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Review

The microbiological efficacy of decontamination methodologies for fresh produce: A review

Caroline Goodburn[a,b,1], Carol A. Wallace[a,*]

[a] International Institute of Nutritional Sciences and Applied Food Safety Studies, School of Sport, Tourism and the Outdoors, University of Central Lancashire, Preston PR1 2HE, United Kingdom
[b] Florette, Florette SAS, Espace d'activities F. Finel, 50430 Lessay, France

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ABSTRACT

Fresh fruits and vegetables are an essential part of the world populations' diet, contributing essential vitamins and minerals, and they are often eaten raw or minimally processed. Fruits and vegetables grown using conventional agricultural methods are at risk from microbiological contamination and foodborne illness relating to the consumption of produce is widely reported throughout the world, as illustrated by recent figures from the USA (at least 713 produce related outbreaks between 1990 and 2005) and UK (88 outbreaks between 1996 and 2006). Better understanding of produce decontamination is essential to support industry in assuring the safety of fruit and vegetable products, thus contributing to consumer health protection.

The purpose of this study was to establish the current state of knowledge on industrial produce decontamination techniques and to identify and prioritise research gaps regarding practical and effective mechanisms to reduce microbial loading of produce with particular reference to industrially cut, washed and prepared fresh produce. Using suitable keywords, a literature review was executed using academic databases and industry sources to identify current literature on different decontamination technologies. Efficacy of approaches was compared to that of chlorine washing, the most common decontamination method used by the fresh produce industry.

Findings indicate that the identified technologies had varying efficacy of microbiological reduction when compared to chlorine, and the reductions achievable across a range of methods are limited, giving rise to food safety concerns. In addition, the results demonstrate that there has been limited consideration given to several key factors, namely industrial application of the technology approaches, organoleptic acceptability of the product, whether the microbiological reduction could be sustained throughout the life of the product and consumer acceptability of the technology. This preliminary study has highlighted concerns about the efficacy of existing produce decontamination techniques and identified research gaps regarding efficacy and industrial application of new decontamination technologies.

Contents

* Corresponding author. Tel.: +44 (0) 1772 893657; fax: +44 (0) 1772 892927.
E-mail addresses: cagoodburn@florette.com (C. Goodburn), cawallace@uclan.ac.uk (C.A. Wallace).
[1] Tel.: +44 7801 608 117; fax: +33 233 46 58 57.

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1. Introduction

Fresh or fresh cut fruits and vegetables with high levels of vitamins and minerals are an essential part of the world's population's diet (World Health Organisation (WHO) 1998). A diet that is rich in fruit and vegetables has been shown to be protective against cancers and chronic illnesses such as coronary heart disease (Codex Alimentarius Commission (CAC), 2010), and the recommended population nutrient intake goal for fruit and vegetable consumption is ≥400 g per day (WHO, 2003a). Both the WHO and the UK FSA have introduced 'Five-a-day' campaigns to encourage consumers to eat at least 5 servings of fruit and vegetables each day (WHO, 2003b; FSA, 2006). Advice and campaigns such as these have contributed to increased consumption of fruit and vegetables over the last 2 decades (CAC, 2010).

Whilst fruit and vegetables are clearly considered part of a healthy diet, foodborne illness relating to the consumption of produce is widely reported (Lynch, Tauxe, & Hedberg, 2009; WHO, 2008). In the United States from 1990 to 2005 the Food Safety Project reported that there were at least 713 produce related outbreaks associated with foodborne disease. In the 1990s at least 12% of all foodborne outbreak illnesses implicated fresh fruits and vegetables (Food Drug and Administration, 2004). Between 1996 and 2004 the Food Drug and Administration (FDA) responded to 14 outbreaks of foodborne illness for which fresh lettuce or tomatoes were confirmed to be the source, where there were 859 cases of reported illness (Smith, De Wall and Bhuiza 2009).

In 2006 in the United States there was a multi-state outbreak of *E. coli* 0157:H7 implicating spinach, 276 cases of foodborne illness and three deaths were reported (Centers for Disease Control and Prevention (CDC, 2006)).

In the United Kingdom between 1996 and 2006 there were a total of 88 reported outbreaks with more than 3435 reported cases of illness relating to fresh fruits and vegetables (WHO 2008). In 2011 the Advisory Committee on the Microbiological Safety of Food (ACMSF) reported that in the UK between 2008 and 2010, there were 531 cases of reported illness relating to the consumption of fruits and vegetables, including one death (ACMSF, 2011).

In May 2011, Germany reported an ongoing outbreak of Shigatoxin producing *E. coli* (STEC), serotype O104:H4. At the end of the outbreak 3785 cases of illness and 45 deaths had been reported in Germany. Other illness and deaths attributed to this outbreak were reported outside of Germany and sprouted seeds were later identified as the outbreak vehicle (EFSA, 2011).

In 2008, the WHO categorized leafy green vegetables as the highest priority in terms of fresh produce safety from a global perspective. This was based on: frequency and severity of disease; the size and scope of production; the diversity of the supply chain and industry; the potential for amplification of foodborne pathogens; the potential for control and the extent of contamination trade and export (WHO, 2008).

Drawn together by investigating environmental factors that could lead to contamination, the following pathogens of concern were identified for fruits and vegetables eaten raw: *Salmonella* spp., *Shigella* spp., *E. coli*, *Campylobacter*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium* spp., *Bacillus cereus*, *Vibrio* spp., Viruses and Parasites (WHO, 1998). Most produce is grown in the natural environment and is therefore vulnerable to contamination from many sources: e.g. from the soil; from irrigation water; from wild animals; from personnel and harvesting equipment and post-harvesting treatment and distribution.

