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Review
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Review

Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions

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Abstract

Fresh produce (processed fruit and vegetables) continues to be the main source of foodborne illness outbreaks implicating pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes* and human parasites (e.g. hepatitis A, *Cyclospora*). Previously, outbreaks were primarily limited to leafy greens, tomatoes, and cantaloupes, but more recently there has been a trend of more diverse produce types (e.g. cucumbers and papayas) being implicated. Although on-farm good agriculture practices (GAP) contribute to preventing pathogens entering the fresh produce chain, it cannot be relied upon completely due to the open nature of farming. As a consequence, there is an identified need for interventions that can remove field-acquired contamination, especially given fresh produce is eaten raw. In the following review, an overview of foodborne illness outbreaks linked to contaminated fresh produce will be described along with potential sources of contamination. Post-harvest washing that was once considered decontamination is now viewed as a high-risk cross-contamination point. The challenges in monitoring the post-harvest wash process will be discussed along with processing factors that need to be considered. A range of alternative, or supplemental, non-aqueous interventions will be described including irradiation, ultraviolet light, high hydrostatic pressure, gas phase (ozone and chlorine dioxide), and hydroxyl radicals generated through advanced oxidative process or gas plasma. All have been proved to be effective at pathogen control on the laboratory scale and are poised to enter commercial application. The current status of these alternative interventions along with challenges of integrating into commercial practice will be described.

Key words: Fresh produce; *Escherichia coli* O157:H7; *Salmonella*; *Listeria*; lirradiation; Ozone; Chlorine dioxide; Advanced oxidative process; Post-harvest; Washing; Leafy greens; Fruit; Vegetable.

Introduction

Fresh produce remains the leading cause of foodborne illness outbreaks implicating virulent pathogens such as Shiga Toxin producing *Escherichia coli* (STEC), *Salmonella*, *Listeria monocytogenes* and, increasingly, human parasites (Callejón *et al*., 2015). The open nature of the fresh produce chain means that contamination can be introduced at various points in production, harvesting and processing, and then passed to the consumer (Nuesch-Inderbinen and Stephan, 2016). Previously, it was assumed that the post-harvest wash step was sufficient to remove field-acquired contamination and, to

this end, much of the research performed focussed on evaluating or formulating effective sanitizers (Feliziani *et al.*, 2016). However, as knowledge accumulated, it became evident that post-harvest washing under commercial conditions has limited decontamination efficacy and, if anything, can potentially lead to cross-contamination events (Barrera *et al.*, 2012; Gombas *et al.*, 2017). Consequently, the current philosophy related to ensuring the food safety of fresh produce is to prevent contamination in the field and to minimize cross-contamination during post-harvest handling. However, preventing contamination in fields or greenhouses is challenging and even good agricultural practices (GAP) are insufficient to ensure human pathogens are not introduced into the fresh produce chain (Francis *et al.*, 2012). A more effective means of control is to apply post-harvest decontamination interventions that can replace or supplement post-harvest washing (Meireles *et al.*, 2016). To this end, research to enhance the microbiological safety of fresh produce has started to identify and develop alternative intervention methods. The majority of approaches tested to date have been on the laboratory scale, although several are advancing towards commercial application. In the following review, an overview of food safety issues within the fresh produce sector will be outlined and limitations of post-harvest washing will be described. A range of alternative intervention approaches will be highlighted along with challenges to implementation on a commercial scale.

Foodborne illness outbreaks linked to fresh produce

Fresh produce remains the leading cause of foodborne illness outbreaks surpassing the typical vehicles for pathogen carriage such as meat, dairy, and seafood (CDC (Center of Disease Control), 2017). There have been over 400 outbreaks linked to fresh produce since 1990. Sprouted seeds such as alfalfa, clover, and mung beans have frequently been implicated in foodborne illness outbreaks linked to *Salmonella*, STEC, or *L. monocytogenes* (Callejón *et al.*, 2015; Nuesch-Inderbinen and Stephan, 2016). For the purpose of this review, sprouted seeds will not be considered, given that the origins of contamination are primarily from the seed and interventions are focussed on disinfection of seeds as opposed to post-harvest (Warriner and Smal, 2014). Of the remaining fresh produce-related outbreaks, leafy greens, tomatoes, cantaloupes, and soft fruits are frequently implicated (Table 1). Yet, in reality, all types of fresh produce have the potential to become contaminated with human pathogens as evident by the diversification of types implicated in outbreaks. For example, cucumbers that hitherto had rarely been associated with outbreaks prior to 2012 have been implicated in *Salmonella* cases with greater frequency (Sharma *et al.*, 2017). Papaya is a further fruit type that has had a relatively good food safety record but yet was implicated in over 200 cases caused by *Salmonella* Kiambu and Thompson in 2017 (CDC, 2017). In contrast, *Salmonella* outbreaks linked to tomatoes that were frequent prior to 2011 have become rare (Sreedharan *et al.*, 2014). The underlying reasons for improvement of tomatoes have been attributed to enhance surveillance, but also close attention to both pre- and post-harvest food safety practices (Ilic *et al.*, 2017). Although improved, there have been sporadic outbreaks of *Salmonella* linked to tomatoes although not at the frequency observed pre-2011 (Table 1).

Over recent years, there has been a shift in produce: pathogen associations. For example, in the case of apples, the pathogen of main concern was *E. coli* O157:H7 (Alegre *et al.*, 2010). However, in 2015, for the first time, there was a *L. monocytogenes* outbreak linked to caramel apples (Salazar *et al.*, 2016). In a similar manner, fresh produce (fruit and leafy greens) outbreaks implicating enteric protozoan such as *Cryptosporidium* and *Cyclospora* have risen along with virial pathogens such as hepatitis A (Dixon, 2016).

The underlying reasons for the diversification of pathogens and fresh produce types implicated in outbreaks are likely linked to several factors: specifically, the increased globalization of the fresh produce supply, aging population, increased consumption, and possibly climate change (Tirado *et al.*, 2010). Changes in production practices and even the introduction of reusable plastic crates could also contribute. Yet, the enhanced sensitivity and selectivity of pathogen diagnostics is a major contribution. Previously, enteric protozoan

Table 1. Examples of foodborne illness outbreaks linked to fresh produce in North America from 2011 to 2017 (source: CDC, 2017).

