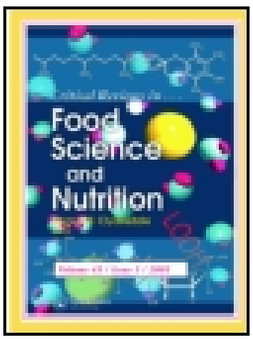

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Sanitization strategies of fresh produce and their limitations

Review: Comparison of the Effectiveness of Decontaminating Strategies for Fresh Fruits and Vegetables and Related Limitations

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Abstract

Given that it should be aware of the nutritional benefits, resulting from the consumption of fresh fruits and vegetables consumed raw and/or minimally processed, comparing the efficacy of different individual sanitizing methods against the major food-borne pathogens localized in fresh commodities is of much importance; these products are easily vulnerable to the microbial contamination. In this review, the current propensity of alternative sanitizing methods was introduced, as well as principal elements for deteriorating the cleaning effects were also discussed. Chlorine-based-sanitizers exhibited the microbial reduction of <1.12 $\log_{10}$ CFU/g on fruits and vegetables. Most of aqueous disinfectants showed ≤3.01 $\log_{10}$-redcutions against a variety of microorganisms inoculated on fresh commodities. Similarly, several physical technologies such as hydrostatic pressure and ultraviolet light were effective for reducing surviving bacterial cells could recover and grow rapidly during the whole processing, posing a potential risk of causing food-borne outbreaks associated with the fresh products. The invasion

and subsequent localization of the organisms into the inner parts of products, interactions between the microbial cell and food-contacting surfaces, as well as development of biofilms could restrict the antimicrobial activity of the currently used approaches.

Keywords

Decontamination, disinfectant, fresh produce, pathogen, sanitization

INTRODUCTION

Since consumers perceive fresh produce as being beneficial for maintaining their health, it has prompted an increase on the consumption of fresh produce worldwide. Consequently, however, the frequency of documented outbreaks associated with consumption of raw fruits and vegetables has increased in recent years (CDC 2012; KFDA 2013). Tomato, lettuce, apple, carrot, spinach, grape and others (Davis et al., 1988; Nguyen-the and Calin, 1994; Burnett and Beuchat, 2002; Rangel et al., 2005; Bhagat et al., 2010) have been reported as causative sources for contamination of these products with various foodborne pathogens. These pathogenic microorganisms isolated from raw vegetables or fruits included *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Shigella* spp., *Bacillus cereus*, *Clostridium* spp., *Campylobacter perfringens*, *Yersinia enterocolitica*, and *Staphylococcus aureus* (Shandera et al., 1983; Brocklehurst et al., 1987; Beuchat, 1996; Fain, 1996; Little et al., 1997; Flessa et al., 2005; Argudín et al., 2010). Generally speaking, fresh produce easily become contaminated with these pathogenic microorganisms by a wide variety of channels; fresh fruits and vegetables served as raw or undercooked may act as a vehicle for potentially microbial contaminations. Food handlers carrying pathogens in their body, soil contacting with animal feces, as well as irrigation water can become the primary route of pathogenic contamination of fresh produce (Beuchat 2002; Steele and Odumeru 2004).

In order to control the microbial behaviors occurred in fresh produce, the food industry has utilized commercial chlorine solutions to sanitize fruits and vegetables extensively. Traditionally, commercial chlorine sanitizers have been used in concentrations of ranging from 50 to 200 ppm (Beuchat, 1992; Brackett, 1992), as well as it is recommended for sanitizing sprouts to dipping

them with the levels of 20,000 ppm chlorine-based-sanitizers by FDA (1999). In spite of these efforts, however, chlorine treatment has exhibited only restrictive effects in reducing the population of pathogenic microbes contaminated on fresh produce; there have been several previous studies reporting ≤ 2 or 3 $\log_{10}$-reductions in the number of pathogens on fresh produce after treatment with chlorine sanitizers (Niemira, 2007; Neal et al., 2012). These results may be due to not only the potential ability of the pathogens to internalize within fresh produce, but also to produce biofilms, lowering the effectiveness of sanitizing process. The internally localized pathogens into the interior tissues of fresh produce are less likely to be removed after sanitization or cleaning, thereby posing a potential risk of causing food-borne illness to humans (Aruscavage et al., 2006). Especially, as microbial biofilms, which are known for the communities of microorganisms attached to a surface, may interrupt the interactive contact between organisms and sanitizers, resulting in the increasing survivals of these pathogens with high levels of pathogenicity on fresh produce (Burnett and Beuchat, 2001), these biofilms are recognized as a detrimental and undesirable substance in the food industry. Then, traditional chlorine treatments may be inconsistent with the appropriate control system of pathogenic contaminations on fresh produce. A number of studies have focused on the development of alternative sanitizing methods, including the use of a new sanitizing agent either alone or in combination with two or more agents, and the utilization of physical methods. All of these approaches applied have shown their own characteristics, but it is clear that their effectiveness may be markedly different, depending on the type of fresh fruits and vegetables involved, the strains of microorganism to be tested in a laboratory scale, as well as other factors. Therefore, scientifically based information should be required to better understand the microbial behaviors in order to facilitate a choice of the most

