2017

Microbial ecology of fresh vegetables

J. Zheng

US Food and Drug Administration

A. De Jesus

US Food and Drug Administration

S. Sahu

US Food and Drug Administration

A. E. Hayford

US Food and Drug Administration

Y. Luo

USDA

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/usfda>

Part of the [Dietetics and Clinical Nutrition Commons](#), [Health and Medical Administration Commons](#), [Health Services Administration Commons](#), [Pharmaceutical Preparations Commons](#), and the [Pharmacy Administration, Policy and Regulation Commons](#)

Zheng, J.; De Jesus, A.; Sahu, S.; Hayford, A. E.; Luo, Y.; Datta, A. R.; Brown, E. W.; and Bell, R., "Microbial ecology of fresh vegetables" (2017). *Food and Drug Administration Papers*. 18.

<http://digitalcommons.unl.edu/usfda/18>

This Article is brought to you for free and open access by the U.S. Department of Health and Human Services at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Food and Drug Administration Papers by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

J. Zheng, A. De Jesus, S. Sahu, A. E. Hayford, Y. Luo, A. R. Datta, E. W. Brown, and R. Bell

CHAPTER 17

Microbial ecology of fresh vegetables

J. Zheng¹, J. Kase¹, A. De Jesus¹, S. Sahu², A.E. Hayford², Y. Luo³, A.R. Datta², E.W. Brown¹, and R. Bell¹

¹ Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, MD, USA

² Center for Food Safety and Applied Nutrition, US Food and Drug Administration, Laurel, MD, USA

³ Agricultural Research Service, US Department of Agriculture, Beltsville, MD, USA

The total consumption of fresh and processed vegetables has exceeded 123 kg per capita in the United States and 118 kg per capita in the European Union (<http://www.helgilibrary.com/indicators/index/vegetable-consumption-per-capita>) in 2009. Changes in lifestyles and consumption trends have prompted the sustained growth of fresh-cut or minimally processed vegetables that are fresh and ready-to-eat (RTE). In 2011, for example, total US fresh-cut produce sales through both food service and retail channels were estimated to surpass \$27 billion (Cook *et al.*, 2012).

1442 illnesses and two deaths (Mody *et al.*, 2011). In addition, contaminated pre-packaged baby spinach caused a devastating *E. coli* O157:H7 outbreak in 2006 in the US, involving 199 cases and three deaths (CDC, 2006). In the wake of these food-borne illness outbreaks, research has begun in earnest to define the complex but critical biological interactions among indigenous microorganisms, human pathogens, and fresh produce. In this chapter, an in-depth review of the microbial ecology of fresh and fresh-cut vegetables and their relationship to the major food-borne bacterial pathogens is presented.

17.1 Introduction

The incidence of food-borne illness outbreaks caused by contaminated fresh and fresh-cut vegetables has increased globally in recent years. In the US, an estimated 34% of all food-borne illnesses that led to hospitalization or death, from 1998 to 2008, were attributable to vegetables (Painter *et al.*, 2013). Among these, contaminated leafy vegetables were the leading causes (22%), followed by vine-stalk (7.9%), and other commodities. The pathogens most frequently linked to vegetable-related outbreaks include bacteria (*Salmonella*, *Escherichia coli*) and viruses (norovirus, hepatitis A) (Painter *et al.*, 2013). As an example, hot peppers were the source for a high profile salmonellosis outbreak in the US and Canada in 2008, leading to

17.2 Prevalence and diversity of microbial communities on fresh vegetables (post-harvest)

Vegetables are known to harbor a diverse and complex array of bacterial communities. Coliforms and fecal coliforms, like generic *E. coli*, are considered indicators of fecal contamination and their presence on food suggests that sanitary quality might be compromised. In 2009, The Consumer Union tested 16 different brands of salad greens ($n = 208$ bags) collected in the New York City Metro area and found about a third of the bags tested had more than 10,000 cfu/g of total coliforms and approximately 5% of the bags contained generic *E. coli* (Consumer Union, 2010). A Canadian-based group conducted a similar retail-level study

across five provinces involving imported herbs and pre-packaged leafy greens and spinach from five countries. The highest prevalence of coliforms were found in imported US leafy greens (mean of 21 samples (66.7% of total tested) – 3.3 log₁₀ cfu/g) followed by US herbs (mean of 12 samples (7% of total tested) – 2.6 log₁₀ cfu/g) (Allen *et al.*, 2013). Although not currently used in the US, hygienic standards for minimally processed vegetables exist in other countries. For example, the European Union guidelines are 100 cfu/g generic *E. coli* in RTE pre-cut fruits and vegetables. A recent field survey of both organically and conventionally grown lettuce grown in Spain found that 20% of samples exceeded this level (i.e., 35% of samples collected tested positive) (Oliveira *et al.*, 2010).

Pathogenic bacteria (e.g., *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*) can also be part of larger microbial communities on fresh produce (Shi *et al.*, 2009; Teplitski *et al.*, 2011). Field surveys on contamination of produce with pathogenic bacteria such as *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* found drastically different results depending on the methods, location, seasons, produce type, and criteria of the survey conducted. Some revealed little or no occurrence of contamination with these pathogenic bacteria (Consumer Union, 2010; Koseki *et al.*, 2011; Sant'Ana *et al.*, 2011; Allen *et al.*, 2013; Althaus *et al.*, 2012), while others showed widespread contamination. Salleh *et al.* (2003) examined 112 samples from four local salad vegetables in Selangor, Malaysia, and found 40 (35%) were contaminated with *Salmonella* with a total of 31 different *Salmonella* serovars being isolated from this study. In Spain, *Salmonella* spp. were detected in 0.7% of lettuce samples ($n=137$) (Sospedra *et al.*, 2013). In 2014, Wijnands *et al.* (2014) conducted a survey to estimate pathogen prevalence and contamination levels of raw produce as well as resulting minimally processed packaged salad as sold in the Netherlands. The overall prevalence for *L. monocytogenes*, *E. coli* O157, and *Salmonella* was 0.11, 0.11, and 0.38%, respectively, across 1800 samples of produce and 1900 samples of RTE mixed salads investi-

gated. Prevalence point estimates for *Salmonella* in specific produce ranged from 0.53% in iceberg lettuce to 5.1% in cucumber. In the Philippines (Vital *et al.*, 2014), 24.7% of retail fresh produce samples, including bell pepper, cabbage, carrot, lettuce, and tomato, were positive for *Salmonella* spp. It is noteworthy that all of these studies indicated that a wide range of vegetables can be contaminated by pathogenic bacteria and are potentially capable of serving as vehicles for human infection. However, manifestation of these capabilities can be greatly influenced by intrinsic and extrinsic ecological factors naturally present in produce or imposed at one or more points during pre-harvest and post-harvest process lines.