Year	Product	Pathogen	No. of cases
2017	Papayas	*Salmonella Kiambu, Thompson, Agona, Gaminara*	173
2016	Frozen strawberries	Hepatitis A	143
2016	Frozen vegetables	*L. monocytogenes*	9
2016	Packaged salads	*L. monocytogenes*	19
2015	Tomato	*Salmonella* Newport	115
2015	Cucumbers	*Salmonella* Poona	907
2014	Caramel apples	*L. monocytogenes*	35
2014	Cucumbers	*Salmonella enterica* Newport	275
2012	Cucumbers	*Salmonella enterica* Saintpaul	84
2012	Mangoes	*Salmonella* Braenderup	
2013	Shredded lettuce	*E. coli O157:H7*	30
2013	Ready to eat salad	*E. coli* O157:H7	
2012	Organic spinach/spring mix blend	*E. coli* O157:H7	33
2012	Romaine lettuce	*E. coli* O157:H7	24
2012	Cantaloupe	*Salmonella enterica* Typhimurium and Newport	261
2012	Mango	*Salmonella enterica* Braenderup	127
2011	Cantaloupe	*L. monocytogenes*	147
2011	Romaine Lettuce	*E. coli* O157:H7	58
2011	Cantaloupe	*Salmonella enterica* Panama	20
2011	Papaya	*Salmonella* Agona	106

could only be detected via microscopy, whereas genetic tools now exist that can detect such pathogens in the field environment (Ganz *et al.*, 2015). In addition, the advent of rapid and affordable DNA sequencing has had a major impact on the ability to detect pathogens and, moreover, identify the vehicles with the enhanced ability to cluster (link) foodborne illness cases (Taboada *et al.*, 2017). Indeed, source attribution of pathogens has increased to over 70%; before the advent of sequencing, this was estimated to be in the order of 20% (Emond-Rheault *et al.*, 2017). The net result is that contaminated produce is more likely to be detected, thereby resulting in recalls, or if an outbreak occurs, the source is more readily found. Therefore, there has been an increased incentive to find effective decontamination methods within the fresh produce sector.

Sources of contamination in fresh produce production

The open nature of fresh produce production makes it susceptible to contamination from multiple sources. There have been several reviews on the source of contamination with soil, water, biological amendments, and activity of wild animals all being cited as routes by which human pathogens can be introduced (Warriner *et al.*, 2009; Olaimat and Holley, 2012; Goodburn and Wallace, 2013; Martínez-Vaz *et al.*, 2014; Nuesch-Inderbinen and Stephan, 2016). Under ideal conditions, the soil, water, and biological amendments would be pathogen-free, thereby preventing contamination. However, in reality, pathogens can survive for extended periods within the environment over extended periods and become widely distributed (Yang *et al.*, 2012; Schwarz *et al.*, 2014). Pathogens such as *Salmonella* can even become established in greenhouse operations that would have been considered an enclosed environment (Holvoet et al., 2014).

In an attempt to reduce the introduction of contamination, there are guides on testing irrigation water and soil along with application of biological amendments (FDA, 2017). However, it is now accepted that pathogens are highly likely to be encountered and tolerance limits for fecal indicator bacteria in water and soil have been proposed (Jongman and Korsten, 2017). However, testing can only go so far in detecting contamination and, in many instances, there are few mitigation strategies available should contamination be encountered (Pagadala *et al.*, 2015). Consequently, a philosophy developed that accepted that contamination via water or manure amendments is highly likely but can be mitigated by allowing sufficient time to elapse to harvest by which point pathogens, if present, would have died off (Astrom *et al.*, 2006; Oliver *et al.*, 2006; Moynihan *et al.*, 2013). In practical terms, this is the basis for the 90–120 day rule, which states that when manure (or biological amendment) is introduced onto land, at least 120 days should elapse before harvest (Xu *et al.*, 2016). In a similar manner, it is also recommended not to irrigate crops 2–7 days prior to harvest (Weller *et al.*, 2015). In both the cases of manure amendments and irrigation water, the assumption is that enteric pathogens will die-off in the environment or on the plant, thereby negating the risk. The wait time periods are largely based on laboratory trials that monitored the rate at which pathogens such as *E. coli* O157:H7 and *Salmonella* decrease over time when introduced into irrigation water or soil. Yet, through studies, it has been reported that a high proportion of a pathogen populations die-off within 1–10 days (Astrom *et al.*, 2006; Oliver *et al.*, 2006; Liang *et al.*, 2011; Erickson *et al.*, 2014; Oladeinde *et al.*, 2014; Généreux *et al.*, 2015). However, in reality, the apparent die-off of pathogens is more related to becoming non-culturable by being induced into a dormant state (Ayrapetyan *et al.*, 2015). The dormancy in bacteria was previously considered to be viable but non-culturable (VBNC), although the term perisister is also referred to. Differentiating between VBNC and persister is open to debate although the latter considers those cells that enter dormancy under conditions that could ordinarily support growth (Kussell *et al.*, 2005). VBNC, in contrast, are cells that enter dormancy under stressed conditions (Dinu and Bach, 2011; Li *et al.*, 2014). The key significance of dormancy is that cells can persist over extended periods (>1 year), but would not be readily detected using standard culture methods. Moreover, dormant cells have an inherent resistance to antimicrobial agents such as sanitizers that are relevant to post-harvest operations (Kussell *et al.*, 2005).

Other less documented sources of pre-harvest contamination are from workers and have been thought to be significant for transferring parasites such as norovirus and enteric protozoan (Bouwknegt *et al.*, 2015; Jensen *et al.*, 2017). Contact surfaces such as knives and reusable crates have also been highlighted with respect to the potential of contamination to be spread between produce batches (Zilelidou *et al.*, 2015). A further route to introduce contamination into the inner plant tissue is via a process referred to as hydrocooling or vacuum cooling (Li *et al.*, 2008). Here, the product is packed into crates and then placed in a sealed chamber before applying vacuum to draw air out of the plant tissue and subliming moisture thereby removing heat. However, contamination on the surface of plants can be internalized through cut edges and stomata (Li *et al.*, 2008). Once internalized, the pathogens can be protected from stresses imposed during post-harvest operations, especially in relation to washing (Jablasone *et al.*, 2005).

