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Strategies to Combat Microbial Hazards Associated with Fresh Produce

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Abstract

Supply of fresh produce on an industrial scale is a major challenge, with foodborne outbreaks from contaminated fresh produce increasingly reported in many parts of the world. Take, for example, the German outbreak of *Escherichia coli* O104:H4 in 2011; an outbreak which caused almost 50 deaths. Although this kind of outbreak is rare, it is surprising that in the modern world, with so many safety nets, it still occurred.

The complex biology of pathogen contamination and survival in the fresh produce supply chain is just beginning to be understood; we are at an interesting time in determining the ecology of bacterial communities and their pathogenesis mechanisms. Pathogen adhesion to the surface of fresh produce and internalization of pathogens can limit the usefulness of conventional processing and chemical sanitizing methods.

Consequently, control strategies that help to significantly reduce the likelihood of pathogen contamination and the susceptibility of fresh produce as a vehicle for transmission of enteric bacteria are still needed: on the farm, during processing, and during packaging. Recent advances in contamination prevention and decontamination techniques bring new possibilities in the search for effective strategies to control pathogenic bacteria associated with fresh produce.

Figure 1 ~ Sprouted seeds in a display cabinet. Source: Maria I. Gil, reprinted with permission of Quality and Safety Lab CEBAS-CSIC.

Relevant Microorganisms and Recent Outbreaks

Modern society is increasingly concerned about lifestyle and diet which translates to a high demand for fresh and healthy food. Advances in agronomics, processing, preservation and distribution, as well as the expansion of global trade, have enabled the produce industry to supply nearly all types of high-quality fresh fruits and vegetables year-round (Sela and Fallik, 2009). This is good news for retailers and consumers, but puts a strain on the large-scale producers who strive to maintain the healthy and safe connotation of fresh produce.

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Globally, we face one of the most difficult times regarding foodborne disease outbreaks related to fresh produce, with an increase in the number of reported outbreaks associated with food of non-animal origin (FnAO) (European Food Safety Agency, (EFSA) Panel on Biological Hazards (BIOHAZ), 2013). This increase has occurred alongside a decrease in the related numbers associated with food of animal origin (FAO) (Figure 2).

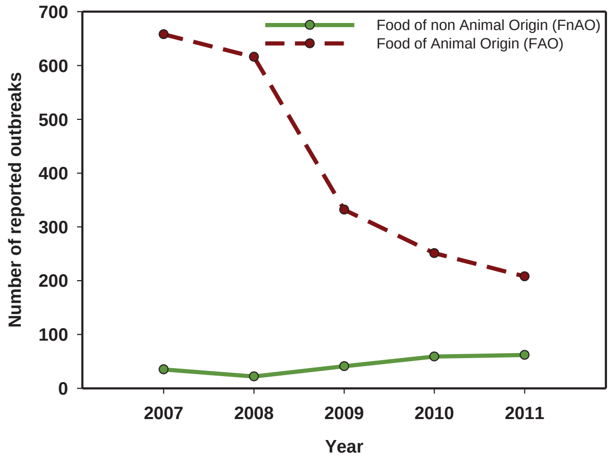

Figure 2 ~ Numbers of reported outbreaks associated with food of non-animal origin (FnAO) and food of animal origin (FAO) between 2007 and 2011. (Adapted from EFSA Panel on Biological Hazards (BIOHAZ), 2013).

In the US, between 1998 and 2007, more outbreaks of illness were associated with fresh produce (684) than those involving poultry (538), beef (428), pork (200) or eggs (< 200) (CSPI, 2009). Also, fresh produce was associated with more cases of illness (26,735) than any of the other food categories most commonly linked to outbreaks. Recent incidents, including the 4,000 cases of infection due to Shiga Toxin-Producing *Escherichia coli* (STEC) *Escherichia coli* O104:H4 in German sprouted alfalfa beans, have emphasised the potential of fresh produce to cause large outbreaks with considerable mortality.

