be altered as a result of oxidation (Kim et al., 2003).

Cold atmospheric plasma is a novel technique that shows significant potential for sanitation of fresh produce. Critzer et al. (2007) studied the efficacy of cold atmospheric plasma at 9 kV to inactivate E. coli O157:H7, Salmonella and L. monocytogenes on fresh-cut apples, cantaloupe and lettuce, respectively. E. coli O157:H7 numbers were reduced by >2 log CFU/10 cm² after 2 min of exposure. Salmonella and L. monocytogenes numbers were reduced by >3 and 5 log CFU/25 cm² after 5 min, respectively. Perni et al. (2008a) found that 16 kV cold atmospheric plasma reduced E. coli on the pericarps of mangoes and melons by >3 log CFU/cm². In another study, E. coli and L. monocytogenes Scott A numbers were reduced by 1.5–2.5 log CFU/cm² on fresh-cut mango or cantaloupe tissues after exposure for 30 s to 8 kV cold atmospheric gas plasma (Perni et al., 2008b). Comparable results were observed by Niemira and Sites (2008) where S. Stanley and E. coli O157:H7 numbers on fresh-cut apples were reduced by 2.9–3.7 and 3.4–3.6 log CFU/ml, respectively, after 3 min exposure to cold plasma.

Pasteurization of fresh produce surfaces using steam, hot water or chlorine dioxide gas has been shown to reduce microbial contamination on hard-surface produce (Stringer et al., 2007). However, fragile fresh produce such as leafy vegetables can be damaged by steam treatment (Sy et al., 2005). Although heat treatment is not used with fresh produce, cooking is clearly a fail-safe method for pathogen elimination from produce (Aruscavage et al., 2006).

Irradiation of fresh produce is generally effective for elimination of microbial contamination at a maximum level of 1.0 kGy (Gomes et al., 2009). The efficacy of irradiation for decontaminating fresh produce is influenced by the target pathogen, produce type, produce condition (whole, cored, peeled or cut) and the atmosphere in which it is packaged (Niemira and Fan, 2005). It has been reported that ionizing irradiation is highly effective for elimination of foodborne pathogens in different vegetables and leafy greens (Gomes et al., 2009; Grasso et al., 2011; Mahmoud, 2010). Mahmoud (2010) studied the efficacy of X-rays for elimination of foodborne pathogens from shredded iceberg lettuce. It was found that treatment with 1.0 kGy reduced the numbers of E. coli O157: H7, L. monocytogenes, S. enterica and S. flexneri by 4.4, 4.1, 4.8 and 4.4 log CFU/5 cm², respectively. Furthermore, more than 5 log CFU/5 cm² reductions of pathogens were achieved with 2.0 kGy X-rays (Mahmoud, 2010). Irradiation using an electron beam up to 3 kGy extended the shelf-life of broccoli heads without any change in the physicochemical properties (Gomes et al., 2008). Grasso et al. (2011) studied the efficacy of electron beam irradiation for inactivation of the native microflora and E. coli on fresh-cut cabbage and found that irradiation at 2.3 kGy reduced both populations more than 4 log CFU/g. Kim et al. (2010) found that the electron beam irradiation at 1 kGy reduced Salmonella numbers more than 3 log on the surfaces of whole or fresh-cut cantaloupe. Chimbombi et al. (2011) showed that exposure of fresh-cut cantaloupe to 1.0 kGy electron beam irradiation reduced S. Typhimurium by 2.65 log CFU/g after 30 h of growth at 23 °C. Shim et al. (2012) found that exposure of contaminated lettuce leaves to 1 kGy gamma irradiation reduced numbers of S. Typhimurium and S. aureus by 3 log CFU/leaf. Lee et al. (2006) found that 1 kGy gamma irradiation reduced S. Typhimurium, E. coli, Staphylococcus aureus and L. ivanovii on peeled sliced cucumber by 3.12, 1.94, 2.11 and 2.97 log CFU/g, respectively; on sliced seasoned spinach by 2.83, 2.53, 2.77 and 2.36 log CFU/g, respectively; and on sliced burdock by 2.51, 5.73, 4.22 and 3.78 log CFU/g, respectively. Gomes et al. (2009) studied the efficacy of irradiation against E. coli and confirmed that irradiation up to 1.0 kGy resulted in 3–4 log CFU/g reductions of E. coli internalized in fresh-cut lettuce leaves. Fan et al. (2008) reported that irradiation at 1 kGy reduced the numbers of pathogens such as E. coli O157: H7 by 3–8 log CFU/g on the surface of fresh-cut produce and by 2–3 log CFU/g when inside. Niemira and Cooke (2010) found that irradiation of intact spinach and lettuce leaves immediately after inoculation at 1 kGy reduced the number of E. coli O157: H7 > 5 log CFU/g, but after 48 h of incubation at 4 °C, this dose reduced its viability by 1.9 and 2.5 log CFU/g in spinach and lettuce, respectively. Biofilm formation was observed on both types of leaves, and in addition, bacterial internalization in stomata was observed on spinach during storage. However, irradiation of fresh produce includes the disadvantage that at >1 kGy the quality of fresh produce may deteriorate through changes in appearance, flavor, color and texture (Fan et al., 2008). In addition, some viruses and fungi can resist irradiation treatment (Fan et al., 2008; Warriner et al., 2009).