Post-harvest wash as an intervention step in fresh produce processing

The post-harvest wash in fresh produce processing serves two main purposes discounting a convenient method for transporting fruit and vegetables through the process. The first function of washing is to remove soils and debris with the second process designed to remove field-acquired contamination (Barrera *et al.*, 2012). Previously, validation of post-harvest washes focussed on demonstrating a 5 log cfu reduction of the relevant pathogen introduced on the vegetable or fruit type of interest (Gombas *et al.*, 2017). As pathogens were used, trials were performed within a laboratory setting using produce inoculated at high levels with the microbe of interest (typically 8–9 log cfu). Under such conditions, it was relatively straightforward to attain a 5 log cfu reduction with numerous studies being published on the efficacy of different sanitizer types (Sapers, 2001; Olaimat and Holley, 2012; Goodburn and Wallace, 2013). Yet, it was noted that under commercial conditions, the actual log reduction was limited to 1–2 log reduction regardless of the sanitizer or washing time applied (Barrera *et al.*, 2012). The finding stimulated research efforts in determining what factors limited the efficacy of post-harvest washing. In addition to attachment and biofilm formation, there was focus on internalization into the inner plant vascular system (Warriner *et al.*, 2009). A range of studies illustrated the internalization of pathogens within young seedlings (sprouts) and within fruit harvested from inoculated blossom (Shi *et al.*, 2007; Jianxiong *et al.*, 2010). Internalization into mature plants was less frequently encountered, although demonstrated by imaging techniques that could detect low levels of target bacteria within the vascular system (Jablasone *et al.*, 2005). In reality, the majority of internalization would occur via the hydrocooling process or through having a temperature difference between produce and water (i.e. warm produce with cold water) (Li *et al.*, 2008).

The nature of attachment or association of human pathogens with plants is one aspect to explain the relative ineffectiveness of post-harvest washing to remove field-acquired contamination. An additional factor is the dynamics of organic loading within wash tanks that physically provides protection of human pathogens against sanitizers but also results in neutralization of antimicrobial action (Shen *et al.*, 2013; Zhou *et al.*, 2014a; ShihChi *et al.*, 2016). This is especially relevant in the case of chlorine that readily interacts with organic and inorganic components (principally ammonia) to form disinfection byproducts that exhibit a fraction of the antimicrobial activity of free chlorine (Boorman, 1999; Cardador and Gallego, 2012). The depletion of free-chlorine within wash tanks has been attributed to disseminating contamination between different batches (Tomas-Callejas *et al.*, 2012; Montibus *et al.*, 2016). This has been clearly demonstrated in several foodborne illness outbreaks with that linked to spinach contaminated with *E. coli* 157:H7 being a prime example (Warriner and Namver, 2013a). The outbreak was caused by spinach that was initially contaminated with *E. coli* O157:H7 via irrigation water and wild animals (wild bores specifically). At the time of harvest of the contaminated batch, there was a failure to maintain free-chlorine levels in the wash tank and hence nothing to prevent cross-contamination between batches. The net result was over 200 confirmed cases with seven deaths. There are further examples where the post-harvest wash process contributed to disseminating pathogens due to the absence or maintenance of sanitizer within wash tanks (Warriner and Namvar, 2013a) that underlines critical nature of the process.

The limitation of post-harvest washing to remove contamination, coupled with the potential to disseminate pathogens during the process, led to a change in the philosophy within the industry. Specifically, there was a transition from attempting to decontaminate fresh produce to preventing cross-contamination occurring (Banach *et al.*, 2015; Gombas *et al.*, 2017). Given previous research has focussed on evaluating sanitizers to decontaminate fresh produce, there was a large knowledge gap with respect to the efficacy of antimicrobials to prevent cross-contamination. This stimulated study to illustrate the extent of cross-contamination within commercial post-harvest was processes using a combination of classic culture techniques but also more advanced DNA typing methods (Banach *et al.*, 2015; Maffei *et al.*, 2017).

Challenges in validation of post-harvest washing process

With the recognition that the main purpose of the post-harvest washing process was to prevent cross-contamination, the main process control variable was considered to be the concentration of active sanitizer within wash tanks (Gombas *et al.*, 2017). It follows that if the concentration of active sanitizer was maintained above a certain value, then the pathogen of concern would be inactivated within the water before contacting fresh produce (Zhou *et al.*, 2014a). Therefore, similar to thermal processing, it was assumed that a set sanitizer concentration or a given time could ensure inactivation of the pathogen target (Gombas *et al.*, 2017). Yet, the underlying assumption that the post-harvest wash process was a static environment was far from reality with changes in microbial, organic, and inorganic loading (Shen *et al.*, 2013; Zhou *et al.*, 2014a; ShihChi *et al.*, 2016). The challenges in validating post-harvest wash processes were illustrated by a working group that was established to define validation procedures (Gombas *et al.*, 2017). The group focussed on developing a validation protocol for a fresh produce post-harvest wash based on hypochlorite as the sanitizer. As with all validation protocols, the first step is to identify the most relevant human pathogens of concern. With respect to fresh produce, the most relevant pathogens have historically been *Salmonella* and *E. coli* O157:H7. Yet, it can be anticipated that *L. monocytogenes* and human parasites will have to be considered in the future. Once identified, the sanitizer concentration and contact time need to be assessed. In thermal processing, this is a relatively simple concept, given that at lower temperatures, there needs to be extended treatment times to get the equivalent level of inactivation compared with that when higher temperatures are applied. In theory, at least the same concept can be used with regards to the inactivation of pathogens with chemical sanitizers. That is, the lower the sanitizer concentration the longer the contact time required to meet the level of inactivation required (i.e. food safety objective) (Gombas *et al.*, 2017). Antimicrobial effectiveness is represented by the 'CT value' which is the antimicrobial concentration (in ppm) multiplied by the contact time in minutes (Van Haute *et al.*, 2013). However, in the dynamic environment of the wash tank, there is a need to know what log reduction of the target pathogen is required and also in what time frame. Typically, in deriving the extent of microbial inactivation required is based on risk assessment that designates a target log reduction to provide a low probability of pathogens being present at a dose that could potentially cause illness. A risk analysis model for fresh produce is still lacking, although a 2–5 log cfu reduction is deemed acceptable (Gombas *et al.*, 2017; Omac *et al.*, 2017). The question to how rapid should pathogens should be inactivated is a further uncertainty. With direct leaf-to-leaf contact, the transfer of pathogens could occur in less than a second. With bacteria released in the water, it would be anticipated that the contact time with sanitizer would be longer. A possible estimate for the time to achieve the target log reduction would be the duration of the wash, given that the target pathogen would be exposed for this period. Yet, currently, the acceptable inactivation kinetics of pathogen inactivation remains to be determined.