The deadly outbreak of *E. coli* in Germany raised doubts about food safety in European countries. It highlighted the importance of a better understanding of the efficacy of current practices, the need to identify potential areas for improvement, determine models of best practice and assess system modifications. Applying a risk assessment process to decide potential control strategies is a very valuable tool currently used by governments and regulatory agencies. In order to identify and rank specific food/pathogen combinations most often linked to human cases of infection originating from FnAO in the EU, the European Food Safety Association BIOHAZ Panel developed a semi-quantitative model using seven criteria (Figure 3) (EFSA Panel on Biological Hazards (BIOHAZ), 2013).

Criteria 1	Strength of associations between food and pathogen
Criteria 2	Incidence of illness
Criteria 3	Burden of disease
Criteria 4	Dose-response relationship
Criteria 5	Prevalence of contamination
Criteria 6	Consumption
Criteria 7	Pathogen growth potential during shelf-life

Figure 3 ~ Risk-ranking model showing seven criteria used to identify and rank food/pathogen combinations linked to human cases (Adapted from EFSA Panel on Biological Hazards (BIOHAZ), 2013).

Based on this model, the EFSA BIOHAZ Panel identified more than 20 food/pathogen combinations that were included in five top ranking groups (Figure 4). Taking into account the output of the risk ranking model based on the reported outbreaks associated with consumption of FnAO in EU between 2007 and 2011, the most relevant food/pathogen combinations were identified. Based on these results, the Panel has been asked to provide scientific opinions on the public health risk posed by pathogens on FnAO regarding risk factors, mitigation options and possible microbiological criteria. One of these opinions has been recently published, focusing on the risk of *Salmonella* and Norovirus in leafy greens eaten raw as salads (EFSA Panel on Biological Hazards (BIOHAZ), 2014).

1. *Salmonella* spp. and Norovirus in leafy greens eaten raw as salads
2. *Salmonella, Yersinia, Shigella* and Norovirus in bulb, stem and root vegetables
3. *Salmonella* spp. and Norovirus in tomatoes
4. *Salmonella* spp. in melons
5. *Salmonella* spp. and Norovirus in berries

Figure 4 ~ Top five ranking groups of food/pathogen combinations identified using the seven criteria ranking model (EFSA Panel on Biological Hazards (BIOHAZ), 2013).

Routes of Microbial Contamination

The large variability in production and processing systems, as well as differing geographical areas, has an impact on the potential sources of microbial contamination. Microbial food safety hazards and sources of contamination vary significantly, not only by the type of crop, production systems and practices but also from one particular context to another, even for the same crop (EFSA Panel on Biological Hazards (BIOHAZ), 2013).

Fresh produce can become contaminated with microorganisms whilst growing in fields; during harvesting, post-harvest handling, processing and distribution, and also during preparation/storage in the home. The main pre-harvest sources of pathogenic microorganisms have been identified as: irrigation water, manure (or compost), animal intrusion, harvest practices and worker hygiene. Post-harvest sources include human handling, transport and processing equipment such as for cutting, washing, centrifugation and packing.

A number of mitigation options could reduce the microbiological risk of foodborne pathogens. Appropriate implementation of food safety management systems, including Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP), should be the primary objective of operators producing fresh produce (Gil *et al.*, 2014). Although some intervention strategies or control measures can be defined to limit the spread or reduce the level of microbial contamination, the main focus for food safety management of fresh produce should be preventive measures (EFSA Panel on Biological Hazards (BIOHAZ), 2014).

One of the main mitigation options highlighted in the available literature is the selection, treatment and quality maintenance of the agricultural water used for irrigation, pesticide application, cooling and washing. Other strategies include: scrutinizing the selection of fields for growing crops to avoid areas where the known or presumptive presence of pathogens would lead to a microbiological risk; the appropriate storage and management of manure; the avoidance of animal intrusion by the use of fences, and the identification of specific hygiene and maintenance requirements for equipment and workers (such as the placement of adequate toilets and washing facilities in the growing fields).