Despite this potential for contamination, market data shows that the quantities of fresh produce consumed remains high. In the UK, the combined fruit, vegetable and potato market was estimated at £14.5 billion in 2011. More than four fifths of this total spend was for salads, potatoes and green vegetables (Mintel Fruits and Vegetables – UK, 2012). When this data is compared with the outbreaks of foodborne disease it can be seen that prevalence of foodborne outbreaks are relatively low (European Commission, 2002), however when they occur the severity can be high, as illustrated by the *E. coli* outbreak in Germany in 2011 (EFSA, 2011).

There have been a number of Good Agricultural Practice (GAP) guidelines written, some Government driven and some led by the produce industry and its customers. Codex Alimentarius, *Code of Good Practice for Fresh Fruits and Vegetables* (2010) is an internationally accepted code setting out requirements for the safe production of fresh produce. Other documents setting out specific practice safety guidance for the produce industry include the Chilled Foods Association, *Microbiological Guidance for Produce Suppliers to* Chilled Food Manufacturers (2007) and Campden and Chorleywood Food Research Association (2004) *Risks of Pathogens in Ready to Eat Fruits, Salads through the production process*. More general requirements are laid down in the Global Gap Standard for Integrated Farm Management (2012), though these standards do not include specific controls for microbiological safety of produce.

Much fresh produce is eaten raw or minimally processed and does not undergo a 'lethal' process treatment such as cooking. Soon, Manning, Davies, and Baines (2012) define minimally-processed foods as those produce commodities that are eaten raw and have not received a formal process or treatment to reduce pathogenic bacteria, their spores or toxins to a safe level. Abadias, Cañamas, Asensio, Angueram, and Viñas (2006) state that safe production methods and proper disinfection or decontamination procedures are critical steps in ensuring food safety of ready to eat foods and vegetables. Whilst preharvest strategies such as the application of GAPs during growing and harvesting may help to reduce the risk of contamination, there is still much reliance on produce decontaminations strategies applied by the processing industry. There are many methods of produce disinfection that have been developed, including chemical washing and spraying procedures, irradiative treatments and natural/biological methods. Much focus in industry has been on chemical techniques and the following range of chemical methods were reviewed by the WHO in 1998 (WHO, 1998):

- Chlorine
- Chlorine dioxide
- Bromine
- Iodine
- Trisodium phosphate
- Quaternary ammonium compounds
- Acids
- Hydrogen peroxide
- Ozone

It was concluded that the efficacy of the methods varied greatly and that there was a lack of scientific data from which to draw firm conclusions concerning the efficacy (WHO, 1998). This has resulted in a range of investigative studies to assess different decontamination techniques on a variety of produce types. The efficacy of decontamination methods is reflected in the microbiological reduction obtained and, even more importantly, the maintenance of this reduction during storage (Abadias, Alegre, Usall, Torres, & Viñas, 2011).

There are many environmental factors that may affect microbiological loading of produce, including growing, harvesting, post-harvest treatment, distribution and storage (Aruscavage, lee, Miller, & Lejeune, 2006). It is usually accepted that preventative measures to avoid and reduce pathogen contamination are the most important steps to safeguard microbiological safety of produce (Beuchat & Ryu, 1997). Processes such as refrigeration and decontamination have long been used to support the preventative measures (WHO 2008).

More recently Olaimat and Holley (2012) reported that some bacterial strains are better able to colonize on produce surfaces than others, and that biofilm formation, tissue damage, plant species as well as level of host maturity, may also have a role to play in pathogen persistence. Understanding the reasons for the increasing contribution of contaminated produce to the overall burden of foodborne illness will shed light on measures likely to be most effective in reversing the incidence of foodborne illness.

Villagomez, Herrera, Orozco, Wild-Padua, and Iturriaga (2010) found that during storage micro-organisms colonizing may produce biofilms that produce protection against disinfection and that levels of naturally occurring microflora may also have an influence on the effectiveness of disinfectant against pathogens of concern. For these reasons further research is needed to understand how industrial decontamination treatments can be used against biofilms.

There are many factors that will affect disinfection, such as initial bacterial colonizing on the surface of produce; pathogen contamination; treatment type; the surface to be treated; the type of disinfectant; whether or not there is any internalisation of contaminating pathogens (Erickson, 2012); and the time and temperature of exposure to the disinfectant (Beuchat, Adler, & Lang, 2004; Beuchat, Pettigrew, Tremblay, Roselle, & Scouten, 2004). Pathogens such as *E. coli* 0157:H7 and *Salmonella* sp. have the ability to internalize, through entering the vascular system of growing plants (Itoh et al., 1998). This is a key area of produce safety of concern, as chemical sanitizers used post harvest are unlikely to reach enteric pathogens in the plant tissue (Little et al., 2011). Research has reported that internalization may be transient and be affected by plant maturity (Olaimat & Holley, 2012). Currently it is not known how consistently pathogens become internalized within plant tissue (Zhang et al., 2009). It is clear that more work into this area is required and currently the best defence against pathogen internalization is good hygiene practices in the growing cycle. Recent calls for HACCP on the farm (Soon et al., 2012) may help to strengthen current GAP procedures and reduce potential for initial produce contamination. Nevertheless, the requirement for effective produce decontamination technologies remains an important weapon in the control of foodborne disease.

The use of chlorine as a produce disinfectant is probably the most common (WHO 1998). It is generally used in the following forms: Chlorine gas, calcium hypochlorite and sodium hypochlorite and there has been much research into the efficacy of chlorine as a sanitizer for produce decontamination (WHO, 1998) and log reductions for various pathogens on a range of produce types have been reported. However, typical mean aerobic mesophilic *counts* on Ready to Eat salad vegetables have been published at the following levels: 5.4×10^6, 1.5×10^7 and 3.7×10^7 cfu/g after 24 h of packing (De Giuisti et al., 2010). This indicates that the overall contamination levels are still relatively high in washed (most likely using chlorine), packed product and raises questions about whether pathogens may also still be present.