A further unknown is the level of free chlorine required to achieve the inactivation of the target pathogen within the time frame (Gombas *et al.*, 2017). Typically, the concentrations are derived from Use Dilutions test, or equivalent, that are applied to assess the efficacy of sanitizers applied to hard surfaces. In the case of chlorine, it has been proposed that the minimum free-chlorine concentration required to inactivate *E. coli* O157:H7 or *Salmonella* varies between 1 and 20 ppm (Gombas *et al.*, 2017; (Paul *et al.*, 2017). The broad range is thought to reside in the inter-strain variation, prior induced stress, and if the cells are growing or non-growing (Paul *et al.*, 2017). Additional factors also relate to water hardness, pH, and presence of organic/inorganic matter (Driss and Bouhelassa, 2014). A greater challenge than identifying the free-chlorine levels is attempting to maintain such concentrations in commercial wash tanks. With the constant introduction of organic and inorganic constituents, the free-chlorine concentration readily depletes even when linked to an oxidation–reduction potential (ORP) feedback system (Gombas *et al.*, 2017). Indeed, ORP, although convenient, is not a reliable predictor of free-chlorine levels within wash tanks (Gombas *et al.*, 2017).

Monitoring quality of water and organic load

As part of a risk control and prevention approach, there is a need to establish a means of monitoring the wash process to demonstrate control with regards to minimizing cross-contamination. Previously, it was considered that ORP measurements were sufficient, although

this has been proved to be limited due to the rapid depletion of chlorine (Gil *et al.*, 2015). Therefore, alternative monitoring methods have been evaluated with respect to determining the chlorine demand of the water so that sufficient hypochlorite can be added to surpass the breakpoint (Barrera *et al.*, 2012; Van Haute *et al.*, 2013; Zhou *et al.*, 2014a, 2014b; ShihChi *et al.*, 2016). A key attribute of an ideal monitoring method is that it must report back the condition of the wash tank water in near real time. Therefore, methods that directly measure microbial numbers in water are not suitable due to the time delay in culturing. Yet, it must be noted that real-time biomass sensors based on impedance have been developed although insufficiently sensitive to measure low levels of microbes (Singh *et al.*, 2014). From studies performed to date, the use of turbidity and conductivity as an indirect measure for chlorine demand is limited due to the lack of correlation to free-chlorine concentration (Barrera *et al.*, 2012). A more reliable measure would be chemical oxygen demand (COD) or biological oxygen demand (BOD), given that both are measures of oxidation capacity of the water. However, COD requires a titration to be performed, whilst BOD analysis takes 5 days to complete. Therefore, until an in-line or rapid off-line monitoring system is developed, alternative methods need to be devised. In this regard, systems are being developed based on spectral analysis to predict the chlorine demand of water (Zhang *et al.*, 2014a). The approach is based on taking spectra of a water sample and predicting the chlorine demand through a machine learning algorithm. The system has yet to be used on a commercial scale but does show promise (Zhang *et al.*, 2014a).

An alternative approach to determining the chlorine demand of wash water is to use alternative sanitizers that are less sensitive to the presence of organic matter. Although there are numerous studies performed on the efficacy of sanitizers to remove contamination from fresh produce, there has been less focus on the ability to prevent cross-contamination (Luo *et al.*, 2012). Chlorine dioxide is considered insensitive to the presence of organics and inorganics in wash water. Yet, studies performed under commercial conditions observed a negative impact on the antimicrobial efficacy of chlorine dioxide in the presence of organic loading (Hassenberg *et al.*, 2017). In contrast, peroxyacetic acid at 50 ppm has been illustrated to be insensitive to the presence of organics and maintains antimicrobial efficacy (Davidson *et al.*, 2017). Acidic electrolysed water (43 ppm) was also effective at preventing cross-contamination, with lactic acid being less so (Jung *et al.*, 2017). Yet, regardless of the sanitizer applied, the actual decontamination efficacy achieved on produce is limited to 1–2 log cfu so that it is not considered as an effective intervention step to remove field-acquired contamination (Warriner and Namvar, 2013b).

Alternative post-harvest decontamination treatments

Through research, it has been illustrated that post-harvest washing is limited as an intervention, and even preventing cross-contamination can represent a challenge. Furthermore, the wash process is dynamic with no single metric that can be used for establishing critical limits and monitoring as a part of an overall food safety plan. Consequently, there is a need for decontamination methods that are effective and, importantly, can be monitored. To this end, there have been a range of techniques evaluated, some of those are new technologies with others being revisited (Meireles *et al.*, 2016) (Table 2). For example, irradiation, UV, ozone, and chlorine dioxide have a long history for decontaminating surfaces with those based on advanced oxidative process (AOP) or gas plasmas being relatively recent advancements. Comparing the efficacy of the different treatments is problematic, given the lack of standard evaluation methodology. Yet, the following section will provide an overview of the most promising methods along with examples of pathogen reduction efficacy (Table 2). The technologies to be described will not go into great detail about antimicrobial active packaging or coatings that are also active areas of research (Arvanitoyannis and Stratakos, 2012; Caleb *et al.*, 2013; Bastarrachea *et al.*, 2015).