17.3 Post-harvest persistence, colonization, and survival on fresh vegetables

Numerous studies have examined the growth or die-off of pathogens under variable temperature conditions experienced during the processing, storage, and shipping of fresh-cut or minimally processed vegetables. Hard conclusions of growth rates and maximum population densities based upon these individual investigations are difficult due to variances in experimental design, test strains (including the use of stressed versus non-stressed bacterial cells), produce type, and distinct packaging materials. It is an accepted fact that native bacterial populations, including any pathogens that might be present, are in constant flux during post-harvest handling and storage. Moreover, variables such as storage temperature and time greatly impact microbial levels and product quality. The US Food and Drug Administration Food Code requires that packaged ready-to-eat fruits and leafy green vegetables be refrigerated at less than or equal to 5°C to minimize the growth of food-borne pathogens. Luo *et al.* (2009) noted increases in native microflora and *E. coli* O157:H7 after storage at ≥8°C within the labeled “Best If Used By” date of bagged baby spinach. In addition, *E. coli* has been noted to grow on

fresh-cut iceberg lettuce an additional 1 log cfu/g over a period of 6 hours at temperatures above 16°C (Rodríguez-Caturla *et al.*, 2012). Similarly, multiple studies have demonstrated *Salmonella*'s ability to persist on whole produce, internally and externally, across a broad range of temperatures from 4 to 25°C for 7 days up to 8 weeks (Liao *et al.*, 2010; Vandamm *et al.*, 2013; Shi *et al.*, 2007; Iturriaga *et al.*, 2007; Kroupitski *et al.*, 2009; Zhou *et al.*, 2014; Beuchat and Mann, 2008). Higher storage temperatures (above 15°C) and longer storage time typically allow *Salmonella* to grow on or within a produce commodity. An examination of growth kinetics (Table 17.1) of *Salmonella* Newport growth on beefsteak and Roma tomatoes shows no significant difference in growth between these two tomato types, and after a lag phase of approximately 6 h, the exponential growth rate reaches almost 0.3 log/h. Pre-storage of the tomatoes at 5°C did not alter the growth kinetics compared to pre-storage at 22°C (Table 17.2), suggesting that pre-storage temperature has little impact on the growth of *Salmonella* once it is held at a higher temperature for storage after slicing. Conversely, storage at lower temperatures (lower than 10°C) suppresses *Salmonella* growth, and with longer storage times, a

decrease in cell number may occur (Pao *et al.*, 2012; Vandamm *et al.*, 2013).

Luo and others (2010) looked at fresh-cut romaine and iceberg lettuce inoculated with *E. coli* O157:H7 and resealed in bags containing the original O₂ levels. At 5°C, *E. coli* O157:H7 populations decreased almost 2 log cfu/g by day 10 even though viable cells were still detected. McKellar *et al.* (2012) monitored and recorded the temperatures of 27 cases of packaged lettuce throughout various stages of storage and shipping from the processor to the retail shelf of three stores. The results indicate a nearly 1 log reduction in viable bacterial cells and the extent of die-off was proportional to the overall time spent in refrigeration. The authors suggested a reduced risk of illness to consumers when fresh-cut lettuce is stored at 5°C or below prior to consumption. *L. monocytogenes*, on the other hand, is psychrotropic and capable of growth at low temperatures due to a variety of intrinsic physiological attributes (Laksanalamai *et al.*, 2011).

Several studies examined the growth potential of *L. monocytogenes* in a variety of fresh produce (Ells and Truelstrup, 2006; Sant'Ana *et al.*, 2012, 2013; Skalina and Nikolajeva, 2010; Tian *et al.*, 2012). In all of these commodities,

Table 17.1 Growth kinetics of *S. Newport* in fresh-cut red ripe tomatoes stored at 22°C.

	pH	a _w	LDT (h)	EGR (log/h)
Beefsteak	4.25±0.03	0.994±0.001	6.40±0.90	0.299±0.010
Roma	4.18±0.09	0.994±0.002	5.77±0.49	0.298±0.014

Table 17.2 Effect of storage temperature before cutting on the growth kinetics of *S. Newport* inoculated in fresh-cut red ripe tomatoes stored at 22°C.

	Storage temperature			
	22°C		5°C	
	LDT (h)	EGR (log/h)	LDT (h)	EGR (log/h)
Beefsteak	2.28±0.95	0.232±0.029	3.81±0.91	0.244±0.036
Roma	2.46±0.73	0.251±0.006	3.34±0.44	0.260±0.005

L. monocytogenes grew 1–2 logs in 7–14 days at 5°C. Growth of 3–4 logs was noticed, however, when the temperature was increased to 10–15°C. In a detailed commodity-specific study of *L. monocytogenes*, the growth potentials of *L. monocytogenes* strains were examined on fresh-cut celery at different temperatures and incubation periods. A cocktail of three outbreak strains of *L. monocytogenes* including serotypes 1/2a, 1/2b, and 4b were used as an inoculum at $3\text{--}5 \times 10^3$ cfu per 5 g of celery. The inoculated samples were stored at 5°C for 30 days, 10°C for 12 days, and 25°C for 7 days. The growth pattern of all three serotypes was comparable at all temperatures; the growth rate was found to be much slower at 5°C followed by 10 and 25°C. The increase in counts at the end of the sampling periods were approximately 3–4 log cfu at 25°C, 2 log cfu at 10°C and 1 log cfu at 5°C per 5 g of celery (Sahu *et al.*, 2014; see Table 17.3). Maximum growth was found to be primarily a function of temperature and duration of incubation while the growth rate was dependent solely on the temperature of storage. These results clearly indicated that *L. monocytogenes* could survive and grow on a variety of fresh vegetables even at refrigerated temperatures. Zeng and others (2014) conducted a comprehensive large-scale study in the US placing temperature sensors in cross-country transport trucks, as well as in the storage rooms and display cases in nine supermarkets to determine temperature fluctuations that fresh-cut bagged

leafy greens might experience during a one to three day storage period in each location prior to purchase by the consumer. Temperature profiles were then reproduced with *E. coli* O157:H7 and *L. monocytogenes* artificially inoculated on to romaine lettuce. Retail storage showed the largest temperature fluctuations (i.e., range of 0.6 to 15.4°C) and, correspondingly, the levels of *E. coli* O157:H7 and *L. monocytogenes* showed the greatest increase, around 3.0 log cfu/g, over the three days of storage. Mean measured temperatures for transport and display in retail cases were rarely above 6°C. While *E. coli* O157:H7 growth was essentially static during this time, *L. monocytogenes* barely proliferated under transport conditions (≤ 0.6 log cfu/g) but increased up to 1.1 log cfu/g during three days of housing in display cases (Zeng *et al.*, 2014). This study illustrated the importance of maintaining an uninterrupted cold chain.

Temperature abuse has not only been noted to occur within the retail environment but can also be a problem within the consumer's home. Somewhat alarming was the notion made by James *et al.* (2008) who reviewed 20 published studies, conducted in parts of Europe, the US, and New Zealand from 1987 to 2006, and found only two studies reporting mean air temperatures of less than 5°C in domestic refrigerators. Most studies showed a great deal of concordance, citing temperatures in >50% of refrigerators above 5°C.

The pH of a produce commodity may also contribute to the ability of pathogens to grow on

Table 17.3 Growth of *Listeria monocytogenes* in artificially contaminated cut celery.

Strain	5°C		10°C		25°C	
	Maximum growth (days)	Doubling time (h)	Maximum growth (days)	Doubling time (h)	Maximum growth (days)	Doubling time (h)
LS806(4b)	2.6×10^4 (30 d)	87.6	1.2×10^5 (12 d)	25.3	8×10^6 (4 d)	4.6
LS814(1/2a)	2.7×10^4 (30 d)	72.8	1.7×10^5 (12 d)	25.9	2.6×10^7 (4 d)	3.8
LS810(1/2b)	4.3×10^4 (30 d)	74.1	2×10^5 (12 d)	23.9	8.2×10^6 (4 d)	4.4

it. For example, the optimal pH range for *Salmonella* is 6.5–7.5. However, the pH range in which salmonellae can survive and grow is much broader (i.e., 3.7–9.5). Tomatoes are considered to be relatively acidic with pH values ranging from 4.37 for round to 4.67 for grape tomatoes (Beuchat and Mann, 2008), but these values are not outside of the pH range to prevent the growth of *Salmonella*. As shown in Table 17.1, *S. Newport* was able to grow in both red round and Roma tomatoes with a pH of 4.2. Inspecting the growth kinetics of *S. Newport* in blended tomatoes with pH adjusted from 3.9 to 4.3 (Table 17.4), growth was observed at all pH levels, albeit at a lower rate and at the lowest pH. This low pH tolerance was also observed by Asplund and Nurmi (1991), where three different *Salmonella* serovars, Enteritidis, Infantis, and Typhimurium, were all able to grow on cut tomatoes with low pH values (3.99–4.37). The ability of *Salmonella* to grow to such high numbers in tomatoes may be due to the major acidulants within tomatoes, citric and malic acids, to which salmonellae may be more tolerant.