Physical and chemical intervention strategies have also been developed for pathogen inactivation on the final product. However, the use of these techniques may be limited by their impact on the quality of the fresh produce. Figure 5 summarizes the most relevant preventive measures, intervention strategies and risk mitigation solutions associated to the risk factors for microbial contamination for the production, processing and distribution of fresh produce.

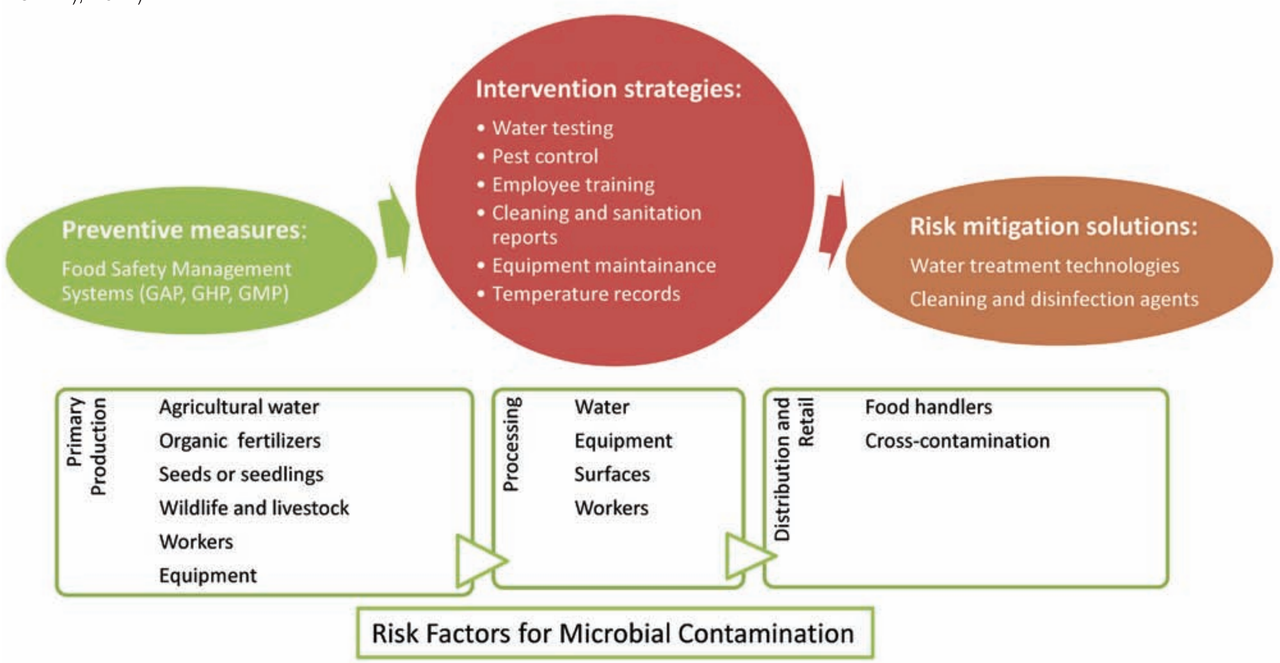

Figure 5 ~ Flowchart of the main risk factors for microbial contamination and preventive measures, intervention strategies and risk mitigation solutions.

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Microbial Attachment, Survival and Proliferation Strategies

In order to be transmitted by fresh produce, foodborne pathogens must attach to plant tissue and survive through production to consumption. Regardless of the environmental source, recent data indicates that foodborne enteric bacteria can attach to growing plant tissue in a relatively rapid fashion, colonize specific microenvironments that may be plant-species specific, coexist with epiphytic bacteria to survive and grow, and persist for significant periods of time (Solomon and Sharma, 2009). Using both microscopy and microbial enumeration tools, enteric pathogens have been shown to enter plant tissues through both natural apertures such as stomata, lateral junctions of roots, flowers and damaged tissue (Erickson, 2012; Gómez-López *et al.*, 2013). Once the microorganisms are attached, bacterial pathogens may be incorporated into biofilms, enhancing their ability to survive on plant tissue (Figure 6).