Irradiation

Irradiation has a long history for non-thermal treatment of foods, although for fresh produce this has largely been to retard ripening, sprout inhibition, and killing insects (Ramos *et al.*, 2013). However, studies have illustrated that irradiation can decrease pathogens such as *E. coli* O157:H7 on spinach or lettuce and is approved by the US FDA for this purpose up to a dose of 1 kGy (Shayanfar *et al.*, 2017). An irradiation dose of 1 kGy can support a 5 log cfu reduction of *E. coli* O157:H7 populations on leafy greens that includes those internalized within leaves and incorporated into biofilms (Gomes *et al.*, 2008) (Table 2). *E. coli* O157:H7 inoculated onto iceberg lettuce appears sensitized to irradiation with a dose of 0.2 kGy required to support a 5 log cfu reduction (Jeong *et al.*, 2010). The sensitivity of *E. coli* O157:H7 to irradiation can also be achieved by dipping leafy green in sanitizer solutions (chlorine, peracetic acid, or quaternary ammonium salt) (Niemira and Cooke, 2010; Moosekian *et al.*, 2014). However, although vegetative bacterial cells are sensitive to irradiation, it should be noted that the viruses, endospores, and enteric protozoa exhibit enhanced resistance (Table 2). For example, rotovirus introduced onto leafy greens and treated with a dose of 2.5 kGy resulted in <1 log reduction of the virial pathogen (Espinosa *et al.*, 2012).

In addition to leafy greens, there has also been interest in treating fruit with irradiation to reduce the carriage of pathogens. For example, cantaloupes inoculated with *Salmonella* Poona could be decreased by 3.6 log cfu by applying a 1.5 kGy dose (Palekar *et al.*, 2015). Applying a 1 kGy irradiation dose to strawberries supported a 4 log cfu reduction on STEC levels (Shayanfar *et al.*, 2017). There are several additional examples of pathogen reduction by means of irradiation and have previously been reviewed (Farkas, 1998; Sikin *et al.*, 2013; Meireles *et al.*, 2016; Pinela and Ferreira, 2017).

Through quantitative risk assessment, it has been estimated that the food safety risk derived from leafy greens contaminated with *Listeria* could be reduced by over 65% by applying irradiation treatment (Omac *et al.*, 2017). However, despite being recognized as an effective intervention step, there are several drawbacks that have restricted commercial use. Specifically, consumer acceptance remains a challenge with little promotional campaigns to illustrate the safety of the process. Instead, it has been proposed to use low-dose irradiation with the assumption that this would not require to bare a label as other irradiated foods (Farkas, 1998; Jeong *et al.*, 2010). However, despite lobbying pressure from industry, the regulatory bodies concluded that any irradiated product must be labelled as such to maintain transparency with consumers. Consequently, there is a reluctance by industry to apply the process with additional factors being highlighted as cost, maintenance requirements, and the lack of assurance that the process can reduce but not eliminate contamination. This is illustrated with viruses, given the inherent resistance to the irradiation process. It has also been proposed that

Table 2. Log count reduction of pathogens of various produce types by different intervention technologies.

Process	Treatment	Produce type	Target pathogen	Log count reduction	Reference
Irradiation	0.7 kGy	Salad spinach	*Salmonella* and *L. monocytogenes*	5	Gomes *et al.* (2011)
	1 kGy	Salad spinach	*E. coli* O157:H7	5	Jeong *et al.* (2010)
	1.5 kGy	Cantaloupe	*Salmonella*	3.6	Palekar *et al.* (2015)
	0.75 kGy	Tomatoes	*Salmonella*	3.7	Mahmoud (2010)
	0.75 kGy	Tomatoes	*E. coli* O157H7	4.2	Mahmoud (2010
	28.7 kGy	Strawberries	Norovirus and Tulane virus	6	DiCaprio *et al.* (2016)
UV	81.6 mJ/cm^2	Lettuce	*E. coli* O157:H7	0.31	Kim *et al.* (2013)
	81.6 mJ/cm^2	Lettuce	Salmonella	0.57	Kim *et al.* (2013)
	81.6 mJ/cm^2	Lettuce	*L. monocytogenes*	1.16	Kim *et al.* (2013)
	6.0 kJ/m^2	Tomato	*E. coli* O157:H7	3.5	Mukhopadhyay *et al.* (2014)
	6.0 kJ/m^2	Tomato	*Salmonella*	2.8	Mukhopadhyay *et al.* (2014)
	11.9 kJ/m^2	Cantaloupe	*E. coli* O157:H7	1.0	Adhikari *et al.* (2015)
			L. monocytogenes	1.0	Adhikari *et al.* (2015)
Pulsed light	40 kJ/m^2	Spinach	*E. coli*	2.2	Aguero et al. (2016)
	12 J/cm^2	Watermelon	*E. coli*	5.0	Ramos-Villarroel *et al.* (2015)
	12 J/cm^2	Watermelon	*L. innocua*	2.79	Ramos-Villarroel *et al.* (2012)
Chlorine dioxide gas	10 ppmv 20 min	Spinach	*E. coli* O157:H7	3.4	Park and Kang (2015)
			Salmonella	3.3	Park and Kang (2015)
			L. monocytogenes	3.4	Park and Kang (2015)
	87 ppm 30 min	Lettuce	*E. coli* O157:H7	3.4	Lee *et al.* (2004)
			Salmonella	4.3	Lee *et al.* (2004)
			L. monocytogenes	5.0	Lee *et al.* (2004)
	5 ppmv 20 min	Tomato	*E. coli* O157:H7	2.3	Park and Kang (2015)
			Salmonella	1.2	Park and Kang (2015)
	5 ppm 5.5 min	Cantaloupe	*E. coli* O157:H7	3	Mahmoud *et al.* (2008)
	5 ppm 4.2 min	Cantaloupe	*Salmonella*	3	Mahmoud *et al.* (2008)
	5 ppm 1.2 min	Cantaloupe	*L. monocytogenes*	3	Mahmoud *et al.* (2008)
Ozone	9 ppm 6 h	Bell pepper	*E. coli* O157:H7	2.9	Alwi and Ali. (2014)
			Salmonella	2.6	Alwi and Ali. (2014)
			L. monocytogenes	3.1	Alwi and Ali. (2014)
	935 ppm 30 min	Spinach	*E. coli* O157:H7	5	Vurma *et al.* (2009)
	3 ppm 5 min	Cantaloupe	*E. coli* O157:H7	1	Rodgers *et al.* (2004)
	5 ppm 10 min	Lettuce	*L. monocytogenes*	1.1	Zhang and Farber (1996)
	10 ppm 20 min	Tomato	*Salmonella*	7.0	Das *et al.* (2006)
Advanced oxidative process	37.8 mJ/cm^2 1.5% hydrogen peroxide 50°C	Iceberg lettuce	*Salmonella*	4.12	Hadjok *et al.* (2008)
		Iceberg lettuce	*E coli* O157:H7	3.87	Hadjok *et al.* (2008)
		Tomato	*Salmonella*	2.22	Hadjok *et al.* (2008)
		Tomato	*E. coli* O157:H7	3.55	Hadjok *et al.* (2008)
Gas plasma	Air plasma 300s	Tomatoes	*E. coli*	3.1	Ziuzina *et al.* (2014)
	Air plasma 10s	Tomatoes	*Salmonella*	6.3	Ziuzina *et al.* (2014)
	Air plasma 120s	Tomatoes	*L. monocytogenes*	6.7	Ziuzina *et al.* (2014)
	Air plasma 300s	Iceberg lettuce	*E. coli*	3.3	Ziuzina *et al.* (2015)
	Air plasma 300s	Iceberg lettuce	*Salmonella*	2.4	Ziuzina *et al.* (2015)
			L. monocytogenes	6.7	Ziuzina *et al.* (2015)
	Helium–oxygen plasma 5s	Mango	*E. coli*	>3	Perni *et al.* (2008)