The use of chlorine washing is becoming increasingly challenged. Considerations have included public health concerns with chlorine and its by-products (Hrudey, 2009; Parish et al., 2003) and an increasing awareness of the negative environmental impact of chlorine (Wei, Cook, & Kirk, 1985). The efficacy of chlorine as a decontaminant for produce has also been questioned. Gomes, Moreira, and Castel-Perez (2011), stated that internalized micro-organisms cannot be eliminated by current procedures of chlorinated washing. Fett (2000) found that the efficacy of chlorine is reduced for *E. coli* 0157:H7 through the forming of biofilms from background bacteria. For these reasons the need to develop alternative technologies to chlorine has become evident.

This paper investigates current literature regarding fresh produce decontamination technologies for industrially cut, washed and prepared produce. It evaluates the different technologies and their efficacies for bacterial reduction and practicality for use in an industrial setting. The technologies are compared with chlorine washing (the most common disinfectant used by the fresh produce industry) and areas for further research relating to fresh produce decontamination are explored.

2. Methodology

A literature search was conducted between January and September 2012. The research was web based, consulting online databases such as *Science Direct, Emerald* and *Ingenta* and considered literature published in the previous 15 years.

Initially, searches using keywords such as ‘produce disinfection’ were undertaken. After the initial searches, the keywords were refined to search for specific technologies. All papers were reviewed for content. Results from the papers are presented and discussed and conclusions drawn on implications for produce decontamination.

3. Results

3.1. Chlorine

Chlorine, delivered as Sodium hypochlorite solution at pH 6.5 is currently the most common sanitizer used in the fresh-cut. 63 produce industry (Shen et al., 2012). For chlorine to be effective it needs to be used in concentrations of 50–200 ppm and at a pH < 8 and to be in contact with the produce for not less than 1 min (WHO 1998).

There has been much research into the efficacy of chlorine. In 1998 the WHO reported that on lettuce leaves log reductions for *Salmonella*, *E. coli* 0157:H7 and aerobic mesophilic population were

1.79, 2.48 and 0.33 respectively, for leaves that were washed at 200 ppm concentration for 10 min Delaquis, Stewart, Toivonen, and Moyls (1999) found log reductions for lettuce washed for 3 min at 100 ppm chlorine at 47 °C and 4 °C were 3.0 and 1.0 cfu/g respectively. Zhang and Faber (1996), using 200 ppm chlorine for 10 min at 4 °C and 22 °C saw log reductions of *L. monocytogenes* of 1.3 and 1.7 on lettuce and 0.9 and 1.2 on cabbage. Lang, Harris, and Beuchat (2004) found similar results with log reductions of 1.42 of *E. coli* 0157:H7 for 200 ppm chlorine for 5 min.

These results show that despite chlorine concentration, pH and immersion time, the typical log reduction is <2 logs. Typical levels of bacteria were reported by De Giusti et al. (2010) where mean aerobic mesophilic *counts* on Ready to Eat salad vegetables were 5.4×10^6, 1.5×10^7 and 3.7×10^7 cfu/g after 24 h of packing. After 7 days after packing this level had increased to 1.1×10^{10}, 3.4×10^9 and 6.0×10^{10} respectively. Although no data were presented for pathogen contamination these high overall levels suggest the need for other technologies to be developed to ensure food safety.

3.2. Chlorine dioxide (CLO_2)

Researchers have focussed on chlorine dioxide as an alternative sanitizer as it has 2.5 times the oxidisation capacity of chlorine and it is less reactive to organic compounds (Beuchat et al., 2004). However chlorine dioxide is unstable, it must be generated on site and it can be explosive when concentrated (WHO 1998). In the United States, a maximum of 5 ppm of chlorine dioxide is permitted for use in the disinfection of whole fresh fruits and vegetables (WHO 1998).

Wu and Kim (2007) state that aqueous chlorine dioxide offers advantages over traditional gaseous chlorine dioxide for decontamination of vegetables and fruits, and it does not need a special chamber for the generation process to be undertaken. Studying the efficacy of aqueous chlorine dioxide (15 ppm of CLO_2) on blueberries, they found, that a log reduction of 4.8 for *L. monocytogenes* was achieved with a treatment time of 2 h; *Pseudomonas aeruginosa* was reduced by 2.16 log after 5 min; *Salmonella typhimurium* saw a 3.32 log reduction after 20 min; *Staphylococcus aureus* achieved a 4.56 log reduction over 30 min; *Yersinia enterocolitica* saw a 3.49 log reduction after 2 h; and yeasts and moulds reduce by a 2.82 logs after 1 h Wu and Kim (2007). Although some of these reductions seem to be encouraging, the level of CLO_2 tested was higher than permitted levels in the USA, making the results less useful to the produce industry.

Du, Han, and Linton (2003) studied the efficacy of chlorine dioxide gas for reducing *E. coli* 0157:H7 on apples and found that log reductions of 3.0 could be achieved after CLO_2 (using concentrations of 1.1–18.0 mg L) with contact times between 10 and 30 min at 21 °C. Similar results were found by Han, Linton, Nielson, and Nelson (2001) where a log reduction of 6.45 on the surface of peppers for *E. coli* 0157:H7 at concentrations of 1.24 ppm for 30 min at 22 °C.

Lee, Costello, and Kang (2004) found that on lettuce leaves the contact time could greatly increase the log reduction. Using chlorine dioxide (4.3, 6.7 and 8.7 mg) at 22 °C for 30 min exposure, a log reduction of 3.4 for *E. coli*, 4.3 for *S. typhimurium* and 5.0 for *L. monocytogenes* was observed. If the same parameters were used, but with an immersion time of 3 h, a 6.9 log reduction of *E. coli,* 5.4 log reduction of *S. typhimurium* and 5.4 log reduction of *L. monocytogenes* was seen.