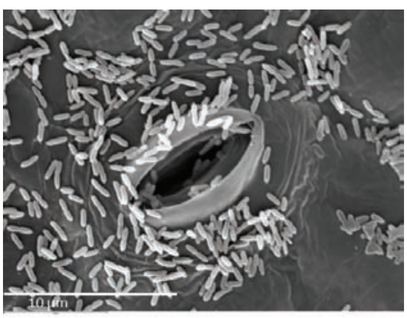

Figure 6 ~ Scanning electron micrograph of microorganisms located on a stoma on the surface of iceberg lettuce.

Biofilms have been described as one of the predominant modes of bacterial growth for many foodborne pathogens. One of the reasons for this is that biofilm formation provides bacterial protection against environmental stress factors, making them less susceptible to antibiotics and disinfectants; it has been demonstrated that biofilm cells exhibit greater resistance when compared to planktonic cells. Joseph *et al.* (2001) observed that biofilm cells in a food processing unit are not usually removed by standard cleaning procedures and therefore could be a source of contamination of foods coming into contact with affected surfaces. Therefore, biofilms seem to also be responsible for limiting the efficacy of sanitizing steps on the vegetable tissue. It has been suggested that higher reductions in bacterial load are not achieved in practice due to the ability of microorganisms to attach strongly to the surface of the produce and embedding of the cells into inaccessible parts of irregular surfaces (Ölmez and Temur, 2010).

Bacteria tend to concentrate in stomata, and other natural irregularities, where there are more binding sites (Sapers, 2001; Parish *et al.*, 2003). This localization, as well as biofilm formation, limits the efficacy of post-harvest sanitizing washes and has to be taken into account when designing new intervention strategies.

It has been reported that between 30% and 80% of bacteria on plant surfaces exist within biofilms (Lindow and Brandl, 2003). The formation of biofilms by bacteria on plant surfaces is likely a survival strategy for the cells to withstand the harsh environment of the plant surface (wide temperature changes, desiccation, ultraviolet rays, and oxidative stress) (Annous *et al.*, 2009). It has been shown that quorum sensing (QS) participates in the control of biofilm processes (de Kievit, 2009; Masák *et al.*, 2014). In fact, many bacteria depend on QS regulated gene systems to establish interactions with eukaryotic hosts or to colonize natural habitats including vegetable tissue. QS has also been recognized as a key mechanism for governing various aspects of biofilm development, including adhesion, motility, maturation, and dispersion (Koutsoudis *et al.*, 2006).

How Do We Control Microbial Contamination?

Outbreaks associated with fresh produce have led to the development of guidelines for producers and processors in order to lower bacterial loading, including specific advice on hygienic production practices and decontamination strategies to control or eliminate the presence of pathogens at all stages of the food supply chain. Most published guidelines agree on the importance of identifying and acting upon specific sources of contamination, such as irrigation and processing water and worker hygiene (EFSA Panel on BIOHAZ, 2013). These critical steps during production, where control or reduction of microbial hazards is possible, may permit implementation of Hazard Analysis and Critical Control Point (HACCP) principles. However, HACCP is not applicable to primary production in an open field (e.g. manure, animal intrusion etc.) because no control measures exist to reduce pathogens to an acceptable level or to completely eliminate them in such an environment (Gil *et al.*, 2014).

Despite the absence of control measures to reduce pathogens to an acceptable level during growth of fresh produce, different post-harvest intervention strategies have been developed in an attempt to reduce the extent of contamination with foodborne pathogens.

Decontamination strategies

Efficient and complete decontamination of fresh and fresh-cut produce is made difficult by the attachment and survival strategies utilized by bacteria (Solomon and Sharma, 2009). Further processing of fresh-cut produce may also make bacterial cells harder to remove or inactivate. Many studies have been

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carried out on the impact of pre- and post-harvest practices on the safety of fresh produce in order to identify intervention strategies for microbial control, such as chemical and physical sanitizers (Allende *et al.*, 2008; Gil *et al.*, 2009; Warriner and Namvar, 2013).