nutrient losses are high in irradiated produce, although research has suggested that this is not the case (Maraei and Elsawy, 2017).

Ultraviolet radiation

Ultraviolet (UV) electromagnetic radiation spans from 100 to 400 nm wavelengths. Photons generated at wavelengths less than 200 nm have poor penetration in air but generate ozone by reacting with oxygen. UV-C at 254 nm has the highest germicidal activity and hence applied for surface disinfection (Gayan *et al.*, 2014). UV-C has been shown to reduce *E. coli* O157:H7 on apple surfaces by 3 log cfu and *Salmonella* on tomatoes by 2 log cfu (Yaun *et al.*, 2004). However, pathogen reduction on uneven surfaces such as cantaloupes and berries is restricted to 1 log cfu with comparable doses (12 kJ/m^2) (Adhikari *et al.*, 2015) (Table 2). This highlights the ultimate limitation of UV in that the light is coherent that cannot penetrate shaded areas on produce surfaces.

Pulsed light can deliver a wide spectrum (200–1100 nm) with a high-intensity flash that achieves 1–3 log cfu reduction of surface bacteria although again limited by cells being located in shaded areas (Kramer *et al.*, 2017). A potential approach to overcome shading is through Light Emitting Diode (LEDs) that are amenable to constructing novel reactors that deliver UV at multiple angles (Chen *et al.*, 2017). Additional advantages of LEDs are the potential to use a range of different wavelengths, thereby providing a synergistic antimicrobial action (Kim *et al.*, 2017). There have been no reports on LED-based reactors for decontaminating fresh produce, although it would probably be an area of interest in the near future.

High-pressure processing

High hydrostatic pressure (HHP), ultra high pressure processing (UHP), or high pressure processing (HPP) are encompassing terms that describe the utilization of elevated pressures, typically in the range of 100–700 MPa, with or without addition of external heat. Although there are variations in the process, the product is vacuum packed then pressurized by direct and indirect methods utilizing a pressure-transmitting medium (normally water). The pressure is isocratic, thereby acting homogenously throughout the chamber and hence independent of product size or dimensions.

In commercial terms, HHP has primarily been used for the non-thermal pasteurization of juices, purees, deli meats, and seafood (Mujica-Paz *et al.*, 2011; Tadapaneni *et al.*, 2014). The processing of whole or processed fresh produce is less commonly encountered, although commercial products such as avocado halves are available (Woolf *et al.*, 2013). The advantage of HHP, especially in relation to avocados, is the inactivation of enzymes and reduction of microbial microflora to extend the product shelf-life up to 60 days (Woolf *et al.*, 2013).

Studies have also been performed using HHP as a pathogen reduction step on fresh produce. For example, *E. coli* inoculated onto lettuce or tomato, then treated with 350 MPa, was reduced by 6 log cfu without changes in sensory quality (Arroyo *et al.*, 1997). A HHP process applied at 600 MPa for 3 min decreases coliform counts on fresh basil to <1 log cfu without any detrimental change in leaf integrity (Koutchma and Warriner, 2017). Therefore, in principle, at least HHP is an effective decontamination step for fresh produce with the added benefit of increasing shelf-life through restricting endogenous enzyme activity (Barba *et al.*, 2012; Tadapaneni *et al.*, 2014; Georget *et al.*, 2015). Yet, there are barriers to commercial application that relate to cost, limited capacity, batch system, and incompatibility with produced packed under Modified Atmosphere Packaging (MAP). In relation to leafy greens, there are also issues of texture loss through plant cell disruption and browning caused by chlorophyll degradation (Seifert and Zude-Sasse, 2016).

Gas-phase treatments

There is interest in gas-phase treatments as an alternative to, or in conjunction with, aqueous-based wash systems (Mahmoud *et al.*, 2007; Shynkaryk *et al.*, 2015). Within the gas phase, issues relating to cross-contamination are minimized with the added advantage of greater penetration into the sub-subsurface of fresh produce (Goodburn and Wallace, 2013).

Fumigation has been practised with ethylene oxide and other potent gases, but are being phased out due to carcinogenic residues (Bononi *et al.*, 2014). Consequently, the main gas-phase treatments used for direct food contact use include acetic acid, hydrogen peroxide vapor, chlorine dioxide, and ozone (Netramai *et al.*, 2016). In terms of commercialization, chlorine dioxide and ozone are considered more feasible given the cost consideration, in addition to negligible effects on sensory characteristics of fresh produce (Shynkaryk *et al.*, 2015). The major advantage of ozone is the availability of generators and also lack of disinfection byproducts. Chlorine dioxide is effective although concerns over chlorite residues persist (Trinetta *et al.*, 2011; Smith *et al.*, 2015).

