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Review

Fresh-cut product sanitation and wash water disinfection: Problems and solutions

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ABSTRACT

It is well known that fresh-cut processors usually rely on wash water sanitizers to reduce microbial counts in order to maintain quality and extend shelf-life of the end product. Water is a useful tool for reducing potential contamination but it can also transfer pathogenic microorganisms. Washing with sanitizers is important in fresh-cut produce hygiene, particularly removing soil and debris, but especially in water disinfection to avoid cross-contamination between clean and contaminated product. Most of the sanitizing solutions induce higher microbial reduction after washing when compared to water washing, but after storage, epiphytic microorganisms grow rapidly, reaching similar levels. In fact, despite the general idea that sanitizers are used to reduce the microbial population on the produce, their main effect is maintaining the microbial quality of the water. The use of potable water instead of water containing chemical disinfection agents for washing fresh-cut vegetables is being advocated in some European countries. However, the problems of using an inadequate sanitizer or even none are considered in this manuscript. The need for a standardized approach to evaluate and compare the efficiency of sanitizing agents is also presented. Most new alternative techniques accentuate the problems with chlorine suggesting that the industry should move away from this traditional disinfection agent. However, the use of chlorine based sanitizers are presented as belonging to the most effective and efficient sanitizers when adequate doses are used. In this review improvements in water disinfection and sanitation strategies, including a shower pre-washing step and a final rinse of the produce, are suggested.

Contents

1. Introduction

Fresh-cut fruits and vegetables are no longer considered low risk in terms of food safety (Bhagwat, 2006; FSA, 2007). Recently, a number of outbreaks have been traced to fresh-cut fruits and vegetables that were processed under less than sanitary conditions. These outbreaks show that the quality of the water used for washing and chilling the produce after harvest is critical (CDC, 2009). It is well known that disinfection is one of the most critical processing steps in fresh-cut vegetable production, affecting the quality, safety and shelf-life of the end product. Washing is designed to remove dirt, pesticide residues and microorganisms responsible for quality loss, as well as to pre-cool cut produce and remove cell exudates that may support microbial growth (Zagory, 1999). The fresh-cut industry has used chlorine as one of the most effective sanitizers to assure the safety of their product. However, there is a trend in eliminating chlorine from the disinfection process because of the concerns about its efficacy on the produce and about the environmental and health risks associated with the formation of carcinogenic halogenated disinfection by-products (Ölmez and Kretzschmar, 2009). Most of the current investigations have been focused on the search for alternative sanitizers based on assuring the quality and safety of the produce.

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However, the majority of the experiments are carried out in unrealistic conditions and the results obtained cannot be compared because of the differing experimental conditions. This review examines the need for a global approach to decontamination strategies in industry to identify solutions for the safety of produce. Additionally, the arguments for or against chlorine derived products are presented considering the sanitation of both the produce and the process water.

2. Fresh-cut product sanitation

In the last few years an important number of papers has been published concerning the efficacy of washing and sanitizing treatments in reducing microbial populations on fresh-cut produce. Some of the results are useless because of the extreme doses and excessive washing times used, the use of an unauthorized substance, e.g. (Zhang et al., 2009). A clear and well-documented comparison of different sanitation methods was compiled in the Food Safety Guidelines for the Fresh-cut Produce Industry (IFPA, 2001) and throughout the Forum on Washing and Decontamination of Fresh Produce (CCFRA, 2002–2008). The efficiency of numerous chemical and physical methods for assuring the microbiological safety of fresh-cut produce has been covered in several reviews (Parish et al., 2003; Sapers, 2003, Allende et al., 2006; Rico et al., 2007; Gómez-López et al., 2009; Ölmez and Kretzschmar, 2009).

Physical methods are effective at removing bacteria from plant surfaces by use of shear forces (Cutler, 2002). Modern aeration 'jacuzzi' washers reduce the bacterial loads on vegetables by between 1 and 2 log units. It should be consider that these reductions were obtained in lab experiments, but they are usually less evident in real processing conditions. Other physical methods include ultrasound, high pressure (HP), high-intensity electric field pulses (HELP), ultraviolet radiation (UV), radio frequency (RF) and ionizing radiation. All of these methods have been shown to be capable of killing or inhibiting bacterial growth. Ultrasound kills by intracellular cavitation but has problems in the presence of solids. It may, however, be useful to combine this technology with other methods such as aqueous ClO_2. Between 2.5 and 4.3 log reductions in *Salmonella* and *E. coli* O157:H7 counts on apples were achieved by combined ultrasound (170 kHz) and ClO_2 (20–40 mg/l) treatments depending on the exposure time (Huang et al., 2006). UV is also a promising technology but its antimicrobial efficacy can be influenced by product composition and soluble solid content of the process water (Selma et al., 2008a). Its application to a re-circulating water stream maintains the water at a reasonable bacteriological quality, but has no effect at all on surfaces either of the process machinery or on the product itself (EHEDG, 2007). As pathogens can survive for relatively long times in water, they can subsequently contaminate the product that passes through it before microbial inactivation with UV occurs. In addition, the efficacy of UV light systems as a wash water disinfectant is significantly impacted by turbidity due to the limited penetration capacity of UV, requiring filtration systems to eliminate suspended solids and absorbing compounds. New UV advanced disinfection technology systems result in a more efficient disinfection as they increase the amount of water that passes close to the UV lamp (Milly et al., 2007). The use of RF is technologically complex and rapidly raises the internal temperature of produce to be disinfected. Ionizing radiation has been shown to greatly reduce potential microbiological risk without damaging the texture/colour of the produce and does not lead to nutrient losses or have an adverse effect on the nutritional status (Niemira et al., 2003; Bari et al., 2004; Dhokane et al., 2006; Mintier and Folley, 2006). However, the long-term consumption of irradiated produce remains a cause of concern to the general public. In August 2008, U.S. Food & Drug Administration (FDA, 2008) gave its approval to use irradiation for killing pathogens on iceberg lettuce and spinach. The move comes in response to a petition filed by The National Food Processors Association, a trade group representing major food companies. Food irradiation uses high-energy Gamma rays, electron beams, or X-rays. Irradiation may be better than most technologies in penetrating fresh produce and it could be a powerful tool if used correctly in different produce items and among different varieties. The technology is publicized as the only solution for destroying internalized pathogens without cooking. In a recent study, Romaine lettuce and baby spinach were immersed in an *E. coli* O157 inoculum solution and vacuum perfusion to internalize the *E. coli* O157 (Niemira, 2008). This study showed that irradiation was effective in reducing *E. coli* O157 on lettuce and spinach, but the obtained reduction was dependent on the leaf type.