Whilst the treatment times and concentrations show varying results, in general the above results show good log reductions of pathogens. Typically a log reduction of ≤6.0 is seen for chlorine dioxide; this is considerably better than that of chlorine, where log reductions of ≤2.0 are generally seen. However, to be effective in log reduction, this technology requires long treatment times from 10 min to 2 h and water temperatures of 22 °C which would be impractical to implement in an industrial setting and which may impact the organoleptic quality and shelf life of the produce, factors which have not been considered as part of these studies. Produce that is submerged in warm water for long periods of time may not have the shelf life and quality parameters of produce which has been washed for less than 1 min at <5 °C for that of chlorine washing.

3.3. Ozone

The treatment of drinking water by ozone for killing pathogenic bacteria has been in use for nearly a century (WHO, 1998). In the United States, the FDA (2001, p. 33829) has approved the use of ozone as an antimicrobial agent for the treatment, storage and processing of food, including raw and minimally processed fruits and vegetables (WHO, 2008). Similar to gaseous chlorine dioxide, ozone has to be generated on site, as it is unstable and decomposes quickly in water. Khadre, Yousef, and Kim (2001) estimated this to be 20–30 min at 20 °C.

The efficacy of ozone was investigated (Kim & Yousef, 2000). Zhang and Faber (1996) who found that lettuce exposed to 5 ppm of ozone for 10 min at 4 and 22 °C gave a log reduction for *L. monocytogenes* of 1.1 and 0.8 respectively. Kim, Yousef, and Chism (1999) found that lettuce immersed for 3 min in 1.3 ppm of ozone saw log reductions of 1.2 and 1.8 for mesophilic and psychotrophic microorganisms respectively.

Ölmez (2010) studied the antimicrobial activity, and effect of incubation times and temperature on the efficacy of ozone treatment against *E. coli*. Similar results to Kim et al. (1999) were observed with lettuce dipped into ozonated water and bubbling ozonated water. With short exposure times of between 2 and 4 min, the temperature of the water did not have a significant effect.

When comparing the log reduction of ozone to that of chlorine as a produce decontaminant, it can be seen that ozone is equal to chlorine. Log reduction for ozone was found to be between 0.8 and 1.8. Typical log reduction values for chlorine are ≤2.0.

In these studies (Khadre et al. (2001), Zhang and Faber (1996), Kim et al. (1999) and Ölmez (2010)) the immersion time and temperature of the water were not considered for industrial practical use and, commercial shelf life and organoleptic qualities of the product. In addition, ozone could cause processors significant challenges in that it has a short life and therefore will need to be generated on site and will need to be capable of being produced in quantities sufficient to meet the needs of the industrial process. Ozone has strong oxidising power which could corrode metal surfaces that are common in industrial processes. The use of ozone could reduce the life of processing equipment, which has a negative impact or practicality of use in an industrial setting.

More recent research into the use of ozone as a decontamination technology for fresh produce (Perry & Yousef, 2011) presents advantages of ozone over chlorine, where due to the short life of ozone by products, the product quickly decomposes into oxygen. It was reported that ozone could be used as an efficient decontamination technology, though concentration of ozone and treatment time could impact on the susceptibility of damage on produce (Perry & Yousef, 2011). These issues would need to be overcome to ensure large scale adoption by the fresh produce industry.

An emerging application for ozone was reported on by Fan, Sokorai, Engemann, Gurtler, and Liu (2012), where ozone was used in pack as an alternative to chemical sanitizers for tomatoes. It was found that *E. coli* 0157:H7 and *Salmonella* sp. were typically reduced by 2–3 log CFU per fruit for 22 days of storage and no negative effect on fruit colour or texture was observed. This technology appears to provide a real alternative to chemical sanitizers and helps overcome some of the issues seen with ozone research on fresh produce to date, in that the in pack system was able to produce high concentrations of ozone in a short time, allowing the ozone to come into contact with the produce surface. Further work is needed to adapt this technology for industrial application and, for the technology to be used across the whole produce sector, feasibility of all packaging formats and produce types would need to be considered.

3.4. Irradiation

The use of gamma irradiation to improve shelf life of foods has been extensively studied (WHO, 1998). The FDA has approved the use of irradiation of fresh foods up to 4× Gy to control foodborne pathogens and extend the shelf life of fresh iceberg lettuce and spinach (FDA, 1999, 2008).

Large scale adoption of this process for the decontamination of produce has not been taken up by the fresh produce industry. This could be due to the need for further research into irradiation for produce, as identified by the WHO (1998), where it stated that there was a need to evaluate the tolerance of most fruits and vegetables to radiation doses required for controlling various pathogenic organisms. Negative consumer perception of irradiated food has been reported by Foster (1991), Gallup (1993), Bruhn (1998) and Junquiera-Gonçalves et al. (2010), and this may also be a factor for adoption of the technology by the produce industry.

Irradiation has been found to be an effective method of reducing the bacterial count on produce (Hsu, Simonne, Jitareerat, & Marshall, 2010). Foley, Euper, Caporaso, and Prakash (2004) found a log reduction of 6.7 log for *E. coli* 0157:H 7 on inoculated coriander at 1.05 kGy. Bari et al. (2005) found that at 1 kGy a log reduction of 5.3, 4.1, 4.9 and 4.6 for *L. monocytogenes* was seen for cabbage, tomatoes, sprouts of broccoli and mung beans respectively. These reports showed microbiological reduction at the time of treatment and further microbiological growth during storage, distribution and consumer life was not investigated. It was not possible to assess, therefore, whether the microbiological reduction could be sustained under commercial conditions.