A challenge in gas-phase treatments is how to apply the gas to large produce batches in a contained area. In the case of ozone, this has been undertaken to date by diffusing low concentrations into storage rooms where long-term exposure can be achieved (Skog and Chu, 2001; Perni *et al.*, 2008; Vurma *et al.*, 2009; Trinetta *et al.*, 2010, 2011; Tabakoglu and Karaca, 2015). Ozone introduced into the headspace of storage rooms has the added advantage of degrading ethylene, thereby delaying ripening although prolonged exposure over days can lead to a decrease in antioxidant content (Rice *et al.*, 1982; Pérez *et al.*, 1999). Yet, as a decontamination treatment, it has a limited efficacy due to the low concentration (3 ppm) that can be introduced into storage rooms along with the lack of penetration of ozone into produce packed in bins or crates (Tzortzakis and Chrysargyris, 2017).

In laboratory-based trials, the application of 50 000 ppm ozone to strawberries or blueberries inoculated with *Salmonella* and *E. coli* O157:H7 supported a 3 log cfu reduction of pathogen numbers (Bialka and Demirci, 2007; Bialka *et al.*, 2008) (Table 2). Ozone gas applied at 10 000 ppm for 30 min controlled *E. coli* O157:H7 on cantaloupes with no detrimental effect on fruit quality (Selma *et al.*, 2008). In a further example, ozone gas treatment has been incorporated into the vacuum cooling process. Here, ozone was introduced into the chamber immediately upon release of the vacuum that facilitated uptake of the antimicrobial gas into the inner leaf structures (Shynkaryk *et al.*, 2016). In a study reported by Yesil et al. (2017), spinach was inoculated with *E. coli* O157:H7, then introduced into a chamber and then a vacuum applied. Upon release of the vacuum, ozone gas was introduced into the chamber at a rate of 1.5 g/h for 30 min. The treatment supported >3 log cfu reduction in *E. coli* O157:H7 levels on spinach, although no mention was made of any detrimental effect on the leafy green (Yesil *et al.*, 2017).

Although ozone has been illustrated to be an effective antimicrobial gas for the decontamination of fresh produce, it is rarely applied in industry. This is primarily due to the difficulty in containing ozone gas but also the extensive corrosion of metal surfaces such as fans, fittings, and condenser coils (Coelho *et al.*, 2015).

Chlorine dioxide is less corrosive than ozone and is generated on site by mixing sodium chlorite with an acid (organic or inorganic) to generate the gas. In laboratory trials, chlorine dioxide introduced at 12 ppm could support a >5 log cfu reduction of *E. coli* O157:H7 inoculated onto apples and importantly could inactivate the pathogen located within the sub-surface of stem scar tissue (Du *et al.*, 2003). Oranges treated with 0.5 ppm chlorine dioxide for 14 min could support a 5 log cfu reduction of *Salmonella* (Bhagat *et al.*, 2011). *Salmonella* on cantaloupes could be reduced by 3 log cfu when exposed to 5 ppm chlorine dioxide for 10 min, thereby illustrating that uneven surfaces could be treated via the gas treatment (Mahmoud *et al.*, 2008).

Although effective, chlorine dioxide treatment has to be balanced due to the strong bleaching action that negatively effects the sensory quality of fresh produce and also the risk of generating toxic byproducts such as chlorite (Kaur *et al.*, 2015). To overcome such limitations there has been focus on novel delivery methods based on slow release of the gas over an extended time frame. For example, it was shown that spraying spinach with sodium chlorite (precursor for chlorine dioxide) followed by hydrochloric acid vapor supported >5 log cfu inactivation of *Salmonella* and *L. monocytogenes* (Hwang *et al.*, 2017). It was thought that the inert chlorite penetrated into the inner leaf tissues then was converted to chlorine dioxide by the reaction with HCl over time. Although the reduction of pathogens was high (>3 log cfu), there was no comment made on the quality changes of the product or risks posed by the chlorite residues. In a further application, slow releasing chlorine dioxide pads have been developed to enable controlled release of the gas into sealed packaging or containers. Similar to the previous example, the chlorite is held within one layer of the pad and acid (tartaric in the current example) diffused through the chlorite layer thereby generating chlorine dioxide gas (Bai *et al.*, 2016). In one application the pads were placed within clam shell packaging containing inoculated tomatoes. The chlorine dioxide maintained a 3.5 ppm headspace concentration for 10 days and supported a 3 log cfu reduction of *E. coli* (Sun *et al.*, 2017).

The introduction of ozone into the headspace of packs has also been evaluated for tomatoes inoculated with *E. coli* O157:H7, *L. monocytogenes*, or *Salmonella*. The pack headspace was filled with 4000 ppm that was able to achieve 2–3 log cfu reduction in pathogen levels (Fan *et al.*, 2012). Given that ozone would be depleted within the pack within minutes, there would be no residual antimicrobial effect as observed for chlorine dioxide gas (Sun *et al.*, 2017).

Advanced oxidative process

The AOP describes the generation of hydroxyl radicals from the decomposition of hydrogen peroxide and/or ozone. The formed-hydroxyl radicals have oxidation potential greater than that of ozone but are short-lived, thereby requiring to be generated at the point of application (Mamane *et al.*, 2007; Assalin *et al.*, 2010). Generation of hydroxyl radicals can be accomplished using a variety of methods. Specifically, the UV-C mediated decomposition of hydrogen peroxide and/or ozone can generate radicals. It is also possible to generate radicals through reacting ozone and hydrogen peroxide in solution and then delivering as a fine mist. The formation of hydroxyl radicals is promoted by Fe ions (Fenton reaction) and at an operating temperature of 48°C (Alaton *et al.*, 2002). The mode by which hydroxyl radicals are generated is thought to influence the rate of formation and hence stability (Zhang *et al.*, 2014b). Although not studied to any great extent, the highest rate of formation occurs by combining ozone, hydrogen peroxide, and UV, whereas a slower rate of hydroxyl radical generation would be using chilled ozone and hydrogen peroxide. In the latter case, there would be a higher probability of hydroxyl radicals being generated slower and hence lower concentration. In contrast, using a combination of hydrogen peroxide, ozone, and UV-C at 48°C, a higher concentration of hydroxyl radicals is formed (Chen *et al.*, 2015).