Chemical methods of cleaning and sanitizing produce surfaces usually involve the application of mechanical washing in the presence of sanitizers, followed by rinsing with potable water (Artés and Allende, 2005). A wide variety of chemical sanitizers have been tested with various degrees of effectiveness. Table 1 shows a review of the literature over the last 7 years on chlorine and alternative decontamination procedures to reduce pathogens and spoilage microorganisms on fresh-cut produce. Most of these studies examined their effect against pathogenic bacteria immediately after washing and only a few of them studied pathogen survival during storage. In general, microbial and visual quality of the washed product was evaluated and few studies examined water characteristics after treatment (Table 1).

Electrolysed Oxidising Water (EOW) has been shown to be a promising alternative decontamination technique with a strong bactericidal effect. This technique has been suggested as a valuable disinfection tool for wash water sanitation in the minimally processed vegetable industry (Ongeng et al., 2006). As an example, Ecodis® technology, based on the principles of anodic oxidation, consists in a highly efficient electrolysis cell equipped with coated permanent titanium electrodes. A direct low-voltage current passing across the electrodes causes the formation of potent oxidising agents principally derived from oxygen, as well as free chlorine when chloride ions are present in the solution. The oxygen and chloride radicals react with each other to form "free oxidants" such as hypochlorous acid (HOCl) and the hypochlorite ion (OCl^-). This technology differs from other physical decontamination technologies in that next to the direct decontamination, a residual disinfection capacity is also generated.

The combination of physical and chemical methods for washing fresh-cut vegetable produce is a useful way forward. The advanced oxidation processes (AOPs) represent the newest development in sanitizing technology, where two or more oxidants are used simultaneously (Selma et al., 2008a). The result is the on-site destruction of even refractory organics without the generation of residues. This is the case of the use of UV and hydrogen peroxide (H_2O_2) for decontaminating fresh produce (Hadjok et al., 2008). Samples of iceberg lettuce were inoculated with *E. coli* O157 and then sprayed with H_2O_2 and subjected to UV light. The same authors observed greater reductions achieved with UV/H_2O_2 treatments than with 300 mg/l chlorine for a range of products including Romaine lettuce, spinach, cauliflower, broccoli, Spanish onion and tomato (Hadjok et al., 2008).

Most of the available literature regarding the use of sanitizers has concluded that washing with water or with disinfectant solutions reduces the natural microbial populations on the surface of the produce by only 2 to 3 log units (Beuchat et al., 2004; Gonzalez et al., 2004; Inatsu et al., 2005; Ukuku et al., 2005; Allende et al., 2007; Gómez-López et al., 2007; Selma et al., 2008b). It was observed that, despite the initial differences, the total bacterial counts after storage were similar when the produce was washed with tap water or when a sanitizing solution was used (Allende et al., 2008a). Some authors have even suggested that washing with antimicrobial solutions initially reduces inoculated strains and the initial total mesophilic population, but they could increase more rapidly and even exceed the level on the water-washed counterpart during extended storage (Park and Lee, 1995; Francis and O'Beirne, 2002; Gonzalez et al., 2004; Beltrán et al., 2005; Gómez-López et al., 2007). The limitations of

Table 1
Experimental procedures used for the evaluation of the efficacy of sanitizing agents on produce and process water.