Packaging may affect the growth of pathogens on fresh-cut vegetables as well. Moisture enters bags of fresh-cut leafy greens as a residual from product washing. Vapor condensation could also lead to the accumulation of water inside the bags. Valentin-Bon *et al.* (2008) suggested that moisture (i.e., condensation) observed at the bottom of many bags may provide a better opportunity for microbial growth including that of any pathogens present. In a subsequent study, Kase *et al.* (2012) noted an

absence of moisture condensation in the bags, which may suggest industry advances in moisture control and bag design (e.g., anti-fog film, perforated versus non-perforated, etc.).

Differences in genetic make-up and efficiency in expression of stress-related pathways most likely dictate the ability of a particular serovar or strain to survive when exposed to various stressors. For example, *Salmonella* possesses the ability to survive in a desiccated state. The desiccation tolerance of several different serovars demonstrated a range of survival from 36 to 80%, depending on the serovar. Additionally, exposing salmonellae to desiccation conditions induced tolerance to several other stressors, including high salts, ethanol, bleach, high temperatures, and UV irradiation (Gruzdev *et al.*, 2011).

17.4 Routes of contamination during post-harvest handling of fresh and fresh-cut vegetables

During post-harvest processing, various routes of contamination with human pathogens may include contaminated water used for washing, chill tanks or sprays and shipping ice, processing equipment and transportation, infected workers, and cross-contamination from food preparation, display, and storage. Recently, Johnston *et al.* (2005) studied the quality of fresh produce at different stages from harvest throughout the packing shed in the southern United States. The group found that total aerobic plate count (APC) levels in cilantro increased from the field and throughout packing, with mean ranges of 5.7 log in the field to 6.7 log cfu/g in the samples obtained from boxes ready for distribution. Total coliforms increased significantly from harvest through packing, with a peak occurring mainly at the rinse step. All of these results suggested that microbiological contamination could either increase or originate during post-harvest processing. Several key areas have been identified as high risk for

Table 17.4 Growth kinetics of *S. Newport* on blended beefsteak and tomatoes as affected by pH incubated at 22 °C.

pH*	LDT (h)	EGR (log/h)
4.3	5.32 ± 0.87	0.189 ± 0.065
4.2	4.92 ± 3.47	0.105 ± 0.088
4.1	6.58 ± 3.11	0.167 ± 0.096
4.0	7.57 ± 2.73	0.146 ± 0.105
3.9	6.9 ± 2.10	0.123 ± 0.024

*pH adjusted with citric acid.

cross-contamination including water used to wash produce, kitchen surfaces, cutting surfaces, and worker hand hygiene, including the use of gloves (Doyle and Erickson, 2008; Todd *et al.*, 2010; Waitt *et al.*, 2013; Zhou *et al.*, 2014).

Water washes are often used immediately after harvest to remove field debris before subsequent processing steps. In the tomato industry, tomatoes are brought from fields to packing houses where they are placed in large dump tanks for washing before sorting and sizing. Contamination of this water can lead to internal and external contamination of the tomatoes. Internal contamination occurs when water and other foreign material, such as *Salmonella*, rush into the tomato via the stem scar due to differences in hydrostatic pressure, differences in temperature, or capillary action (Bartz, 1982; Smith *et al.*, 2006). Recent findings have demonstrated that even with a 10 °F positive temperature differential (i.e., washwater 10 °F warmer than incoming tomatoes) *Salmonella* was still able to internalize (Zhou *et al.*, 2014).

Common kitchen surfaces are another mechanism for transfer of pathogens to fresh produce. In one large study examining many common kitchen surfaces, such as ceramic, glass, plastic, and stainless steel, it was demonstrated that *Salmonella* can readily be transferred from contaminated surfaces to fresh produce, with higher transfer rates from wet surfaces (79–97% transfer) (Jensen *et al.*, 2013). The potential for fresh produce to contaminate the kitchen surface also exists, albeit at significantly lower rates of transfer. This directional tendency of pathogens to move from the kitchen surface to produce is attributed to the limited availability of nutrients and suitable attachment sites on abiotic surfaces. Additionally, microbial attachment to produce commodities is facilitated by the presence of complex carbohydrates (Jensen *et al.*, 2013). *S. Montevideo* was transferred from the surface of a tomato to the interior by cutting with a sterile knife (Lin and Wei, 1997). The amount transferred and the depth of transfer into the tomato pulp increased

in a dose-dependent manner. Additionally, when the same knife was used to cut subsequent tomatoes, transfer was noted well into the interior of the next tomato. A key example of cutting surface involvement leading to an outbreak situation happened in Queensland, Australia, where an outbreak of *S. Bovismorbificans* linked to cut lettuce leaves occurred. Investigators discovered the outbreak organisms on the surface of the cutting equipment used to process the lettuce and sited inadequate cleaning and sanitation of the cutting equipment leading to the contamination of lettuce products (Stafford *et al.*, 2002). *Listeria* has been isolated in several food processing environments. *Listeria*, specifically *L. monocytogenes*, has the ability to grow on different food contact surfaces where it is capable of establishing biofilms (Silva *et al.*, 2008). The organism has been isolated from various locations on food premises including drains, abattoirs, conveyor belts, freezers, smoke houses, slicing blades, packaging machines, floors and walls, footbaths, air ducts, and others (Moretro and Langsrud, 2004; Sofos, 2008). Biofilms can protect the embedded bacteria from antibacterial treatments such as sanitization, desiccation, UV radiation, concentrated disinfectants, etc., allowing the persistence of *L. monocytogenes* for long periods of time in the processing environment. These biofilm-coated surfaces become sources of frequent contamination when food products come into contact with them (Carpentier and Cerf, 2011; Hall-Stoodley *et al.*, 2004).

Good worker hygiene is critical to prevent the transfer of pathogens to fresh produce as well. The use of gloves as a barrier can be an effective means to reduce the risk of transfer. Unfortunately, glove use can lead to a false sense of security and unsafe practices are adopted because workers believe that gloves will prevent any cross-contamination (Todd *et al.*, 2010). Gloves were shown to aid in the transfer of *S. Enteritidis* to the edible portions of living lettuce during harvest (Waitt *et al.*, 2013). Moreover, high transfer rates were seen from the point of contamination to the subsequent three heads of

lettuce. Thus, infrequent glove changes can lead to the contamination of large numbers of produce items.

17.5 Microbial adaptation on produce commodity

It has become clear that natural selection plays a significant role in the adaptive change now observed among *Salmonella* strains associated with the food supply, produce notwithstanding. The recent application of whole-genome sequencing, with both fragment-based and long read technologies included, has yielded important clues into the specific genetic determinants that give rise to adapted phenotypes in *Salmonella*. Moreover, these data have provided substantial insight into the genetic mechanisms that underpin adaptive change among salmonellae now thriving in produce and other post-harvest processing niches including the mobilome (i.e., high-frequency horizontally transferrable elements such as phage, transposable elements, and other recombination hotspots across the genome) of *Salmonella*. The intersect of adaptive change and horizontal transfer is not insignificant as it has been postulated that those changes which are honed by selection are then transferred rapidly across the population by means of laterally enhanced regions of the genome (Allard *et al.*, 2012).