A combination of UV and hydrogen peroxide was demonstrated to inactivate *E. coli* and *Salmonella* introduced on the surface or sub-surface of a range of produce types such as lettuce, cauliflower, and onion (Hadjok *et al.*, 2008) (Table 2). From optimization studies, the efficacy of the AOP-based treatment was dependent on the hydrogen peroxide concentration (1.5% v/v), UV-C dose (37.8 mJ/cm^2), and operating temperature (48°C). An AOP-based process has also been successfully applied to inactivate *Listeria* and *E. coli* on mushrooms without any negative sensory effects (Guan *et al.*, 2013; Murray *et al.*, 2015).

Although the AOP process is an effective treatment, the major barriers to commercialization relate to throughput given treatment times of 30 s are required. Contacting all the fresh produce surface is a further challenge, especially with leafy greens that tend to layer. Still, it is possible that the treatment will find utility in decontaminating whole produce where material transfer issues would be less significant.

Gas plasma

Similar to AOP process, gas plasma is a mixture of ions, electrons, radicals, and UV photons (Pignata *et al.*, 2014; Pignata *et al.*, 2017). Gas plasmas are generated by passing a high voltage through a gas phase that can be composed of oxygen, helium, hydrogen, and argon, amongst others (Perni *et al.*, 2008). The antimicrobial effects of gas plasmas have been known since the latter part of the 19th century, however, need for high voltage generators, excessive heat generation, use of noxious working gases (e.g. hydrogen peroxide or peracetic acid based mixture), and requirement for treatment to work under low pressure, has restricted commercial application. Yet, through advances, atmospheric gas plasmas have been developed that can operate under atmospheric conditions at working temperatures around 50°C (Mir *et al.*, 2016).

The composition of a plasma is largely dependent on gas composition and electric field strength (Pavlovich *et al.*, 2013). There are different configurations for generating plasma that includes partial discharge, dielelectric barrier discharge, corona discharge, microwave discharge, and atmospheric plasma jet (Pignata *et al.*, 2017). The additional variables are related to voltage, gas flow, and composition, and in addition if the sample is placed within or distant from the plasma. From studies performed to date, it has been found that different plasma treatments result in a 1–3 log cfu reduction of pathogens introduced onto a range of fresh produce with treatment times ranging from 300 s to 20 min (Pignata *et al.*, 2017). Clear limitations of the technology are the extended treatment times and need for the sample to be in close proximity to the plasma source. Despite such limitations, there are gas plasma applications that could potentially be incorporated into fresh produce processing. Specifically, gas plasmas have been generated within modified atmosphere-packed fresh produce, thereby generating antimicrobial radicals *in situ*. Here, the packaging acts as a dielectric layer through which electric discharge is passed and by so doing ionizing the gas in the pack headspace (Moon *et al.*, 2016). Laboratory studies reported a 3 log

cfu reduction of the total aerobic count of strawberries within packs containing air (Misra *et al.* 2014). *Salmonella* and *Listeria* have been reported for a 5 min treatment (Min *et al.*, 2016). Gas plasmas generated in an oxygen-rich atmosphere have been shown to inactivate *E. coli* O157:H7 on a lettuce pile of 7 layers thick (Min *et al.*, 2017). This would suggest that the ionized gas generated has good penetration characteristics. However, given that fresh produce is sensitive to headspace gases, it would be unlikely that oxygen-rich MAP would be applied in place to the typical oxygen:carbon dioxide mix. Trials performed involving generating gas plasma with typical MAP gas composition have proven less effective with <1 log cfu reduction in *E. coli* O157:H7 on lettuce being reported (Min *et al.*, 2017).

A further promising approach is generating plasma jets that as the name suggests, providing a stream of radicals akin to misting or fogging. In one example, a plasma was generated by aerosolized hydrogen peroxide (7.8%) through an electrode gap polarized at 17 kV (Jiang *et al.*, 2017). A 45 s treatment resulted in >5 log cfu reduction of *Salmonella*, *Listeria*, and *E. coli* O157:H7 on tomatoes with up to a 4 log cfu with spinach. Plasma jets therefore could provide an effective treatment for produce decontamination, although the hardware to accomplish this on a commercial scale is unfeasible at the current time.

Conclusions and future outlook

Fresh produce continues to be responsible for the highest number of foodborne illness outbreaks. In the majority of cases, the contamination is brought into the processing environment and subsequently disseminated over different batches. Given the limitation of pre-harvest controls, there is a need for effective post-harvest decontamination interventions. Although the post-harvest wash was considered as an intervention, the process has subsequently been found to have a limited efficacy with an increased risk of cross-contamination if sanitizer concentrations are not maintained. With the limitations of post-harvest washing along with the need to implement risk prevention and control steps, there has been interest in alternative decontamination methods. In this regard, there are several methods available with those based on irradiation, ozone, chlorine dioxide, and AOP showing promise. In contrast, UV, pulsed light, and gas plasma may find a limited utility due to limited decontamination efficacy and/or practicality in implementing on a commercial scale. For commercial adoption of the alternative interventions, there would be a need to appreciate the high throughput most processing lines operate and also the cost along with seeking regulatory approval or letters of no objection. It will also be important to note that one technology will not fit all given diverse nature of fresh produce with certain interventions being more suitable to fruit and vegetable types. The target pathogens should also be considered especially in relation to human parasites that are frequently being implicated in fresh produce-related outbreaks. In this regard, there is a knowledge gap on the efficacy by which alternative technologies can inactivate human parasites which are typically more resistant than vegetative bacterial pathogens. A further research need is to identify suitable surrogates that can be applied for in-plant validation and verification trials. Finally, a combination of interventions, which includes post-harvest washing, should be applied in a hurdle concept to control foodborne pathogens. It is likely by applying multiple hurdles a synergistic antimicrobial effect will be achieved without significant detrimental effects on produce sensory quality.

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