Experimental procedures	Sanitizer treatment/storage conditions	Objectives	Microorganism	Product	Reference
Produce after washing	-Calcium hypochlorite (1900–18,000 mg/l) for 5, 10, 15 and 20 min.	-Efficacy of chlorine at different temperatures and pH.	*E. coli* O157:H7 and *Salmonella* spp	Alfalfa seeds	Fett (2002a)
	-Calcium hypochlorite (1900–18,000 mg/l) for 5, 10 and 15 min.	-Efficacy of chlorine at different pH.	*E. coli* O157:H7 and *Salmonella* spp	Mung bean seeds	Fett (2002b)
	-Chlorine dioxide (5, 7.5 and 10 mg/l) for 10, 30 and 60 min.	-Efficacy of chlorine dioxide.	Hepatitis A	Process wash water	Li et al. (2002)
	-Lactic acid (15 g/l) and hydrogen peroxide (H_2O_2) (15 g/l) for 15 min; lactic acid (15 g/l) and H_2O_2 (20 g/l) for 5 min; H_2O_2 (20 g/l) for 60 or 90 s.	-Synergetic effect of heat, lactic acid and H_2O_2.	*E. coli* O157:H7, *Salmonella* spp and *L. monocytogenes*	Lettuce	Lin et al. (2002)
	-Chlorine (25–200 mg/l) for 5 min.	-Efficacy of chlorine at different pH.	Natural microflora	Artichokes and borages	Sanz et al. (2002)
	-Chlorine (100 mg/l) and peroxyacetic acid (80 mg/l) for 30 s.	-Comparison of efficiencies using chlorine and peroxyacetic under simulated commercial processing conditions.	*L. monocytogenes*	Iceberg and romaine lettuce	Beuchat et al. (2004)
	-Irradiation (0.56, 1.05, 1.15 and 1.40 kGy) and chlorine (200 mg/l) for 1 min.	-Synergetic effect of chlorination and irradiation.	*E. coli* O 157:H7	Cilantro	Foley et al. (2004)
	-Citric acid (1000 mg/l), sodium hypochlorite (100 mg/l) with and without acidification (citric acid: 1000 mg/l), sodium chlorite (500 and 1000 mg/l) with and without acidification (citric acid: 1000 and 10000 mg/l and succinic, malic, tartaric, acetic, lactic and propionic acids at 5 mM) for 15 min.	-Comparison of efficiencies using sodium hypochlorite and sodium chlorite with and without acidification.	*E. coli* O 157:H7	Chinese cabbage leaves	Inatsu et al. (2005)
	-Ozonated water (1, 3, 5 mg/l) for 0.5, 1, 3, 5 min and ozonated water (3 mg/l) combined with organic acids (acetic, lactic and citric acids) (10 g/l) for 1 min.	-Synergetic effect of ozone and organic acids.	*E. coli* O157:H7 and *L. monocytogenes*	Lettuce	Yuk et al. (2006)
	-Chlorine (100 mg/l) and lactic, citric, acetic and ascorbic acids (5 and 10 g/l) for 2 and 5 min.	-Comparison of efficiencies using organic acids and chlorine.	*E. coli* and *L. monocytogenes*	Iceberg lettuce	Akbas and Ölmez (2007)
	-Acidified sodium chlorite (1200 mg/l), chlorine (50 mg/l) and acidic electrolysed water (30–35 mg/l) for 60 s and 90 s.	-Comparison of efficiencies using chlorine, acidified sodium chlorite and acidic electrolysed water.	*E. coli* O157:H7, *Salmonella* spp and *L. monocytogenes*	Leafy greens	Stopforth et al. (2008)
	-Sodium hypochlorite (200 mg/l), sodium chlorite (1000 mg/l), acidified sodium chlorite (100, 250, 500 and 1000 mg/l) and citric acid (6000 mg/l) for 1 min.	-Comparison of efficiencies using sodium hypochlorite, sodium chlorite with and without acidification, and citric acid.	*E. coli* O157:H7, total plate count and yeasts and moulds	Fresh-cut cilantro	Allende et al. (2009)
	-Sodium hypochlorite (300 and 600 mg/l) for 3 min, and irradiation doses of 0.25–1.5 kGy at a rate of 0.098 kGy/min.	-Comparison of efficiencies using sodium hypochlorite and irradiation.	*E. coli* O157:H7	Lettuce varieties	Niemira (2008)
	-Combined treatment of UV/H_2O_2 (UV at 0.63 mW/cm^2 for 60 s and H_2O_2 at 1.5% v/v sprayed at a rate of 480 ml/min for 60 s) and chlorine (200 mg/L) for 3 min.	-Comparison of efficiencies using the combined treatment of UV and H_2O_2 at 50 °C and chlorine.	*E. coli* O157, *Salmonella* spp and *Pseudomonas fluorescens*	Iceberg and romaine lettuces, spinach, cauliflower, broccoli, Spanish onion and tomato	Hadjok et al. (2008)
Produce after washing and during storage	-Chlorous acid (268 mg/l), sodium hypochlorite (200 mg/l) and lactic acid (20 g/l). Product stored at 5 °C for 9 days.	-Comparison of efficiencies using chlorous acid, sodium hypochlorite and lactic acid.	*Salmonella Typhimurium* and *L. monocytogenes*	Mung bean sprouts	Lee et al. (2002)
	-Chlorine (100 mg/l), citric acid (10 g/l) and ascorbic acid (10 g/l) for 5 min. Product stored for 14 days at 3 and 8 °C.	-Comparison of efficiencies using chlorine, citric and ascorbic acids.	*L. innocua* and *E. coli*	Lettuce and coleslaw mix	Francis and O'Beirne (2002)
	-Anolyte water (electrolysed sodium chloride salt at 50 g/l) for 5, 10 and 20 min and chlorine (100 mg/l) for 20 min. Product stored for 16 days at 1 °C.	-Comparison of efficiencies using anolyte water and chlorine.	Total plate counts, coliforms, yeast and moulds.	Carrots	Workneh et al. (2003)
	-Chlorinated water (10, 100, 200 mg/l), hydrogen peroxide (10, 20, 30 ml/l), peroxyacetic acid (40, 60, 80 mg/l) for 2 min and sodium bicarbonate (1, 5, 10%) for 5 min. Product stored for 21 days at 4, 25 and 37 °C.	-Comparison of efficiencies using chlorine, hydrogen peroxide, peroxyacetic acid and sodium bicarbonate.	*E. coli* and F-specific coliphage MS2	Lettuce and cabbage	Allwood et al. (2004)
	-Warm (48 °C) chlorinated water (100 mg/l) for 30 s followed by cold chlorinated water (100 mg/l) for 25 s. Product stored for 18 days at 4 °C.	-Synergetic effect of chlorine and heat under pilot scale wash flume.	Natural microflora	Lettuce	McKellar et al. (2004)
	-Sodium hypochlorite (200 mg/l) and acidified sodium chlorite (100, 250, 500 and 1,000 mg/l). Product stored for 14 days at 5 °C.	-Comparison of efficiencies using sodium hypochlorite and acidified sodium chlorite.	*E. coli* O157:H7 and natural microflora	Shredded carrot	Allende et al. (2008c)