Adaptation among *Salmonella* strains may account for the novel genetic changes now emerging among certain produce vehicles. *S. Saintpaul*, for instance, recently associated

with one of the largest fresh produce contamination events ever documented in North America, appears to have acquired a number of nucleotide substitutions that distinguish it from other non-tomato/pepper associated Saintpauls. A recent study by Hayford *et al.* (2015) (Figure 17.1) documents a variety of non-synonymous single nucleotide changes across the genome when compared to other isolates from other produce- and non-produce-related sources. Interpretation of these nucleotide substitutions in the context of the amino acid PAM matrix pointed to several amino acid changes with functionally relevant differences in the host protein (Mount, 2008). That is, several non-synonymous changes were conserved biochemically and may play a role in the enhanced survival of *S. Saintpaul* strains in pepper and tomato and potentially in other members of the *Solanaceae*. Surprisingly, these changes have provided a substantial clue to potential areas of adaptation having occurred in genes responsible for propanediol utilization (*pduF*) and propanediol diffusion facilitator (*pudB*) genes. Interestingly, propanediol is a metabolite resulting from ripening or rotting of plant tissues (Bobik *et al.*, 1997; Brandl *et al.*, 2013; Goudeau *et al.*, 2013). Previous studies revealed that populations of mutants deficient in propanediol utilization were several logs lower than wild-type strains grown in cilantro (Goudeau *et al.*, 2013). In the light of *S. Saintpaul*'s documented association with at least Jalapeno and Serrano peppers, these changes may be of significance, representing key adaptive changes among produce-specific *Salmonella*. Moreover, these changes may signal

Figure 17.1 Genetic Map of *Salmonella* Saintpaul propanediol operon (see GenBank sequence gb|AOXY01000026.1). Regions of two propanediol genes (*pduF* and *pduB*) with location and positions of nonsynonymous and synonymous SNP mutations.

emerging alleles in produce niches such as Jalapeno peppers, yielding an adaptive metabolic premium allowing for survival of specific *S. Saintpaul* in harsh and relatively narrow produce settings.

Adaptive change among bacteria in the produce and produce-processing environment is likely to be further driven by intrinsic genetic factors known to enhance evolutionary change and the acquisition of adaptive change among enteric pathogens. Such evolution may be explained in part by the hypermutable phenotype (LeClerc *et al.*, 1996) caused by defects in the bacterial methyl-directed mismatch repair (MMR) system. Up to 73% of the MMR defects found in feral settings are due to lesions within the *mutS* gene, resulting in increased nucleotide substitution rates, enhanced DNA transposition, and, perhaps most importantly, a relaxation of the internal barriers that normally restrict homologous recombination following horizontal gene transfer (HGT) of foreign DNA (LeClerc and Cebula, 1997). The now incontrovertible connection between HGT and MMR gene evolution has led to the thesis that genetic exchange of *mutS* alleles could simultaneously quiet the mutator phenotype while rescuing adaptive changes from the population (LeClerc *et al.*, 1996; Brown *et al.*, 2001). Consistent with this hypothesis, the *mutS* gene is evolutionarily scrambled by HGT in subspecies I *S. enterica*, which has been documented in our laboratories (Brown *et al.*, 2002, 2003).

In *L. monocytogenes*, two cell–cell communication systems, *luxS* orthologous (Challan *et al.*, 2006) and virulence regulator, the *arg* system (Riedel *et al.*, 2009; Rieu *et al.*, 2007), have been reported to be involved in the regulation of biofilm formation. *luxS* mutants in *L. monocytogenes* strains are reported to form denser biofilms than parental strains (Sela *et al.*, 2006). Flagellum-mediated motility also plays an important role in biofilm formation in *L. monocytogenes* (Lemon *et al.*, 2007). Virulence genes like *prfA* and *inlA* are also reported to have important roles in biofilm development in *L. monocytogenes*. The *prfA* mutant is reported

to present a defective biofilm compared with the wild type (Lemon *et al.*, 2010), whereas truncated *InlA* showed enhanced biofilm formation compared with the full length one (Franciosa *et al.*, 2009). It is interesting to note that many proteins in *L. monocytogenes* have evolved to serve both for environmental adaptation such as biofilm formation and adaptation in human hosts leading to disease manifestation.

17.6 Effective post-harvest intervention technologies

The produce industry faces unique challenges for eliminating pathogen contamination when compared to other types of foods. In 2013, the US FDA Food Safety Modernization Act (FSMA) proposed rules for produce safety to set standards in identified routes of microbial contamination of produce, including: (1) agricultural water; (2) biological soil amendments of animal origin; (3) health and hygiene; (4) animals in the growing area; and (5) equipment, tools, and buildings (<http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm>). However, even with the proper plan and systems in place, it may still be possible for some microbial contamination to occur.

A better understanding of microbial ecosystems on the surface of raw vegetables would be extremely useful when developing interventions to minimize contamination, prevent the growth of pathogens, and kill or remove pathogens at various stages of pre- and post-harvest. The composition and abundance of microbial ecosystems unique to various types of produce (Leff and Fierer, 2013; Barak *et al.*, 2008) can be greatly influenced by changes in practice and field conditions prior to harvest and alterations in conditions of various stages after harvesting.

After weeks of sun and ambient temperature exposure, vegetables typically undergo a pre-cooling step soon after harvest to reduce the field heat. Pre-cooling is generally done through forced air cooling, hydrocooling (cold water

Table 17.5 Chemical and physical interventions on fresh and fresh-cut vegetables in post-harvest.

Disinfection technique	Dose/concentration	Investigated vegetable commodity	Targeted microorganism	Results	Reference
<i>Chemical-based disinfection</i>					
Chlorine	50–200 ppm	Carrots, lettuce, cabbage, bell peppers, artichokes, borages, cilantro, and coleslaw mix	Bacteria, virus	1–2 log reduction	Gonzalez et al. (2004), Akbas and Olmez (2007), Allwood et al. (2004), Foley et al. (2004), Hadjok et al. (2008), Sanz et al. (2002), Uyttendaele et al. (2004)
Chlorine dioxide	Aqueous: 100 ppm Gaseous: 1.2–4.1 mg/l	Cucumber, lettuce, carrot, tomato, onion, and cabbage	Bacteria, yeast, molds	1.5–5.8 log reduction	Singh et al. (2002), Chung et al. (2011), Sy et al. (2005)
Organic acids	0.5–1.0%	Iceberg lettuce, spinach	Bacteria	1–2 log reduction	Akbas and Olmez (2007), Neal et al. (2012)
Hydrogen peroxide	1–3%	Mushrooms, tomatoes, red bell peppers, lettuce, spinach, cucumbers, zucchini, and bell peppers	Bacteria	1–3 log reduction	Back et al. (2014), Moore et al. (2011), Kim et al. (2007), Sapers and Simmons (1998)
Peroxyacetic acid	80–100 ppm	Lettuce, spinach, celery, cabbage, and leek	Bacteria, yeast, molds, and virus	1–2 log reduction	Neal et al. (2012), Fraisse et al. (2011), Vandekinderen et al. (2009)
Calcium-based solutions	1.5%	Lettuce	Bacteria	1–2 log reduction	Martin-Diana et al. (2005)
Electrolyzed oxidizing water	10–50 ppm available chlorine	Lettuce, Chinese celery, bell pepper, Japanese radish, potato, carrots, spinach, cucumber, and kale	Bacteria, yeast, molds, virus, and fungus	Up to 2.9 log reduction	Buck et al. (2002), Hati et al. (2012), Issa-Zacharia et al. (2011), Izumi (1999), Tamaki et al. (2014), Mansur et al. (2014), Koide et al. (2011)
Ozone	Up to 10 ppm O ₃	Lettuce, potato, carrot, spinach, cucumber, tomato, baby leaf, brassica, cabbage, rocket leaf, pepper,	Bacteria, yeast, molds, and fungus	Up to 2.6 log reduction	Singh et al. (2002), Garcia et al. (2003), Beltran et al. (2005a, 2005b), Horvitz and Cantalejo (2014)
Plant extracts		Cilantro, parsley, spinach, carrot, and tomato	Bacteria	Up to 4 log reduction	Singh et al. (2002), Orue et al. (2013), Lu and Wu (2010), Mattson et al. (2011)
<i>Physical-based treatment</i>					
Thermal treatments	85–100 °C	Lettuce, green bell pepper	Bacteria, yeast, molds and parasite	1 log reduction	Rico et al. (2007), Duhaïn et al. (2012)
High-pressure processing	300–600 mPa	tomato	Bacteria	0.5–3.6 log reduction	Maitland et al. (2011)
Irradiation	≤1.0 kGy	Cilantro, lettuce	Bacteria, molds, yeast	Up to 6.7 log reduction	Foley et al. (2004), Goularte et al. (2004)
Ultraviolet light	1.18 kJ/m ² , 2.37 kJ/m ²	Lettuce	Bacteria, yeast, and molds	1–2 log reduction	Allende et al. (2006)