More recent research (Villagomez et al., 2010) focussed on the efficacy of irradiation on *Salmonella enterica* on coriander throughout storage and under different conditions. It was found that the efficacy of irradiation was dependent on a number of factors. Irradiation dose will affect the microbiological reduction; the higher the dose, the greater the destruction. Large numbers of microorganisms reduce the effectiveness of a given radiation dose. The type of microorganism can influence efficacy of irradiation and storage temperatures can also play a part in irradiation efficacy during shelf life. For example, in coriander stored a 5 °C, the level of pathogen reduction throughout storage remained similar to that after treatment. However if samples were stored at 22 °C, after two days of storage pathogen re-growth was seen (Villagomez et al., 2010).

Niemira and Cooke (2010) investigated the efficacy of irradiation on *E. coli* 0157:H7 biofilm formation on romaine lettuce and spinach leaf. The study found that allowing time for biofilm formation reduced the efficacy of irradiation. This demonstrated that, for irradiation to be effective, it needed to be carried out soon after harvest.

Further research into irradiation and its effects on pathogens in relation to organoleptic acceptability of the product is needed. Research needs to be undertaken as to where in the supply chain irradiation could be best used and how it can be combined with other decontamination or preservation processes to be most effective. Research undertaken by Niemira and Cooke (2010) suggests that optimum efficacy is seen shortly after harvest, whilst Villagomez et al. (2010) found that *Salmonella* could grow after irradiation. These contrasting findings suggest that irradiation could be used as a post harvest treatment, but that irradiation cannot be used in isolation and needs to be part of a number of controls or hurdles in the decontamination process.

Consumer acceptability of irradiation would need further investigation if the technology was to be more widely accepted for use by the fresh produce industry. Health concerns remain with the consumption of irradiated foods: Papers by Ashley et al. (2004) and Neimera and Fan (2005) identified the need for additional research to fully address health issues associated with the consumption of irradiated foods. Recent consumer research (Teisl, Fein, & Levy, 2009) found that the mechanisms for supply of information to consumers can affect their attitude and behaviour and influence the market. It was found the more information supplied to the consumer regarding irradiation, the more the technology becomes acceptable to the consumer.

3.5. Combined technologies

Studies have been undertaken to use a combination of technologies or change the process steps within industry approved processes. This is referred to as hurdle technology; its purpose is to attack the microorganism in different ways, leading to an overall effective reduction in contamination. It is widely accepted within the fresh produce industry that the use of refrigeration, a decontamination step and modified atmosphere packing can inhibit or slow down growth and reduce bacterial count (WHO, 2008).

Foley, Rodriguez, Caporaso, and Prakash (2002) conducted a study combining the effects of chlorination and low dose irradiation (0.55 kGy) on *E. coli* 0157:H7 on shredded iceberg. This combined process gave a 5.4 log reduction. It can be seen that when compared to chlorination alone, the log reduction can be considered to be significantly better.

Garcia, Mount, and Davidson (2003) conducted a study to see the effects of ozone (7.5 ppm) and chlorine (150 ppm) on aerobic plate count on shredded lettuce. This combined process gave a log reduction of 1.45–2.5. When compared with chlorination it can be seen that the log reduction is similar and no benefits were observed by the use of ozone in addition to chlorine.

Nou and Lou (2010) conducted a study to evaluate if a sanitizer wash before cutting improves efficacy compared to traditional methods of sanitization post cut. Chlorine at 70 ppm was used to determine the log reduction that could be achieved for *E. coli* 0157:H7 on romaine and lollo rossa leaves. Compared to traditional processing. It was found that whole leaf washing improved pathogen reduction by 1 log for *E. coli* 0157:H7 and that background microflora saw similar reductions. The effects of process cross-contamination were also studied, and this method showed 0.3 log cross-contamination reduction in process. However, it is unlikely that this level of log reduction would be viewed positively by the produce industry as to substantiate the level of investment required for this additional process step to be implemented.

3.6. New and emerging technologies

3.6.1. Electrostatic sprays

Electrostatic spraying is an emerging technology and there is currently little published research on the topic for produce decontamination. Electrostatic spraying is a method that can be used for fine coating the surface of a food with a substance. An electrostatic spray is where an intense electric field is applied to the surface of a liquid that induces an electrostatic force sufficient to overcome the surface tension and disrupts the liquid so that it becomes a spray of charged particles. This disruption of the droplet surface becomes a cloud of charged droplets which are attracted to the surface (Bailey, 1974).

Ganesh et al. (2010) studied the antimicrobial effects of organic and inorganic acids applied conventionally and electrostatically to observe the effect on *Salmonella typhimurium* inoculated spinach. The study found that malic acid and lactic acid and malic acid and grape seed combinations demonstrated antimicrobial activities against *Salmonella typhimurium* on spinach with log reductions of 4.3 and 3.3 respectively when sprayed electrostatically.

When the efficacy of electrostatic spraying is compared with that of chlorine, chlorine dioxide and ozone, it can be seen that the log reduction is better than, or comparable to these decontamination methods. Benefits of this technology are that organic acids that are less corrosive and will cause less damage to processing equipment. In addition, organic acids have occupational health benefits for processing staff in that they are easier to use and are more stable. Consumer perceptions of organic acids and spray technology may be viewed more positively than other chemical or non-chemical technologies, in that organic acids could be viewed as natural. This is demonstrated by Rozin (2005) where consumers have positive association when the word “natural” is suggested.

As stated above, electrostatic spraying is a new and emerging technology and further research is required both to understand if electrostatic spraying can increase the efficacy of other decontaminants used for fresh produce and to determine if the reduction can be sustained during the shelf life of the product. Further work is also needed to understand the practicalities of use in an industrial setting.