(continued on next page)

Table 1 (*continued*)

Experimental procedures	Sanitizer treatment/storage conditions	Objectives	Microorganism	Product	Reference
Produce after washing and process water	-Ozonated water (0.5, 1, 1.6, 2, 2.2 and 5 mg/l) for 0–60 s and ozone (2 mg/l) activated with UV-C for 0–5 min.	-Comparison of efficiencies using ozone and ozone activated with UV-C and the synergetic effect of the combination of both treatments	*Shigella sonnei*	Lettuce	Selma et al. (2007)
	-Ozonated water (3 mg/l), chlorine dioxide (3 and 5 mg/l), peroxyacetic acid (80 mg/l) and chlorinated trisodium phosphate (100 and 200 mg/l chlorine) for 0–5 min. Product stored for 9 days at 4 °C.	-Comparison of efficiencies using ozone, chlorine dioxide, peroxyacetic acid and chlorinated trisodium phosphate.	*L. monocytogenes* and *E. coli* O157:H7	Apples, lettuce, strawberries and cantaloupe	Rodgers et al. (2004)
	-Chlorine (200 mg/l), citric acid-based sanitizer (10 g/l), peroxyacetic acid (80 mg/l) and acidified sodium chlorite (1000 mg/l) for 2 min. Product stored for 14 days at 5 °C.	-Comparison of efficiencies using chlorine, citric acid, peroxyacetic acid and acidified sodium chlorite under simulated process water conditions	*E. coli* O157:H7, total viable count, yeast and moulds	Fresh-cut carrots	Gonzalez et al. (2004)
	-Sodium hypochlorite (100 mg/l) for 1 min. Product stored for 14 days at 5 °C.	-Efficacy of chlorine in process water containing different concentrations of organic matter.	Natural microflora	Fresh-cut romaine lettuce	Luo (2007)
	-Chlorine (0, 0.1, 0.2, 0.25, 0.3, 0.4, and 0.5 mg/l) for 0, 10, 20, 40, 60, 90, and 120 min, lactic acid (10 and 20 g/l) for 1, 5, 15, and 30 min and 24 h. Carrots stored for 7 days at 4 °C and 5 days at 7 °C. Peppers stored for 6 days at 7 °C.	-Comparison of efficiencies using chlorine, lactic acid and thyme essential oil.	*Enterobacteriaceae* and *Aeromonas* spp	Grated carrots, mixed lettuce, chopped bell peppers	Uyttendaele et al. (2004)

postharvest sanitizing washing in removing contamination are well established and the microbiological quality of the raw produce entering processing is, therefore, of great importance (Warriner, 2002). To date it is thought that biofilms are responsible for limiting the efficacy of sanitizing step. It has also been suggested that higher reductions are not achieved in practice due to the ability of microorganisms to attach strongly on the surface of the produce due to embedding of the cells into inaccessible parts of irregular surfaces (Ölmez and Kretzschmar, 2009). Bacteria tend to concentrate where there are more binding sites (Sapers, 2001; Parish et al., 2003). Attachment can also occur in stomata (Seo and Frank, 1999), indentations, or other natural irregularities on the intact surface (Fig. 1). Bacteria can also attach at cut surfaces (Takeuchi and Frank, 2000; Liao and Sapers, 2000) or in punctures or cracks in the external surface (Burnett et al., 2000). Bacteria can also be protected from inactivation by being internalized within growing plant tissue (Sapers, 2001). Studies using inoculated seed types demonstrated that *E. coli* could be recovered from the outer surface of 30-day old lettuce and coriander plants but not from watercress or celery (Warriner, 2002).