Because deterioration of produce occurs with irradiation treatment at >1 kGy, efforts have been made to reduce the dose of irradiation and still maintain produce safety. This can be achieved by combining irradiation with other preservative methods such as modified atmosphere packaging. Gomes et al. (2011) studied the irradiation sensitivities of Salmonella spp. and Listeria spp. in intact baby spinach leaves packaged under high oxygen. They found that irradiation sensitivities of organisms were increased with increased oxygen in packages, which yielded from 16% to 25% reductions in D10-values. Irradiation at 0.7 kGy with 100% oxygen at room temperature reduced the number of tested organisms by 5 log CFU/g. Irradiation of produce in the presence of oxygen may lead to the formation of ozone, which is also antimicrobial. However, packaging materials for this use should not yield toxic reaction products after irradiation and affect food sensory properties. Although several packaging materials have been approved by the FDA for use during irradiation of packaged food, some materials like polyolefins may contain additives that have not been approved for this use (Fan et al., 2008).

Ultraviolet light (UV) light can be divided into three classes based on its wavelength: UV-A (400–320 nm), UV-B (320–280 nm) and UV-C (<280 nm); with the most effective range for produce decontamination being 200–280 nm. It is established that bacterial spores and stationary phase cells are more resistant to UV-C than vegetative and exponential phase cells (Warriner et al., 2009). Erkan et al. (2001) reported that UV at a wavelength of 190–280 nm for 10–20 min eliminated microbial contamination from zucchini squash slices. Yau et al. (2004) reported that more than a 9 mW/cm² dose of UV-C light reduced microbial numbers by 2 log/lettuce and tomato and 3 log/apple. There are several advantages associated with the use of ultraviolet light to treat food; it does not leave any residue; it does not have legal restrictions; it is easy to use; it is lethal to most types of microorganisms, and it does not require installation of extensive safety equipment (Yau et al., 2004). Its limited penetration is a disadvantage.

Use of antagonistic bacteria, particularly lactic acid bacteria (LAB), as biocontrol agents against human pathogens on fresh produce has been evaluated in number of studies (Teplitzki et al., 2011). Scolari and Vescovo (2004) reported that Lactobacillus casei added to mechanically damaged escarole lettuce reduced numbers of E. coli O157:H7, A. hydrophila and S. aureus to undetectable levels after 4 to 6d, while L. monocytogenes was reduced by 2.4 log CFU/g over 6d. Trias et al. (2008) found that LAB isolated from fresh produce reduced the growth of S. Typhimurium and E. coli O157:H7 on apple wounds and fresh-cut lettuce by 1–2 log CFU wound or g, whereas L. monocytogenes growth was completely inhibited after 2d. Janisiewicz et al. (1999) found that P. syringae reduced the growth of E. coli O157:H7 on damaged apples by 10–1000-fold after
2d. Different mechanisms can be responsible for pathogen inhibition by biocontrol agents, including production of inhibitory compounds, competition for nutrients, space or even colonization sites (Whipps et al., 2008). Matos and Garland (2005) evaluated an undefined mixed culture of bacteria for controlling Salmonella on sprouted alfalfa seeds and found that Salmonella numbers were reduced more than 5 log CFU/g within 7d. Cooley et al. (2006) found that Enterobacter asburiae decreased the survival of E. coli O157:H7 by 20–30 times on intact lettuce foliage after 10d.

Bacteriocins are cationic antimicrobial peptides produced by variety of bacteria including LAB which can be used as hurdles for controlling foodborne pathogens. They are considered to be safe biopreservatives because they are degraded by the proteases in the gastrointestinal tract (Cleveland et al., 2001). Allende et al. (2007) evaluated the effect of bacteriocin-containing washing solutions on survival of L. monocytogenes in fresh-cut lettuce packaged in macro-perforated polypropylene bags. Washing fresh-cut lettuce with these solutions decreased the viability of L. monocytogenes by 1.2–1.6 log CFU/cm² immediately after treatment, but they were not able to prevent subsequent L. monocytogenes proliferation. The usefulness of bacteriocins is influenced by the breadth of their spectrum of antibacterial action and upon the chemical and physical properties of foods. Bacteriocins produced by LAB are generally not active against Gram-negative pathogens (E. coli and Salmonella). In addition, they are of most value when used in combination with other hurdle techniques (Allende et al., 2007).