3.6.2. Silver and hydrogen peroxide

Silver nitrate in low concentrations has been used as a disinfectant in swimming pools and for drinking water (Batterman, Zhang, & Wang, 2000; Christensen, Tronnes, Vollan, Smidsrod, & Bakke, 1990). Gopal, Coventry, Wan, Roginski, and Ajlouni (2010) investigated the use of silver and hydrogen peroxide as possible alternatives to chlorination. The study investigated the following: the effect of silver (from silver nitrate) and chlorine on fresh cut lettuce; the effect of silver (from silver nitrate) and hydrogen peroxide on fresh-cut lettuce; and the effect of electrochemically generated silver and hydrogen peroxide on fresh fruit and lettuce for viable count, presumptive Pseudomonas, yeasts and moulds and Enterobacteriaceae over a seven day period (Gopal et al., 2010). It was found that washing with silver was more effective on all of the organisms than chlorine used at comparable concentrations (Gopal et al., 2010).

Little work has been performed on the residual uptake of silver by lettuce. It is therefore not known what levels of silver remain in the lettuce, and thus the potential toxicological effect remains unknown. The study only considered lettuce and it is also not known if the use of silver could be effective on other produce. In 2003, the WHO investigated the toxicological effect of silver for use in water disinfection. It stated that all foods contain traces of silver (10–100 micron per Kg) and gave an acute lethal dose of silver nitrate of 10 g, reporting an estimate that, for a human, 10 g is the maximum oral intake for life (WHO, 2003c).

Gopal et al. (2010) reported a need for potential development of silver as a disinfectant for produce. The toxicological effect and the uptake of silver by produce will need to be considered as part of this development, given that the lethal dose for a human is 10 g. This work would need to be developed before this technology could be used in an industrial setting. It is likely, given the toxicology of silver, that intervention of regulation would be needed. In addition, the organoleptic effects were not considered as part of the study (Gopal et al., 2010) and consumer perception of the use of silver as a produce disinfectant is not known. These areas would also need to be developed if silver was to be adopted as a disinfectant by the fresh produce industry.

3.6.3. Pulsed light

The application of intense pulsed light is an emerging technology for the use of food preservation for the inactivation of microorganisms. This technique has also been investigated as a decontamination agent on food contact equipment (Rajkovic et al., 2010). On produce, Ramos-Villarroel, Aron-Maftei, Martín-Belloso, and Soliva-Fortuny (2012) investigated *E. coli and Listeria innocua* reduction on fresh water melon using pulsed light full spectrum treatments. A log reduction for *L.innocua* of 2.79 cfu/g was achieved and for *E. coli* a log reduction of more than 3 cycles was seen. Similar results were seen by Oms-Oliu et al. (2010) where pulsed light was seen to reduce the natural flora on mushrooms from 0.6 to 2.2 log, which was sustained over a period of 15 days. For this technique to be determined successful, further work is needed to understand the effect of spectral range on specific pathogens. Consideration should also be given to the impact of the pulsed light on the nutritive value of produce, in particular its effect on water soluble vitamins that are known to be sensitive to UV light.

3.6.4. Electrolysed water

The potential of electrolysed water as a decontamination step for fresh produce has been researched. Gómez-López et al. (2007) investigated the effect of neutralised electrolysed water to extend shelf life of shredded cabbage, when stored under equilibrium modified atmosphere. In this study, electrolysed water was found to be beneficial for improvement in visual acceptability and gave a shelf life extension of at least 5 and 3 days. However there was no significant difference in the microbiological parameter tested between treated and non-treated samples. Rico et al. (2008) investigated the effect of neutral electrolysed water for shelf life extension of minimally processed lettuce. Electrolysed water was compared with standard chlorine washing methods. The research found that both treatments showed a significant mesophilic reduction (2.2–2.4 log) after one day and after 1 day of storage there were no significant differences in bacterial counts between the samples treated with electrolysed water and chlorine. The use of electrolysed water was shown to have a negative effect on browning and a loss of turgor and mineral content was also observed.

More recently the use of electrolysed water has been in combined technologies. Issa-Zacharia, Kamitano, Muhimbula, and Ndabikunze (2010) investigated the effect of slightly acidified electrolysed water (SAEW) against pathogens on ready to eat vegetables and sprouts compared to a traditional chlorine wash. It was found that SAEW treatment significantly reduced the total aerobic mesophilic bacteria from Chinese celery, lettuce and daikon sprouts by 2.7, 2.5 and 2.45 log 10 CFU/g, respectively relative to untreated. The results demonstrate that SAEW could be developed as an alternative to chlorinated washing since the same microbial reduction as chlorine washing is obtained. However in the Issa-Zacharia et al. (2010) research the samples were submersed in electrolysed water for 5 min and further work would need to be undertaken to find an industrial solution to this hurdle. In addition, issues such as nutritive impact and oxidising potential on organoleptic acceptability need to be investigated further.

3.6.5. Biological control – bacteriocins and bacteriophages

Bacteriocins are cationic antimicrobial peptides produced by many types of bacteria and which may have applications in food biopreservation (Settanni & Corsetti, 2008). Lactic acid bacteria (LAB) decontamination technology is a new and emerging technology for decontamination. Lactic acid bacteria are generally recognised as safe (GRAS) by the USA Food Drug and Administration (Cleveland, Montville, Nes, & Chikindas, 2001; FDA, 1998). Historically LAB have been used to preserve meat and dairy products (Stiles & Holzapfel, 1997). A new area of research has been into the use of LAB as a bioprotective technology in fruits and vegetables. Trias, Bañeras, Badosa, and Montesinos (2008) developed 18 isolates of LAB and found that when applied to Golden Delicious Apples and Iceberg Lettuce, that the strains reduced the cell count of *S. typhimurium* and *E. coli* by 1 or 2 log/cfu respectively and inhibited the growth of *L. monocytogenes*. Allende et al. (2007) using LAB found that *L. monocytogenes* on fresh cut lettuce was reduced by 1.2–1.6 log cfu and that this inhibitory activity depleted over long term storage. The use of *Lactococcus lactis* for biopreservation on Iceberg lettuces was reported by Randazzo, Pitino, Scifò, and Caggia (2009); it was found that the treatment did not completely eliminate *L. monocytogenes* but a log reduction of 2.7 cfu was sustained for 7 days.