Relatively little work has been published regarding the effectiveness of decontamination treatments against viruses. Seymour and Appleton (2001) have previously reported that viruses are relatively resistant to chlorine decontamination. According to them, other studies on the effect of sanitizers on the survival of viruses have demonstrated that they are more resistant than bacteria (Allwood et al., 2004).

3. Wash water disinfection

The importance of the maintenance of water quality during washing has attracted much attention as it is now specified that "antimicrobial chemicals, when used appropriately with adequate water quality, help to minimize the potential microbial contamination of processing water and subsequent cross contamination of the product" (FDA, 2008). Many of the most recent findings about fresh-cut washing agree with this approach (Allende et al., 2008b; López-Gálvez et al., 2009). It is assumed that if produce is washed without the use of sanitizers, large quantities of water are required to achieve the same level of microbial reduction.

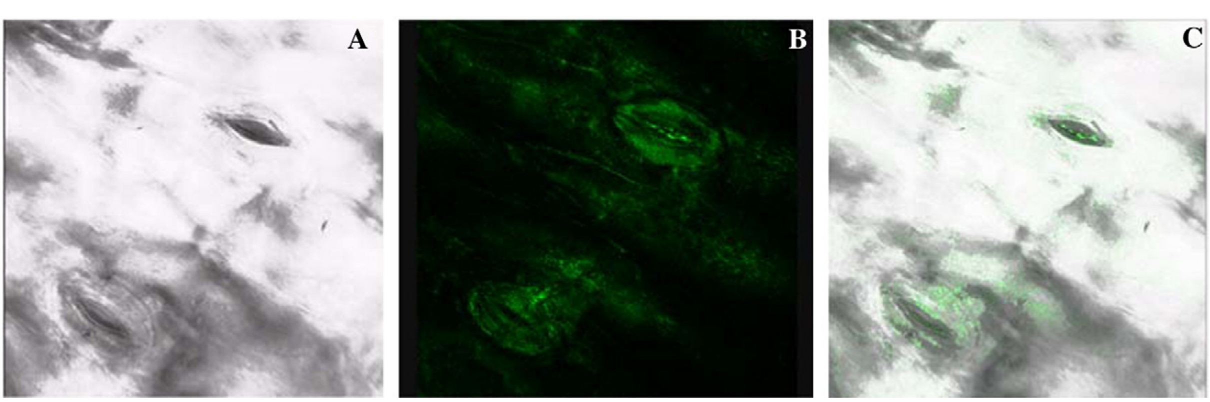

Fig. 1. Confocal micrographs showing stomata of lettuce tissue (A), distribution of GFP-labelled *E. coli* strain (B) and green fluorescent protein (GFP)-labelled *E. coli* on lettuce stomata (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

However, it should be clearly stated that water serves as a source of cross-contamination as re-using processing water may result in the build-up of microbial loads, including undesirable pathogens from the crop. Thus, sanitizing agents should be used to maintain the quality of the water and prevent cross-contamination of the product, in spite of their limited direct microbial benefit on the produce. In general, it could be assumed that the cleaning action of the washing process removes microorganisms from the product and the sanitizing agent eliminates them in suspension.

Washing and disinfection have economic and environmental implications mainly because of the large amount of water needed to assure that the water quality is adequate for its intended use, both at the start and the end of all washing processes. One challenge for the food industry is the minimization of water consumption and wastewater discharge rates (Ölmez and Kretzschmar, 2009). One method used to reduce water usage is to disinfect the water with a suitable sanitizer. The amount of wastewater generated per unit mass of product is dependent on the disinfection technique employed. A technique capable of disinfecting efficiently both the process water and the product would allow a high ratio of recycling and thereby would reduce the wastewater rates and would have less impact on the environment (Ölmez and Kretzschmar, 2009).

In a recent study, it was observed that the quality of the process water impacted the effectiveness of washing (Allende et al., 2008b). It was also confirmed that a low amount of contaminated product in a batch impacted the safety of the entire lot that passed through the washing tank. The risk of cross-contamination was not eliminated by using large quantities of water (López-Gálvez et al., 2009). This confirms the importance of using a sanitizing agent in the process water to kill microbes before they attach or become internalized in the produce, avoiding cross-contamination. Nevertheless, water treatment should be managed with the goal of minimizing the effective dose of sanitizer used for microbial disinfection (Suslow, 2001).