dunk or rinse), icing (direct or indirect contact with produce), vacuum cooling, or hydrovac cooling (i.e., water is sometimes sprayed on the produce prior to vacuum cooling). Vegetables in close contact with the ground usually undergo a washing step following harvest to remove dirt and other debris taken from the agricultural fields. Typically washwater contains a disinfectant which, if used correctly, can reduce the populations of both human pathogens and spoilage microorganisms. Care should be taken to ensure the presence of a sufficient amount of sanitizers in the washwater in order to prevent pathogen cross-contamination. Traditionally, chlorine in the form of a sodium hypochlorite solution or as a dry, powdered calcium hypochlorite is used in hydrocooling or washwater as a disinfectant. However, the reaction of chlorine with other organic compounds in perishable produce may lead to the formation of halogenated by-products in the presence of organic matter, giving rise to toxicity concerns. In some European countries, including Germany, the Netherlands, Switzerland, and Belgium, the use of chlorine in RTE products is prohibited (Rico *et al.*, 2007). Moreover, the efficacy of chlorine to reduce microbial pathogens on vegetables is often limited by pH (i.e., hypochlorous acid – the form with the most antimicrobial activity – is present at pH 6.5 to 7.5), temperature, exposure to light, levels of soil and organic matter, initial and residual free chlorine concentration, and length of exposure (Gonzalez *et al.*, 2004; Shen *et al.*, 2013). Several innovative approaches both chemically and physically have been explored for the decontamination of fresh or fresh-cut vegetables (Table 17.5). Effective washing and decontamination of fresh-cut vegetables is difficult to achieve due to the different types of vegetables, the inadequate efficacy of individual treatments alone (Table 17.5), the presence of biofilms on vegetables and on processing equipment (Jahid and Ha, 2012; Somers *et al.*, 1994), and internalization/infiltration of bacteria within produce (Zhuang *et al.*, 1995; Zheng *et al.*, 2013; Takeuchi and Frank, 2000). Therefore, a combination of

different disinfection methods (e.g., hurdle technology) (Rico *et al.*, 2007; Joshi *et al.*, 2013) is necessary to increase the efficacy of disinfectants against microbial population reduction. Novel biocontrol strategies using environmentally and ecologically friendly bacterial epiphytes, designed and now being evaluated with human health end points (i.e., prevention of salmonellosis and other produce-borne illnesses), may also play a significant role in the reduction of pathogen loads on fresh produce. Given the lack of effective technologies to eliminate pathogens from produce surfaces and the potential for pathogen cross-contamination during produce washing and post-harvest handling, preventing pathogen proliferation via temperature control is critical to mitigate food safety risks. Overall, technology advances spanning the detection, monitoring, and tracking of food-borne pathogens along with more highly effective preventive control and kill-step measures will be crucial to maintaining a safe fresh and fresh-cut produce supply for consumers in the US and around the world.

References

- Akbas, M.Y., and Olmez, H. 2007. Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. *Letters in Applied Microbiology*, **44**, 619–624.
- Allard, M.W., Luo, Y., Strain, E., Li, C., *et al.* 2012. High resolution clustering of *Salmonella enterica* serovar Montevideo strains using a next-generation sequencing approach. *BMC Genomics*, **13**, 32.
- Allen, K.J., Kovacevic, J., Cancarevic, A., Wood, J., Xu, J., Gill, B., Allen, J.K., and Mesak, L.R. 2013. Microbiological survey of imported produce available at retail across Canada. *International Journal of Food Microbiology*, **162**, 135–142.
- Allende, A., McEvoy, J.L., Luo, Y.G., Artes, F., and Wang, C.Y. 2006. Effectiveness of two-sided UV-C treatments in inhibiting natural microflora

- and extending the shelf-life of minimally processed "Red Oak Leaf" lettuce. *Food Microbiology*, **23**, 241–249.
- Allwood, P.B., Malik, Y.S., Hedberg, C.W., and Goyal, S.M. 2004. Effect of temperature and sanitizers on the survival of feline calicivirus, *Escherichia coli*, and F-specific coliphage MS2 on leafy salad vegetables. *Journal of Food Protection*, **67**, 1451–1456.
- Althaus, D., Hofer, E., Corti, S., Julmi, A., and Stephan, R. 2012. Bacteriological survey of ready-to-eat lettuce, fresh-cut fruit, and sprouts collected from the Swiss market. *Journal of Food Protection*, **75**, 1338–1341.
- Asplund, K. and Nurmi, E. 1991. The growth of salmonellae in tomatoes. *International Journal of Food Microbiology*, **13**, 177–182.
- Back, K.H., Ha, J.W., and Kang, D.H. 2014. Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. *Food Control*, **44**, 78–85.
- Barak, J.D., Liang, A., and Narm, K.-E. 2008. Differential attachment to and subsequent contamination of agricultural crops by *Salmonella enterica*. *Applied and Environmental Microbiology*, **74**, 5568–5570. doi: 10.1128/AEM.01077-08.
- Bartz, J.A. 1982. Infiltration of tomatoes immersed at different temperatures to different depths in suspensions of *Erwinia carotovora* subsp. *carotovora*. *Plant Diseases*, **66**, 302–305.
- Beltran, D., Selma, M.V., Tudela, J.A., Gil, M.I. 2005a. Effect of different sanitizers on microbial and sensory quality of fresh-cut potato strips stored under modified atmosphere or vacuum packaging. *Postharvest Biology and Technology*, **37**, 37–46.
- Beltran, D., Selma, M.V., Marin, A., Gil, M.I. 2005b. Ozonated water extends the shelf life of fresh-cut lettuce. *Journal of Agriculture and Food Chemistry*, **53**, 5654–5663.
- Beuchat, L.R. and Mann, D.A. 2008. Survival and growth of acid-adapted and unadapted *Salmonella* in and on raw tomatoes as affected by variety, stage of ripeness, and storage temperature. *Journal of Food Protection*, **71**, 1572–1579.
- Bobik, T.A., Xu, Y., Jeter, R.M., Otto, K.E., and Roth, J.R. 1997. Propanediol utilization genes (pdu) of *Salmonella typhimurium*: three genes for the propanediol dehydratase. *Journal of Bacteriology*, **179**, 6633–6639.
- Brandl, M.T., Cox, C.E., and Teplitski, M. 2013. *Salmonella* interactions with plants and their associated microbiota. *Phytopathology*, **103**, 316–325.
- Brown, E.W., LeClerc, J.E., Li, B., Payne, W.L., and Cebula, T.A. 2001. Phylogenetic evidence for horizontal transfer of mutS alleles among naturally occurring *Escherichia coli* strains. *Journal of Bacteriology*, **183**, 1631–1644.
- Brown, E.W., Kotewicz, M.L., and Cebula, T.A. 2002. Detection of recombination among *Salmonella enterica* strains using the incongruence length difference test. *Molecular Phylogenetic Evolution*, **24**, 102–120.
- Brown, E.W., Mammel, M.K., LeClerc, J.E., Cebula, T.A. 2003. Limited boundaries for extensive horizontal gene transfer among *Salmonella* pathogens. *PNAS*, **100**, 15676–15681.
- Buck, J.W., van Iersel, M.W., Oetting, R.D., Hung, Y.C. 2002. *In vitro* fungicidal activity of acidic electrolyzed oxidizing water. *Plant Diseases*, **86**, 278–281.
- Carpentier, B. and Cerf, P. 2011. Persistence of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology*, **145**, 1–8.
- Centers for Disease Control and Prevention (CDC). 2006. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach – United States, September 2006. *Morbidity and Mortality Weekly Report*, **55**, 1045–1046.
- Challan, B.S., et al. 2006. Assessment of the roles of LuxS, S-ribosyl homocysteine, and autoinducer 2 in cell attachment during biofilm formation by *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology*, **72**, 2644–2650.
- Chung, C.-C., Huang, T.-C., Yu, C.-H., Shen, F.-Y., and Chen, H.-H. 2011. 2011 International Conference on Food Engineering and Biotechnology, Singapore, pp. 107–112.