For development into industrial applications, each LAB strain will need to be studied for safety as LAB strains have been linked with sepsis, endocarditis and bacteraemia (Daniel et al., 2006). In addition, due to the inhibition ranges towards pathogens associated with fresh vegetables and fruit, research will need to be conducted to ascertain whether the LAB strains have a long term effect on bacterial reduction in the shelf life.

Bacteriophages have also been investigated as potential antipathogen agents on fresh produce (Parish et al., 2003; Ye, Kostrzynka, Dunfield, & Warriner, 2009, 2010). Leverentez et al. (2003) used *Salmonella*-specific bacteriophages on melon slices and achieved a 3.5 log reduction at 5 or 10 °C, however the same bacteriophages were not effective on apple slices and it was postulated that this may have been due to the lower pH of apple (pH 4.2). Kocharunchitt, Ross, and McNeil (2009) describe a study on *Salmonella*-specific bacteriophages on sprouted seeds where a 1 log reduction of *Salmonella* was achieved. In this study a temporary phage-resistance was seen, which it was suggested may thwart the potential for use of bacteriophages in biological control (Kocharunchitt et al., 2009). In a study on the potential for bacteriophages against *E. coli* 0157:H7, Viazis, Akhtar, Feirtag, and Diez-Gonzalez (2011) applied a cocktail of *E. coli*-specific phages (BEC8) both alone and in combination with trans-cinnameldehyde essential oil (TC). They report no detection of survivors when leaves were treated individually with BEC8 or TC at low *E. coli* 0157:H7 inoculum levels after 24 h at 23 and 37 °C. At higher inoculum levels or lower incubation temperatures the efficacy of BEC8 and TC individually decreased, however when used in combination, no survivors were detected after 10 min at all temperatures and inoculum levels (Viazis et al., 2011). These results seem encouraging, however the practicality of this technology in an industrial setting needs to be evaluated, e.g. with regard to incubation temperatures and their likely impact on produce quality. The specificity of bacteriophages further complicates their potential for use where multiple pathogens may be present as contaminants on fresh produce and it is unlikely that a bacteriophage cocktail could be produced that would be effective against all likely pathogens.

3.6.6. Essential oils

Natural antimicrobials have been identified in herbs and spices and recent studies have looked at the impact of natural oils for microbiological spoilage of fresh produce. Tzortzakis (2009) reported the efficacy of cinnamon oil for reduction of microbiological spoilage, finding that essential oils may possess antifungal activity. The use of myrtle oil against *S. typhimurium* on fresh produce was investigated (Gündüz, Gönül, & Karapinar, 2009) and log reductions seen were 1.66 cfu/g to 1.89 cfu/g for tomatoes and iceberg lettuce. This log reduction was achieved using 1000 ppm of myrtle oil without any rinsing treatment. The impact of sumach water extract and oregano oil for the inactivation of *S. typhurmurium* was investigated in a further study by Gunduz et al. (2010). Using 100 ppm oregano oil, the maximum log reduction for *S. typhurmurium* was 2.78 cfu/g and for 4% sumach extract it was 2.38 cfu/g, both on tomatoes. The washing processes examined used water at 20 °C for 5, 10, 15 and 20 min (Gündüz, Gönül, & Karapinar, 2010). This method would not be acceptable for an industrial process, for both productivity and organoleptic reasons.

Whilst the levels of inactivation differ between oils used, the log reduction seen for natural oil is similar to that of chlorine. For natural oils to be an innovative tool for microbiological reduction, further work is need on understanding which oil can be effective against the target pathogen. Research will also need to be conducted on the organoleptic acceptability, in terms of tainting and toxicity. The water temperature and washing times would also need to be reviewed for industrial acceptability.

4. Discussion

Washing is an accepted step in decontamination for fresh produce as, in addition to any antimicrobial effect, it removes soil and pesticide residues. Produce decontamination often shares technology with water treatment and much of the research has taken technologies used in water treatment and transferred it to produce washing. Such technologies include chlorine, chlorine dioxide, ozone, silver and hydrogen peroxide.

Many studies have identified that chlorine remains the most popular disinfection method for fresh produce contamination (Food and Agriculture Organisation (2010); Gil, Selma, López-Galvez, and Allende (2009); Issa- Zacharia et al. (2010); Rico, Martin-Diana, Barat, and Barry-Ryan (2007); Sapers (2001); Shen et al., 2012; and WHO, 1998). Reasons for this are that it has a proven track record in water disinfection; it is easy to use in an industrial setting; the contact time with the produce is short; it is effective when used in chilled water (which is necessary to maintain quality throughout shelf life) and it is cost effective.

However, there are trends to eliminate chlorine from the disinfection process because of concerns regarding efficacy. Gomes et al. (2011) reported that internalized micro-organisms cannot be eliminated by chlorine, and other concerns about the use of chlorine relate to its environmental impact and occupational health issues regarding the possible by products left after the washing process (Hrudey, 2009; Parish et al., 2003; Wie et al., 1985). Abidias et al. (2011) reviewed a number of alternative sanitizers in use within the produce industry and found the efficacy in reducing food borne pathogens on fresh cut apple was either equal to or less than that of chlorine.