4. Evaluation of the efficacy of disinfection technologies

Evaluation of the disinfection efficacy of different technologies is greatly affected by several factors such as physicochemical properties of process wash water and the type of produce (Table 2). Additionally, the methods used to apply the decontamination treatments and the characteristics of the procedure (duration, washing sequence, e.g.) also affect the recovery of native microbiota and pathogenic microorganisms (Pirovani et al., 2004; Ukuku and Fett, 2004). It is also difficult to compare decontamination agents because of the differences in the inoculation procedure, drying times prior to washing and the method of detection with special emphasis in the detection limit (Beuchat et al., 2001; Singh et al., 2002; Beuchat et al., 2004; Burnett et al., 2004; Lang et al., 2004) (Table 2). Moreover, the recovery of the inoculum will depend on the number of times the produce is washed in the sanitizing solutions. For all treatments, dip inoculation method showed the greatest reduction in numbers of microorganisms, followed by spot and then by spray inoculation (Lang et al., 2004). The effectiveness of washing decreases if the time interval between the contamination event and washing is increased (Sapers, 2001). The efficacy of the disinfection treatments was also dependent on the type of vegetable, the characteristics of the produce surfaces (cracks, crevices, hydrophobic tendency and texture) and even the tissue location (inner and outer leaves) (Kondo et al., 2006). As an example, some disinfection treatments on inoculated strains of *Listeria innocua* and *E. coli* were more effective on lettuce than on coleslaw (Francis and O'Beirne, 2002) and broccoli (Behrsing et al., 2000). Tremendous variation in weight/surface area (g/cm^2) has been remarked among various types of products such as lettuce (two-sided plane) and tomato (sphere) (Beuchat et al., 2001). These authors described that a decontamination process designed to achieve, for example, a 3-log reduction in CFU/g of lettuce or tomato would result, respectively, in approximately 0.114- and 18-log reductions in CFU/cm^2.

Table 2
Factors that affect the experimental procedures of decontamination treatments.

Factors	Factors
Water quality	pH
	Temperature
	Turbidity
	Organic matter
Sanitizer	Concentration
	Contact time
Decontamination treatment	Application method (dipping, spraying and agitated, rubbed or static condition during exposure)
	Produce/water ratio
	Single or multiple batches
	Rinse after sanitation
	Multiple washings
Target microorganisms	Choice of microbial strain
	Single strain or a cocktail of strains
	Physiological states of the bacterial cells
	Natural or inoculated microorganisms
	Population size
Inoculation procedure	Inoculation method (dip, spray or spot inoculum)
	Incubation time (time of attachment prior to washing)
Method of detection	Detection and enumeration media
	Confirmation procedures
Produce	Type of vegetable
	Characteristics of the product surfaces (cracks, crevices, hydrophobic tendency and texture)
	Inner and outer leaves
	Relation weight and surface area
Time interval	Time between contamination and washing

In most of the cases, the concept of a highly efficacious disinfection strategy is supported by laboratory studies where various sanitizing agents and methods are used, yielding impressive results (Fonseca, 2006). An often forgotten aspect is the use of simulated commercial processing conditions as significant differences are found when the sanitizing agents are assessed at a lab, pilot or factory scale (Sapers, 2001; Beuchat et al., 2004). In general, the majority of the research studies concerning the evaluation of sanitizing agents on the reduction of pathogenic microorganisms during washing do not take into account the presence of organic matter (Allende et al., 2007; Beuchat et al., 2004; Stopforth et al., 2008). In fact, wash water quality deteriorates rapidly during produce washing and it usually contains high organic loads including soil, leaves, and other debris as well as microorganisms associated with the produce (Allende et al., 2008a). When potable water is used to evaluate different sanitizing agents, it might lead to unrealistic results with no practical applications. Further studies should be conducted in real situations in which the water consumption and wastewater discharge rates are reduced as well as the quality of the produce should be evaluated after storage. The lack of a standardized methodology and validation procedure makes it difficult to select the most adequate sanitizing strategies for the disinfection of fresh-cut produce.

5. Legislation of substances used for produce decontamination

The regulation of substances that are used to reduce the microbial load of fresh fruits and vegetables is complex and in some areas uncertain. In each country, the regulatory status of sanitizing solutions is different. The definition of the product used to disinfect wash water depends on 1) the type of product to be washed, and in some cases, 2) to the location where the disinfectant is used (IFPA, 2001). In the USA, the wash water disinfectants used for fresh-cut produce are regulated by the FDA as a secondary direct food additive, unless they are considered to be Generally Recognized As Safe (GRAS). If the product is a raw agricultural commodity that is washed in a food processing facility, such as a fresh-cut facility, both the EPA (Environmental Protection Agency) and the FDA have regulatory jurisdiction and the

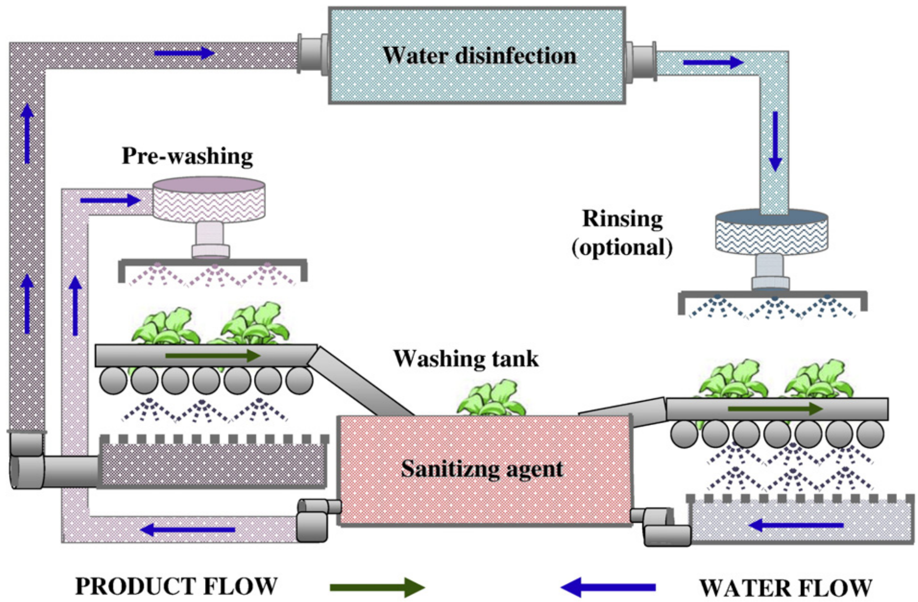

Fig. 2. Efficient water disinfection process with a re-circulation system proposed as an alternative for traditional fresh-cut washing.