Surface-sanitizing agents have been shown to fail to completely eliminate foodborne pathogens from leafy tissue surfaces, mainly due to the internalization of pathogens within vegetable tissue or the formation of biofilms on the surface of produce (Seo and Frank, 1999; Ölmez and Temur, 2010). In fact, it is generally accepted that no intervention strategy, with the exception of irradiation, is capable of completely inactivating or removing attached bacteria from raw produce. The reasons for this are complex, but answers may be found in the interaction between bacteria and their plant host (Solomon and Sharma, 2009). Therefore, the main goal of surface decontamination techniques during harvest and processing of fresh produce is to reduce the microbial load and to avoid cross-contamination during washing, by keeping process water contamination-free, rather than having a preservative effect on the produce itself.

Some chemical and physical sanitation treatments have been shown to be effective at reducing foodborne pathogens to a degree, giving the industry opportunity to reduce the extent of foodborne outbreaks caused by fresh produce.

Chemical washing

A number of chemical sanitizers are currently used or being investigated as a way to limit pathogen cross-contamination of produce during washing (López-Gálvez *et al.*, 2009; Luo *et al.*, 2011). Among those shown in Table 1, chlorine is the most widely used option, which is capable of reducing the bacterial load in fresh produce by up to 3.0 log CFU/g. Chlorine dioxide, peroxyacetic acid, hydrogen peroxide and ozone have all been shown to be effective at reducing microbial growth in fresh produce. Despite the efficacy of these chemical sanitizers in reducing microbial populations, side effects such as the formation of by-products, safety concerns, and a detrimental effect on fresh produce quality, limit their use.

Other chemical sanitizers include organic acids, quaternary ammonium compounds, trisodium phosphate, sucrose esters, iodine compounds, alcohols, anionic and non-ionic surface-active agents, aldehydes, phosphoric acids, cysteine, methyl jasmonate and bioflavonoids (EFSA Panel on Biological Hazards (BIOHAZ), 2014). However, most of these sanitizing treatments are not currently applied by the food industry for a variety of reasons, such as their impact on the quality of leafy greens and a lack of application experience other than in experimental settings.

Physical treatments

Physical treatments offer an alternative to chemicals for disinfection of recycled or re-circulating process water and fresh produce sanitization. High pressure, pulsed electric fields, oscillating magnetic fields, ultrasound treatments, ionizing radiation and pulsed UV-C light, have all been investigated to reduce or eliminate microorganisms from fresh produce. Only ionizing radiation has been shown as a non-thermal kill step that effectively eliminates foodborne pathogens internalized in fresh produce such as lettuce (Niemira, 2008). However, it is known that differences in the supporting food matrix can significantly influence the radiation sensitivity of pathogenic bacteria. Additionally, there have been problems regarding the consumer acceptance of this technology.

Non-ionizing radiation, such as UV-C light, has been implemented by areas of the food industry such as water and air disinfection. The inactivation of microorganisms as a result of UV radiation is almost entirely attributable to photochemical reactions that are induced within their cells. However, UV-C light has a very low penetration capacity which has limited its efficacy for wider use and hindered its implementation in fresh produce.

Sanitizer	Log Reductions	Limitations	Side Effects
Sodium hypochlorite	0.2-3	Interacts with organic matter	By-products formation (trihalomethanes and chlorates among others)
Chemical electrolytes			
Chlorine dioxide	0.2-5	'*In situ*' generation or use of stabilized solutions	By-products formation (chlorates)
Peroxyacetic acid	0.5-3.5	Expensive	Corrosive for equipment
Hydrogen peroxide	0.5-4	Residual levels may vary	Detrimental effects on vegetable tissue
Ozone	0.5-4	Dangerous for operators, highly affected by the organic load	By-products formation

Table 1 ~ Comparison among different sanitizing agents including the active principle, the expected log reductions, their limitations and side effects.

Other physical methods have already been implemented for pasteurized juices and dairy products but they have a very limited application in fresh fruits and vegetables. In most cases, the high capital expenditure together with the expensive process of optimization and water treatment limits the appeal of these technologies to the fresh produce industry. However, most of these physical methods such as ultrasound, high pressure, high-intensity electric field pulses, and radio frequency have been proven to prevent cross contamination in process wash-water.