Studies on chlorine dioxide have demonstrated that it has been effective in reducing pathogens (Han et al., 2001; Mahmoud, Bhagat, & Linton, 2007; Rodgers, Cash, Siddiq, & Ryser, 2004; Zhang & Faber, 1996). Contact time with chlorine dioxide ranged from 5 to 30 min in water of up to 22 °C. Compared with the temperature of chlorinated water at 5 °C and a contact time of 1 min (WHO, 1998), These studies did not take into account the impracticality of long contact time for manufacturing processes or, more importantly, the negative impact on the quality and potential shelf life reduction of fresh produce if in contact with water at 22 °C for up to 30 min.

Ozone was found to be effective in reducing pathogens on produce with log reductions of up to 5.0 (Kim and Yousef, 1999; Zhang & Faber, 1996). However, for ozone to be effective, the optimum factors are contact time of 30 s and water temperature of 25 °C. Ozone gives good log reduction, with a contact time similar to that of chlorine. The suitability of this method in an industrial setting is questionable, as ozone would need to be generated on site since it decomposes within 20–30 min (Khadre et al., 2001). In addition it would be costly to maintain ozone levels and temperature in large volumes of water. The quality aspect of the product due to the impact of the water temperature was not considered in the studies and would need to be investigated. This is important as putting fresh produce into water for up to 30 s at temperatures of up to 25 °C, will give a reduced shelf life and give poor visual quality. The use of in-pack ozonation systems reported by Fan et al. (2012) may help overcome these issues and needs further investigation.

Irradiation is probably the most effective method for decontamination with log reductions seen up to 7.0 (Gomes et al., 2011), however the technique of irradiation for the use with food has not been consistently adopted. For example, the European Union has not reached an agreement on a guideline for the use of irradiation on food. Irradiation is, however, approved for use in some European countries for some products such as herbs and spices in the United Kingdom, France and the Netherlands, but not in Germany (Nordian, 2011). The irradiation process could not be used in isolation, as a washing step would still be needed in order to remove soil and chemical residues from growing. Irradiation would be costly to implement and maintain, because of the necessary investment in equipment and safety checks. Consumer acceptability of the technology remains an area for further development. The health concerns associated with the consumption of irradiated foods reported by Ashley et al. (2004) and Niemira and Fan (2005) remain areas for further research. More positively it has been found that irradiation becomes more acceptable to consumers the more informed they are of the benefits of irradiation (Teisl et al., 2009).

Combined technologies (or hurdle technology) have been used to improve log reduction. Their success is variable and, for the combinations reported (Foley et al., 2004; Garcia et al., 2003; Nou & Lou, 2010), does not provide log reductions seen in that of irradiation or ozone. Garcia et al. (2003) combined chlorine and ozone technologies and demonstrated no benefit to the use of chlorine in isolation.

Electrostatic application of sanitizers to cut produce is a new and emerging technology with little published research. Ganesh et al. (2010) found log reductions exceeding that of chlorine. This is technology that could easily be transferred to an industrial setting as the process is similar to that of chlorine washing. Further work on efficacy and practicality is needed.

Gopal et al. (2010) investigated the use of silver and hydrogen peroxide, which is technology that has been successful in water treatment. As this is emerging technology, further research needs to be undertaken into the toxicology of the residues left on the surface. Also, little is known about consumer acceptability of the process and this would need to be determined.

Since many of the technologies studied originate in water treatment, it is logical to look at new developments in water treatment for options that may also work on produce. A further emerging technology for water treatment is the use of ultrasound, although this technique has not yet been used for produce treatment. This technology has been found to have germicidal effects in the treatment of water, suggesting that this is a potential area of future research for fresh produce decontamination (Mahvi, 2009; Mahvi, Maleki, Rezaee, & Safari, 2009).

Of the new and emerging technologies reviewed in this paper. Further research is needed to understand how these technologies can be adapted for industrial use, from both a practical point of view and from the organoleptic aspect. All of the technologies reviewed gave similar results to chlorine (within one log). None of the new or emerging technologies reviewed were able to eliminate the presence of pathogens.

For the research examined for this paper, all of the fresh produce samples were artificially inoculated. The levels of pathogens on the inoculated samples are not at levels routinely found, as described by De Giusti et al. (2010) where *E. coli* was found to be at levels of <10 cfu/g to 8.0×10^1 cfu/g, and *Listeria* and *E. coli* 0157:H7 was not found in 964 samples of fresh produce. Factors such as growth of spoilage bacteria naturally on fresh produce surfaces and the effect this has on pathogen multiplication through the shelf life were not considered. The effect of biofilm production on the surface is an influencing factor that can affect the efficacy of decontamination and this was not investigated fully in the research identified. These areas all deserve further attention in the understanding of produce decontamination efficacy.

The organoleptic and shelf life factors are areas where there was little consideration given in any of the research. If decontamination technology is to be successful, this vital step must be considered, along with practicality in the industrial setting.

Understanding of consumer acceptability of decontamination technology must be developed further. Irradiation has been viewed negatively by consumer and consumer groups and has received much attention through the media. Similarly, there have been occupational health concerns raised for the use of chlorine as a disinfectant.

5. Conclusion

There has been much research into fresh cut produce decontamination. Many studies have focussed on finding an alternative to chlorine, which remains the most popular method used by the fresh produce industry for decontamination. Much published research focuses on pathogen reduction in isolation. Practical application of technology for industry such as, for example, process time, water usage and energy consumption, has not been considered. The impact on shelf life, product quality and consumer acceptability of technology is also not addressed. These factors must be taken into consideration for further developments for decontamination technology.

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