disinfectant products must be registered as pesticides with the EPA. A selected list of wash water disinfectants and sanitizing solutions approved by the FDA is reported in the Code of Federal Regulations 21 CFR. Sections: 173.315 and 178.1010 (FDA, 2003a,b).

Hammond (2004) outlined the situation in Europe and made some considerations on changes that may be forthcoming. The *European Council Directive (89/107/EEC)*, on food additives comprises the lists of substances which may legally be added to food if they perform a useful purpose, are safe and do not mislead the consumer. The detailed controls made under the Framework Directive are implemented into the national law of each EU member state and stipulate which food additives are permitted for use, the specific purity criteria and conditions of use, including maximum levels for specific additives. There are opportunities to use other substances for produce decontamination, providing that function as '*processing aids*' which are defined as: 'any substance not consumed as a food itself, intentionally used in the processing of raw materials, foods or their ingredients to fulfil a certain technological purpose during treatment and processing and which may result in the *unintentional* but technically *unavoidable presence of residues* of the substance or its derivatives in the final product, *provided that these residues do not present any health risk and do not have any technological effect on the finished product*'. Chlorine and chlorine dioxide used for fruit and vegetable washing would normally be regarded as "processing aids". Thus, they would appear to be outside the scope of the biocide controls because they are "defined" in the Directive 89/107/EEC.

Whether a wash water chemical is an additive or processing aid is of great importance, since it is unlikely that the consumer will accept a 'natural' agricultural product (such as leafy salad) which carries the name of a chemical additive on the label. Therefore, in practice, wash water decontaminants must be able to be classed as processing aids, which requires they have no lasting technological effect on the produce, a key challenge for the chemical sciences (RSC, 2009). The European Commission is planning to develop more detailed regulations governing the use of processing aids. Although at a very early stage of development, one possibility being considered is that the definition of a processing aid will be tightened, so that residues of processing aids in a final food will no longer be acceptable, unless the substance in question is specifically authorized for food use. Legislation on processing aids is not yet harmonised at European Community level, and so processing aids that may legally be used in some European countries like the UK and France are not permitted in other member states. A global approach to processing aids is needed to control the agents which are essential for the minimization of the potential transmission of pathogens from water source to produce. The risk is not eliminated by using large quantities of water; the risk of pathogen cross-contamination is only avoided using processing aids.

6. Problems with the use of chlorine and their solutions

The efficiency of chlorine and chlorine based derivatives, providing adequate water disinfecting capabilities, has been well proven over the past 30 years (Suslow, 1997, 2001; IPFA, 2001; Sapers 2003, 2005; Gómez-López et al., 2008). The use of chlorinated water as a decontamination stage in the washing of fresh-cut produce is widespread throughout the fresh produce industry. Without chlorine, there probably would not be a market for fresh-cut salads and vegetables. Approximately 76% of respondents in an industry survey reported the use of hypochlorite (Seymour, 1999), although it was apparent that many of the important aspects of chlorine chemistry e.g. pH control, were not fully understood. As a consequence, many users of hypochlorite were not using it under optimum conditions and therefore not achieving maximum effectiveness. Most of the chemical companies offering alternative technologies to chlorine emphasise the negative reports showing the undesirable by-product residues and suggest that the industry is moving away from this traditional disinfection method. The sensitivity of detection of residues has also increased by orders of magnitude in the last 10 years; however, the fact that one can detect something does not mean that it is a major risk (Russell, 2005). Nevertheless, the results from toxicity studies do not indicate any cause for concert regarding safety with respect to the use of chlorine washes. Klaiber et al. (2005) determined that the by-product formation due to chlorination of minimally processed carrots with tap water containing 200 mg/l free chlorine was negligible (<0.2 µg/l). In the UK, a statement has been issued by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment regarding the commercial survey that investigated the occurrence of disinfectants and their by-products in prepared salads (COT, 2007). Chlorine based disinfectants can react with organic matter in water and form by-products like trihalomethanes, haloacetic acids, haloketones and chloropicrin. There is no published research investigating the formation of these halogenated compounds on fresh produce when washed in chlorinated water. Therefore, the Fresh Prepared Salads Producer Group undertook a study to analyse a range

Table 3
Parameters to monitor for assuring the optimum disinfection procedure in fresh-cut washing.