- Consumer Union. 2010. Bacteria and bagged salads: better standards and enforcement needed. Available at: <http://www.consumersunion.org/pdf/BaggedSaladReport.pdf>.
- Cook, R. 2012. Trends in the marketing of fresh produce and fresh-cut products. University of California [Online]. Posted on September 18, 2012.
- Doyle, M.P. and Erickson, M.C. 2008. Summer meeting 2007 - the problems with fresh produce: an overview. *Journal of Applied Microbiology*, **105**, 317–330.
- Duhain, G.L.M.C., Minnaar, A., and Buys, E.M. 2012. Effect of chlorine, blanching, freezing, and microwave heating on *Cryptosporidium parvum* viability inoculated on green peppers. *Journal of Food Protection*, **75**, 936–941.
- Ells, T.C. and Truelstrup, H.L. 2006. Strain and growth temperature influence *Listeria* spp. attachment to intact and cut cabbage. *International Journal of Food Microbiology*, **111**, 34–42.
- Foley, D., Euper, M., Caporaso, F., and Prakash, A. 2004. Irradiation and chlorination effectively reduces *Escherichia coli* O157:H7 inoculated on cilantro (*Coriandrum sativum*) without negatively affecting quality. *Journal of Food Protection*, **67**, 2092–2098.
- Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., Vialette, M., Morin, T., and Perelle, S. 2011. Comparison of chlorine and peroxyacetic-based disinfectant to inactivate feline calicivirus, murine norovirus and hepatitis A virus on lettuce. *International Journal of Food Microbiology*, **151**, 98–104.
- Franciosa, G., et al. 2009. Expression of internalin A and biofilm formation among *Listeria monocytogenes* clinical isolates. *International Journal of Immunopathology and Pharmacology*, **22**, 183–193.
- Garcia, A., Mount, J.R., and Davidson, P.M. 2003. Ozone and chlorine treatment of minimally processed lettuce. *Journal of Food Science*, **68**, 2747–2751.
- Gonzalez, R.J., Luo, Y.G., Ruiz-Cruz, S., and McEvoy, J.L. 2004. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *Journal of Food Protection*, **67**, 2375–2380.
- Goudeau, D.M., Parker, C.T., Zhou, Y., Sela, S., Kroupitski, Y., and Brandl, M.T. 2013. The *Salmonella* transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. *Applied and Environmental Microbiology*, **79**, 250–262.
- Goularte, L., Martin, C.G., Morales-Aizpurua, I.C., Destro, M.T., et al. 2004. Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L.). *Radiation and Physical Chemistry*, **71**, 157–161.
- Gruzdev, N., Pinto, R., and Sela, S. 2011. Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. *Applied Environmental Microbiology*, **77**, 1667–1673.
- Hadjok, C., Mittal, G.S., and Warriner, K. 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *Journal of Applied Microbiology*, **104**, 1014–1024.
- Hall-Stoodley, L., Costerton, J.W., and Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *National Review of Microbiology*, **2**, 95–108.
- Hati, S., Mandal, S., Minz, P.S., Vij, S., Khetra, Y., and Singh, B.P. 2012. Electrolyzed oxidized water (EOW): non-thermal approach for decontamination of food borne microorganisms in food industry. *Food and Nutrition Sciences*, **3**, 760–768.
- Hayford, A.E., Brown, E.W., Zhao, S., Mammel, M.K., et al. 2015. Genetic and resistance phenotypic subtyping of *Salmonella* Saintpaul isolates from various food sources and humans: Phylogenetic concordance in combinatory analyses. *Infection, Genetics and Evolution*, **36**, 92–107.
- Horvitz, S. and Cantalejo, M.J. 2014. Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, **54**, 312–339.
- Issa-Zacharia, A., Kamitani, Y., Miwa, N., Muhimbula, H., and Iwasaki, K. 2011. Application of slightly

- acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. *Food Control*, **22**, 601–607.
- Iturriaga, M.H., Tamplin, M.L., and Escartin, E.F. 2007. Colonization of tomatoes by *Salmonella* Montevideo is affected by relative humidity and storage temperature. *Journal of Food Protection*, **70**, 30–34.
- Izumi, H. 1999. Electrolyzed water as a disinfectant for fresh-cut vegetables. *Journal of Food Science*, **64**, 536–539.
- Jahid, I.K. and Ha, S.D. 2012. A review of microbial biofilms of produce: future challenge to food safety. *Food Science and Biotechnology*, **21**, 299–316.
- James, S.J., Evans, J., and James, C. 2008. A review of the performance of domestic refrigerators. *Journal of Food Engineering*, **87**, 2–10.
- Jensen, D.A., Friedrich, L.M., Harris, L.J., Danyluk, M.D., and Schaffner, D.W. 2013. Quantifying transfer rates of *Salmonella* and *Escherichia coli* O157:H7 between fresh-cut produce and common kitchen surfaces. *Journal of Food Protection*, **76**, 1530–1538.
- Johnston, L.M., Jaykus, L.A., Moll, D., Martinez, M.C., Anciso, J., Mora, B., and Moe, C.L. 2005. A field study of the microbiological quality of fresh produce. *Journal of Food Protection*, **68**, 1840–1847.
- Joshi, K., Mahendran, R., Alagusundaram, K., Norton, T., and Tiwari, B.K. 2013. Novel disinfectants for fresh produce. *Trends in Food and Science Technology*, **34**, 54–61.
- Kase, J.A., Borenstein, S., and Feng, P.C.H. 2012. Microbial quality of bagged baby spinach and romaine lettuce – effects of top vs. bottom sampling. *Journal of Food Protection*, **75**, 132–136.
- Kim, H.J., Fonseca, J.M., Kubota, C., Choi, J.H. 2007. Effect of hydrogen peroxide on quality of fresh-cut tomato. *Journal of Food Science*, **72**, S463–S467.
- Koide, S., Shitanda, D., Note, M., and Cao, W. 2011. Effects of mildly heated, slightly acidic electrolyzed water on the disinfection and physicochemical properties of sliced carrot. *Food Control*, **22**, 452–456.
- Koseki, S., Mizuno, Y., Kawasaki, S., and Yamamoto, K. 2011. A survey of iceberg lettuce for the presence of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Japan. *Journal of Food Protection*, **74**, 1543–1546.
- Kroupitski, Y., Pinto, R., Brandl, M.T., Belausov, E., and Sela, S. 2009. Interactions of *Salmonella enterica* with lettuce leaves. *Journal of Applied Microbiology*, **106**, 1876–1885.
- Laksanalamai, P., Burall, L., and Datta, A.R. 2011. *Adaptation Mechanisms of Psychrotolerant Bacterial Pathogens. Extremophiles Handbook* (ed. Horikoshi, K.). Springer.
- LeClerc, J.E. and Cebula, T.A. 1997. Hypermutability and homeologous recombination: ingredients for rapid evolution. *Bulletin of Institute of Pasteur*, **95**, 97–106.
- LeClerc, J.E., Li, B., Payne, W.L., and Cebula, T.A. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science*, **274**, 1208–1211.
- Leff, J.W. and Fierer, N. 2013. Bacterial Communities Associated with the Surfaces of Fresh Fruits and Vegetables. *PLoS One*, **8**, e59310. doi: 10.1371/journal.pone.0059310.
- Lemon, K.P., Higgins, D.E., and Kolter, R. 2007. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *Journal of Bacteriology*, **189**, 4418–4424.
- Lemon, K.P., Freitag, N.E., and Kolter, R. 2010. The virulence regulator PrfA promotes biofilm formation by *Listeria monocytogenes*. *Journal of Bacteriology*, **192**, 3969–3976.
- Liao, C.-H., Cooke, P.H., and Niemira, B.A. 2010. Localization, growth and inactivation of *Salmonella* Saintpaul on jalapeno peppers. *Journal of Food Science*, **75**, M377–M382.
- Lin, C.-M. and Wei, C.-I. 1997. Transfer of *Salmonella* Montevideo onto the interior surfaces of tomatoes by cutting. *Journal of Food Protection*, **60**, 858–863.
- Lu, Y.J. and Wu, C.Q. 2010. Reduction of *Salmonella enterica* contamination on grape tomatoes by washing with thyme oil, thymol, and carvacrol as compared with chlorine treatment. *Journal of Food Protection*, **73**, 2270–2275.

- Luo, Y., He, Q., McEvoy, J.L., and Conway, W.S. 2009. Fate of *Escherichia coli* O157:H7 in the presence of indigenous microorganisms on commercially packaged baby spinach, as impacted by storage temperature and time. *Journal of Food Protection*, **72**, 2038–2045.
- Luo, Y., He, Q., and McEvoy, J.L. 2010. Effect of storage temperature and duration on the behavior of *Escherichia coli* O157:H7 on packaged fresh-cut salad containing romaine and iceberg lettuce. *Journal of Food Science*, **75**, M390–397.
- Maitland, J.E., Boyer, R.R., Eifert, J.D., and Williams, R.C. 2011. High hydrostatic pressure processing reduces *Salmonella enterica* serovars in diced and whole tomatoes. *International Journal of Food Microbiology*, **149**, 113–117.
- Mansur, A.R., Wang, J., Park, M.S., and Oh, D.H. 2014. Growth model of *Escherichia coli* O157:H7 at various storage temperatures on kale treated by thermosonication combined with slightly acidic electrolyzed water. *Journal of Food Protection*, **77**, 23–31.
- Martin-Diana, A.B., Rico, D., Barry-Ryan, C., Frias, J.M., Mulcahy, J., and Henehan, G.T.M. 2005. Calcium lactate washing treatments for salad-cut Iceberg lettuce: effect of temperature and concentration on quality retention parameters. *Food Research International*, **38**, 729–740.
- Mattson, T.E., Johnny, A.K., Amalaradjou, M.A.R., More, K., Schreiber, D.T., Patel, J., and Venkitanarayanan, K. 2011. Inactivation of *Salmonella* spp. on tomatoes by plant molecules. *International Journal of Food Microbiology*, **144**, 464–468.
- McKellar, R.C., LeBlanc, D.I., Lu, J., and Delaquis, P. 2012. Simulation of *Escherichia coli* O157:H7 behavior in fresh-cut lettuce under dynamic temperature conditions during distribution from processing to retail. *Foodborne Pathogens and Disease*, **9**, 239–244.
- Mody, R.K., Greene, S.A., Gaul, L., Sever, A., et al. 2011. National outbreak of *Salmonella* serotype Saintpaul infections: importance of Texas restaurant investigations in implicating Jalapeno peppers. *PLoS One*, **6**.
- Moore, K.L., Patel, J., Jaroni, D., Friedman, M., and Ravishankar, S. 2011. Antimicrobial activity of apple, hibiscus, olive, and hydrogen peroxide formulations against *Salmonella enterica* on organic leafy greens. *Journal of Food Protection*, **74**, 1676–1683.
- Moretro, T. and Langsrud, S. 2004. *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. *Biofilms*, **1**, 107–121.
- Mount, D.W. 2008. Using PAM matrices in sequence alignments. *Cold Spring Harbor Protocols*, **2008**. doi: 10.1101/pdb.top38.
- Neal, J.A., Marquez-Gonzalez, M., Cabrera-Diaz, E., Lucia, L.M., et al. 2012. Comparison of multiple chemical sanitizers for reducing *Salmonella* and *Escherichia coli* O157:H7 on spinach (*Spinacia oleracea*) leaves. *Food Research International*, **45**, 1123–1128.
- Oliveira, M., Usall, J., Viñas, I., Anguera, M., Gatiús, F., and Abadías, M. 2010. Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiology*, **27**, 679–684.
- Orue, N., Garcia, S., Feng, P., and Heredia, N. 2013. Decontamination of *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7 from leafy green vegetables using edible plant extracts. *Journal of Food Science*, **78**, M290–M296.
- Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., and Griffin, P.M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, **19**, 407–415.
- Pao, S., Long III, W., Kim, C., and Rafie, A.R. 2012. *Salmonella* population rebound and its prevention on spray washed and non-washed jalapeno peppers and roma tomatoes in humid storage. *Foodborne Pathogens and Disease*, **9**, 362–366.
- Rico, D., Martin-Diana, A.B., Barat, J.M., and Barry-Ryan, C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science and Technology*, **18**, 373–386.