Taking into account that decontamination techniques used during harvest and minimal processing are unlikely to reach enteric pathogens residing within plant tissue, it is imperative that paths for pathogen internalisation be recognized and minimized. New strategies that inhibit QS systems and virulence of pathogenic bacteria to prevent attachment and colonization of fresh produce represent promising tools to enhance the efficacy of conventional pre- and post-harvest intervention strategies in eliminating pathogens.

Novel strategies: inhibition of QS in foodborne pathogens

Until now, most research studies carried out to avoid survival and growth of foodborne pathogens in fresh produce have focused on the evaluation of decontamination techniques. However, these strategies are only able to reduce microbial risks associated with fresh produce but not eliminate them. Therefore, it is time for the development of a new strategy. The regulation of virulence of foodborne pathogens via QS confers a strategic advantage, which might play a role to increase safety of fresh produce. A compound, metabolite or bacterium capable of blocking QS is likely to increase the susceptibility of the infecting organism to host defences and its removal from the host (Bjarnsholt and Givskov, 2007). The use of QS signal blockers to attenuate bacterial pathogenicity, rather than bacterial growth, is therefore highly attractive, particularly with respect to enhancing fresh produce defence against pathogens by the natural microbiota of the plant. Until now, there have been a limited number of studies exploring compounds that inhibit QS.

Preliminary experiments have shown that *Bifidobacterium* spp., which are part of the natural microbiota of the human gut, significantly attenuated the virulence of STEC O157:H7, resulting in a 36% reduction in biofilm formation by this organism (Kim *et al*., 2012). In addition to this, microbiota metabolites (urolithins) have also been identified which may be applied to fresh produce as another pathogen control strategy (Truchado *et al*., 2012a,b; Giménez-Bastida, 2012). These findings propose a novel function of natural microbiota in repressing the virulence of STEC O157:H7 and other foodborne pathogens. Currently, a very well-known biocontrol bacterium, *Bacillus thuringiensis*, is widely used as a larvicide and is considered an environmentally friendly method of mosquito control in fresh produce. Spores and crystalline insecticidal proteins produced by *B. thuringiensis* have been commonly used to control insect pests in fresh produce since the 1920s. The use of bacterial strains which act as QS signal blockers to attenuate bacterial pathogenicity is a new strategy to enhance fresh produce safety and is an approach that should be investigated in the future.

Conclusions

Previous findings highlight that attachment, survival and growth of foodborne pathogens, such as *Salmonella* spp., in fresh produce is possible and might be affected by pre- and post-harvest conditions. Recent studies show that internalization of *Salmonella* spp. into baby spinach leaves can occur during washing but may be minimized under specific post-harvest handling conditions. Once attached and internalized, foodborne pathogens cannot be completely removed from the vegetable tissue by any of the currently available decontamination techniques. In fact, the combination of physical and chemical treatments for washing fresh produce has been tested to minimize microbial risk but it does not seem to be the right answer to enhance safety of fruit and vegetables. Sanitizing agents significantly reduce initial microbial loads but result in enhanced survival and/or growth during storage. Therefore, the main goals of sanitizing treatments for washing fresh produce are to reduce the microbial load and keep process water free from contamination rather than having a preservative effect.

New control strategies that aim to reduce the risk of microbial contamination of fresh produce may focus on the attenuation of bacterial pathogenicity, rather than bacterial growth. Several bacterial virulence factors have been related to QS regulated gene systems, such as bacterial attachment and biofilm formation. Taking into account the ability of leafy vegetables to support a vibrant and essential microbial community, it seems quite understandable that attachment and survival of bacterial pathogens is also determined by their ability to compete with the epiphytic bacterial community. Therefore, a better understanding of the natural microbiota of edible plants and the potential interactions with pathogenic microorganisms during pre- and post-harvest handling, which probably involves QS systems, would help in the development of new control strategies.

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