Parameter to monitor	On-line control
Adequate pre-wash	No
Water flow	Yes
Level of free oxidants	Yes
pH	Yes
Temperature	Yes
Turbidity	No
Chemical oxygen demand	No
Oxidation reduction potential	Yes
Conductivity	Yes
Rapid microbial detection method	Yes

of salads for the presence of disinfectant by-products on fresh produce. The conclusions were that produce subjected to typical chlorination processes contained less chlorine and chlorinated by-products than permissible in a glass of tap water and concluded that there is no cause for concern regarding the presence of chlorinated compounds on salads (COT, 2007). To achieve amounts of trihalomethane residues approaching toxicological limit values in typical hypochlorite-treated lettuce, one would have to consume many kilograms of such lettuce per day (Russell, 2005).

Chlorine has been very badly abused and over-used in the past to such an extent that its use could be prohibited if we do not control it properly. Suslow (1997) and Dawson (2002) provided background information on the use of chlorine and the best practice for chlorine-based washing of fresh fruits and vegetables. They explain the significance of pH control for efficient use of chlorine and the measurement of different forms of chlorine, i.e. total, combined and free chlorine. The effect of introducing organic matter into the chlorine-based washing system is also covered. In general, microbial reduction increases as both initial chlorine concentration and water-to-product ratio increases (Pirovani et al., 2004). However, washing time has no effect on microbial reduction as increasing the washing period above 1 or 2 min showed no improvement on the reduction of the bacteria (Adams et al., 1989; Beuchat et al., 1998). It was also demonstrated that a low level of free chlorine could be used for fresh produce washing, maintaining this level using a controlled dosing system. In fact, the most effective disinfection systems are those where a specific level of residual is maintained at the outlet of the washer, which is in fact after the disinfection has occurred. These systems ensure that there is always sufficient oxidant in solution to prevent microbial contamination and cross contamination. Chlorine specific sensors based on amperometric techniques, and pH sensors, ensure that residual levels are continuously monitored and controlled to give optimum disinfection conditions at all times.

There is a tremendous uncertainty if chlorine has to be replaced. It is widely thought that the majority of processed vegetables can achieve the desired shelf life in relation to microbiological level, if washed correctly with clean water. The market for minimal processors without chlorine could disappear or certainly shrink to the level of other European countries like Germany or Switzerland, where chlorine is banned (Stead, 2004).

7. Future trends in fresh-cut washing

An appropriate washing process must involve a shower as a pre washing step to remove dirt and cell exudates from the cut surfaces (Fig. 2). This step must be followed by the immersion of the product in a washing tank which contains the sanitizing agent (Fig. 2). A rinse step will be optional depending on the sanitizing agent. If a chlorine derivative agent is used at a concentration similar to that of the tap water, the rinsing could be suppressed. It is recommended that water flows in the opposite direction to the movement of produce through the different unit operations. Thus, water in the sanitizing tank could be re-circulated for use in the pre-washing step (Fig. 2). The same applies to the rinse water which could be incorporated into the sanitizing tank after the shower in a continuous process. Water disinfection remains an essential activity in the fresh-cut industry and is possible with an efficient disinfection strategy such as chlorine, ozone and AOPs in a re-circulated system (Fig. 2). Additionally, fresh-cut processors should include systems for the on-line monitorization of the process to maintain the control of the washing process whenever possible (Table 3).

In order to control the safety of the end product, it is desirable to measure the microbial quality of the water to detect potential pathogens, not only the presence of viable bacterial cells but also the non viable cells in the washing tank. If a contaminated product passes throughout the washing tank, the pathogens can be detected in the water, and then the commercialization of the product can be stopped before distribution to the market. Traditional culture-based methods for pathogen detection in foods are time consuming and limited by their poor sensitivity and specificity, frequently leading to uncertain identification results. As an alternative, rapid nucleic acid amplification and detection technologies have rapidly been replacing the more traditional assays; in fact, the polymerase chain reaction (PCR) and more recently real-time PCR (RTi-PCR) are increasingly applied techniques for pathogen detection.

8. Conclusions

The combination of physical and chemical treatments for washing fresh-cut produce seems to be a very promising tool to reduce microbial risk. Sanitizing agents significantly reduce initial microbial loads but result in enhanced survival and/or growth during storage. The maintenance of the quality of the process water is very important as it might serve as a source of cross-contamination. In fact, the main effect of sanitizing treatments for washing fresh-cut produce is to reduce the microbial load and keep process water free from contamination rather than having a preservative effect. A standardized experimental approach to study the efficacy of different sanitizing treatments is needed considering as much as possible the commercial processing conditions. Most of the studies on disinfection agents for the fresh-cut food industry have been focused on alternative disinfection treatments to chlorine because of its excessive use (hyperchlorination), which causes several environmental and human health effects. Considering the advantages and the disadvantages of the use of chlorine, this study shows that chlorine derived products have a greater potential for the disinfection of vegetables when organic matter is eliminated. Modern produce washers should be designed to assist with the disinfection process by incorporating different stages such as showers to remove fluids and exudates from cut surfaces before the disinfection. The last stage before packaging should be the disinfection step which requires very low doses of disinfecting agent to achieve good results. This wash can not be done with clean water without disinfectants because its inability to prevent cross contamination from one batch of product to the next. Regulations should be re-examined for a global harmonization of processing aids for water disinfection.

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