Bacteriophages have been used as effective methods to control pathogens in the environment and on foods including fresh produce (Teplitski et al., 2011). Bacteriophages have been used to control E. coli O157:H7, L. monocytogenes and Salmonella on different types of fresh produce. Kochurchett et al. (2009) reported that bacteriophages reduced the number of Salmonella on alfalfa seeds 10-fold after 3 h at 25 °C. Similarly, Pao et al. (2004) found that bacteriophage reduced the number of Salmonella 1.37, 0.55 and 1.02 log CFU/g in soaked mustard seeds, broccoli seeds and soaking water, respectively, after 24 h at 25 °C. Bacteriophage on intact lettuce leaves reduced L. monocytogenes numbers by 2 log CFU/g during storage for 3d at 6 °C, while the pathogen was undetectable on sliced cabbage leaves under the same conditions (Guenther et al., 2009). Leverentz et al. (2003) reported that bacteriophage reduced L. monocytogenes up to 4.6 logs and below 0.4 logs on fresh-cut honeydew melons and apples, respectively, stored at 10 °C for 7d. In another study, they found that application of bacteriophage 1 h before contamination of honeydew melons with L. monocytogenes resulted in a 6.8 log reduction over 7d at 10 °C (Leverentz et al., 2004). Abuladze et al. (2008) tested the efficacy of a commercial cocktail of phages to control E. coli O157:H7 on intact tomatoes, broccoli and spinach and found the organism was reduced 99%, 99.5% and 100% after 1d, respectively. Sharma et al. (2009b) evaluated the efficacy of a cocktail of three E. coli O157:H7 phages to eliminate the pathogen from fresh-cut lettuce and cantaloupe and found that phage treatment reduced E. coli O157:H7 numbers by 1.6–2.5 log CFU/g after 2d. In another work, E. coli O157:H7 was not recovered 24 h after its inoculation on intact lettuce or spinach leaves which were treated with bacteriophage and stored at 23 or 37 °C. A combination of bacteriophage with trans-cinnamaldehyde was more effective; the organism was undetectable after 10 min at 4, 8, 23 or 37 °C (Viazis et al., 2011).

Recent studies suggested that the use of bacteriophages in combination with antagonistic bacteria could increase microbial inhibitory effects (Ye et al., 2009, 2010). Ye et al. (2010) reported that the combination of a bacteriophage cocktail with antagonistic bacteria (Enterobacter isolated from mung bean sprouts) reduced the numbers of Salmonella by 6 log CFU/ml in broth culture. When they treated inoculated, mug beans sprouted over 4d, Salmonella was detected by enrichment only; indicating levels present were less than 2 log CFU/g.

It is evident that absolute, dependable and consistent inactivation of pathogens on or in produce does not seem possible yet with currently available physical, chemical or biological control technologies, except possibly for the use of low dose, high energy irradiation at elevated O2 concentrations.

7. Future research

Understanding the ecology of pathogens on fresh produce is essential for development of methods to eliminate them from these products; therefore, future research should focus on factors affecting survival, attachment and internalization of human pathogens in fresh produce. In addition, understanding the physiological changes or behaviors of human pathogens during the transition to colonization of fresh produce may identify unsuspected vulnerabilities. Conventional sanitizing methods are ineffective for removing internalized bacteria, and thus improved treatments will need to address this issue where it has been shown to be problematic. Improved sanitizing may develop from studies examining synergistic interactions between sanitizers. It has been demonstrated that biocontrol methods have some efficacy in eliminating pathogens from fresh produce, so the development of biocontrol procedures against human pathogens before harvest as well as during processing is needed. Of methods for decontamination currently available, irradiation holds the most promise and its combination at 1 kGy with high oxygen packaging is an attractive alternative. Interventions to reduce pathogenic microorganisms on fresh produce are important elements in decreasing the risk of foodborne illness because it appears to be almost impossible to prevent produce contamination in a predictable manner.

Attention is being drawn to the linkages among animal feed contamination, food animal carcass contamination, manure and produce contamination. The link between feed and carcass contamination is well established, but debate continues regarding the relationship between animal feed contamination by toxigenic (VTEC) E. coli or Salmonella and produce contamination by these organisms. Continuous challenge of the agriculture production system with these pathogens in feed when animal and plant production are poorly segregated in most jurisdictions, enhances the probability that produce will be contaminated at harvest (Holley, 2010). Further attention must be afforded this complex issue.

8. Conclusions

Increased global production, distribution and consumption of fresh produce in conjunction with more intensive production methods and inconsistent application of good agricultural practices explain the high incidence of foodborne illness linked to this food category. However, environmental factors, both during pre-harvest and after harvest play significant roles as sources of foodborne pathogens. It is clear that E. coli O157:H7 and Salmonella can survive in soil as well as on fresh produce for long periods, particularly at cool temperatures. Bacterial harborage in leaf pores (stomata), at surface imperfections, and biofilm formation by bacteria on plant surfaces complicate efforts to consistently sanitize contaminated produce. Internalization of human pathogens within the plant vascular system and its influence on pathogen persistence in plants and on the overall extent of produce contamination is incompletely known. It is evident that current options in commercial use to sanitize produce are prone to failure and thus emphasis must continue to be placed upon prevention of produce contamination.
References


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Statistics Canada. 2011. Tables by subject: food and nutrition, food available, by major food groups. Available at: http://www40.statcan.ca/l01/ind01/t1_920_eng.html#humi (accessed 02.08.11).