- Riedel, C.U., *et al.* 2009. AgrD-dependent quorum sensing affects biofilm formation, invasion, virulence and global gene expression profiles in *Listeria monocytogenes*. *Molecular Microbiology*, **71**, 1177–1189.
- Rieu, A., *et al.* 2007. Agr system of *Listeria monocytogenes* EGD-e: role in adherence and differential expression pattern. *Applied and Environmental Microbiology*, **73**, 6125–6133.
- Rodríguez-Caturla, M.Y., Valero, A., García-Gimeno, R.M., Zurera, G. 2012. Development of a risk-based methodology for estimating survival and growth of enteropathogenic *Escherichia coli* on iceberg-lettuce exposed at short-term storage in foodservice centers. *Journal of Microbiology Methods*, **90**, 273–279.
- Sahu, S.N., Zink, D., and Datta, A.R. 2014. Growth potential of *Listeria monocytogenes* in artificially contaminated chicken salad. IAFP Annual Meeting, Indianapolis, IN, USA. Abstract.
- Salleh, N.A., Rusul, G., Hassan, Z., Reezal, A., Isa, S.H., Nishibuchi, M., and Radu, S. 2003. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. *Food Control*, **14**, 475–479.
- Sant'Ana, S.A., Landgraf, M., Destro, M.T., and Franco, B.D.G.M. 2011. Prevalence and counts of *Salmonella* spp. in minimally processed vegetables in Sao Paulo, Brazil. *Food Microbiology*, **28**, 1235–1237.
- Sant'Ana, A.S., *et al.* 2012. Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life. *International Journal of Food Microbiology*, **157**, 52–58.
- Sant'Ana, A.S., *et al.* 2013. Growth potential of *Salmonella* and *Listeria monocytogenes* in ready-to-eat lettuce and collard greens packaged under modified atmosphere and in perforated film. *Journal of Food Protection*, **76**, 888–891.
- Sanz, S., Gimenez, M., Olarte, C., Lomas, C., and Portu, J. 2002. Effectiveness of chlorine washing disinfection and effects on the appearance of artichoke and borage. *Journal of Applied Microbiology*, **93**, 986–993.
- Sapers, G.M. and Simmons, G.F. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. *Food Technology*, **52**, 48–52.
- Sela, S., *et al.* 2006. A mutation in the luxS gene influences *Listeria monocytogenes* biofilm formation. *Applied and Environmental Microbiology*, **72**, 5653–5658.
- Shen, C., Luo, Y., Nou, X., Wang, Q., and Millner, P.D. 2013. The effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157:H7 and non-O157 STEC. *Journal of Food Protection*, **76**, 386–393.
- Shi, X., Namvar, A., Kostrzynska, M., Hora, R., and Warriner, K. 2007. Persistence and growth of different *Salmonella* serovars on pre- and post-harvest tomatoes. *Journal of Food Protection*, **70**, 2725–2731.
- Shi, X., Wu, Z., Namvar, A., Kostrzynska, M., Dunfield, K., and Warriner, K. 2009. Microbial population profiles of the microflora associated with pre- and postharvest tomatoes contaminated with *Salmonella typhimurium* or *Salmonella montevideo*. *Journal of Applied Microbiology*, **107**, 329–338.
- Silva, S., *et al.* 2008. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. *Journal of Food Protection*, **71**, 1379–1385.
- Singh, N., Singh, R.K., Bhunia, A.K., and Stroshine, R.L. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *Lebensm.-Wiss. Technology*, **35**, 720–729.
- Skalina, L. and Nikolajeva, V. 2010. Growth potential of *Listeria monocytogenes* strains in mixed ready-to-eat salads. *International Journal of Food Microbiology*, **144**, 317–321.
- Smith, S.M., Scott, J.W., and Bartz, J.A. 2006. The effect of time after harvest on stem scar water internalization in tomato. *Proceedings of Florida State Horticultural Society*, **119**, 272–274.

- Sofos, J.N. 2008. *Listeria monocytogenes* – Enemy No. 1 for the ready-to-eat industry. *Troy: The National Provisioner*, pp. 70–72.
- Somers, E.B., Schoeni, J.L., and Wong, A.C.L. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella*-Typhimurium. *International Journal of Food Microbiology*, **22**, 269–276.
- Sospedra, I., Rubert, J., Soriano, J.M., Manes, J. 2013. Survey of microbial quality of plant-based foods served in restaurants. *Food Control*, **30**, 418–422.
- Stafford, R.J., McCall, B.J., Neill, A.S., Leon, D.S., Dorricott, G.J., Towner, C.D., and Micalizzi, G.R. 2002. A statewide outbreak of *Salmonella* Bovismobificans phage type 32 infection in Queensland. *Communications in Disease Intelligence*, **26**, 568–573.
- Sy, K.V., Murray, M.B., Harrison, M.D., Beuchat, L.R. 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *Journal of Food Protection*, **68**, 1176–1187.
- Takeuchi, K. and Frank, J.F. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *Journal of Food Protection*, **63**, 434–440.
- Tamaki, S., Bui, V.N., Ngo, L.H., Ogawa, H., and Imai, K. 2014. Virucidal effect of acidic electrolyzed water and neutral electrolyzed water on avian influenza viruses. *Archives of Virology*, **159**, 405–412.
- Teplitski, M., Warriner, K., Bartz, J., and Schneider, K.R. 2011. Untangling metabolic and communication networks: interactions of enterics with phyto bacteria and their implications in produce safety. *Trends in Microbiology*, **19**, 121–127.
- Tian, J.Q., et al. 2012. Survival and growth of food-borne pathogens in minimally processed vegetables at 4 and 15 degrees C. *Journal of Food Science*, **77**, M48–M50.
- Todd, E.C.D., Michaels, B.S., Greig, J.D., Smith, D., and Bartleson, C.S. 2010. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 8. Gloves as barriers to prevent contamination of food by workers. *Journal of Food Protection*, **73**, 1762–1773.
- Uyttendaele, M., Neyts, K., Vanderswalmen, H., Notebaert, E., and Debevere, J. 2004. Control of *Aeromonas* on minimally processed vegetables by decontamination with lactic acid, chlorinated water, or thyme essential oil solution. *International Journal of Food Microbiology*, **90**, 263–271.
- Valentin-Bon, I., Jacobson, A., Monday, S.R., and Feng, P.C.H. 2008. Microbiological quality of bagged cut spinach and lettuce mixes. *Applied and Environmental Microbiology*, **74**, 1240–1242.
- Vandamm, J.P., Li, D., Harris, L.J., Schaffner, D.W., and Danyluk, M.D. 2013. Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery. *Food Microbiology*, **34**, 151–157.
- Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., and Van Camp, J. 2009. Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiology*, **26**, 882–888.
- Vital, P.G., Dimasuay, K.G.B., Widmer, K.W., and Rivera, W.L. 2014. Microbiological quality of fresh produce from open air markets and supermarkets in the Philippines. *The Scientific World Journal*, **2014**, article ID 219534, 7 pp. doi: 10.1155/2014/219534.
- Waitt, J.A., Kuhn, D.D., Welbaum, G.E., and Ponder, M.A. 2013. Postharvest transfer and survival of *Salmonella enterica* serotype enteritidis on living lettuce. *Letters in Applied Microbiology*, **58**, 95–101.
- Wijnands, L.M., Delfgou-van Asch, E.H.M., Beerepoot-Mensink, M.E., van der Meij-Florijn, A., Fitz-James, I., van Leusden, F.M., and Pielaat, A. 2014. Prevalence and concentration of bacterial pathogens in raw produce and minimally processed packaged salads produced in and for the Netherlands. *Journal of Food Protection*, **77**, 388–394.

- Zeng, W., Vorst, K., Brown, W., Marks, B.P., Jeong, S., Pérez-Rodríguez, F., and Ryser, E.T. 2014. Growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in packaged fresh-cut Romaine mix at fluctuating temperatures during commercial transport, retail storage, and display. *Journal of food protection*, **77**, 197–206.
- Zheng, J., Allard, S., Reynolds, S., Millner, P., Arce, G., Blodgett, R.J., and Brown, E.W. 2013. Colonization and internalization of *Salmonella enterica* in tomato plants. *Applied and Environmental Microbiology*, **79**, 2494–2502.
- Zhou, B., Luo, Y., Nou, X., Yang, Y., Wu, Y., and Wang, Q. 2014. Effects of postharvest handling conditions on internalization and growth of *Salmonella enterica* in tomatoes. *Journal of Food Protection*, **77**, 365–370.
- Zhuang, R.Y., Beuchat, L.R., and Angulo, F.J. 1995. Fate of *Salmonella*-Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, **61**, 2127–2131.