effective sanitization method to achieve high levels of food safety, regarding some distractions leading to decreasing efficacies of a most of sanitizing methods. The objective of the present study was to; (1) introduce various sanitizing methods, (2) evaluate/compare the effectiveness and efficacy of each sanitizing method, and (3) investigate some causes inhibiting antimicrobial activities of the sanitizing methods mentioned above.

USE OF CONVENTIONAL CHLORINE-BASED-SANITIZERS

In response to the recent public health concerns regarding whether fresh produce is being safe from contaminations by pathogenic and spoilage microorganisms, and how these products can be prevented from the prevalence of food-borne outbreaks, many previous studies have been performed to evaluate the efficacy of various sanitizing methods for reducing the microbial populations on surface of fresh produce before and after storage. A wide variety of chemical solutions with varying ranges of concentrations have been used to decontaminate fresh fruits and vegetables, and then these results were collected in the present manuscript. Table 1 shows a list of effectiveness of chlorine-based-sanitizers, including commercial chlorine, sodium hypochlorite, and calcium hypochlorite, against pathogenic and spoilage microorganisms on fresh produce. It is generally recognized that washing is the primarily fundamental procedure for gaining good hygiene practices in the fresh fruits and vegetables during the whole cleaning process, since it might physically detach all sorts of impurities (ca. soils and dusts) and microorganisms from the surface, and then dipping fresh produce in an aqueous solution containing one or more antimicrobial agents may ensure product safety (Ruiz-Cruz et al., 2007). As shown in Table 1, there were decreases of 1.3 and 1.8 $\log_{10}$ CFU/g in the number of *E. coli* O157:H7 inoculated in spinach and lettuce, respectively, after treatment with 600 ppm chlorine

for 3 min (Niemira and Cooke, 2010). Reductions in the levels of ≤ 1.0 $\log_{10}$ CFU/g were also observed when *E. coli* O157:H7 in spinach and lettuce was treated with at least 100 ppm chlorine for several minutes (Niemira, 2007; Hadjok et al., 2008; López-Gálvez et al., 2010; Neal et al., 2012). In addition, treatment of *E. coli* O157:H7 on spinach with 100 ppm chlorine for 5 min showed a < 1.9 $\log_{10}$-reduction (Lee and Bae, 2008; Nei et al., 2009). It has been shown that the use of chlorine-based-sanitizers with higher concentrations of 200-600 ppm did not yield more than 2.0-$\log_{10}$ reductions in eliminating *E. coli* O157:H7 on fresh produce. Similarly, it appeared that effectiveness of chlorine-based-solutions for sanitizing fresh produce was lower against *Salmonella* spp. Hadjok et al. (2008) observed declines in the number of *Salmonella* spp. on spinach, broccoli, onion, and tomato by 0.5, 0.3, 0.3, and 0.3 $\log_{10}$ CFU/g, respectively, after treatment with 200 ppm chlorine for 3 min. In the same manner, sanitizing these bacterial pathogens in spinach, sesame leaf, and cabbage with chlorine-based-sanitizers exhibited ≤ 0.9 $\log_{10}$-reductions in the microbial load (Yeon et al., 2005; Choi et al., 2008; Neal et al., 2012). Of importance, there were a few reports in which microbial reductions of more than 2.0 $\log_{10}$ CFU/g occurred after chlorine treatment of spinach and bell pepper (Yuk et al., 2006a; Rahman et al., 2010). The number of *Salmonella* spp. in bell pepper was decreased by 2.0 $\log_{10}$ CFU/g, followed by exposure to 200 ppm chlorine for 2 min. Dipping spinach in 100 ppm chlorine for 5 min also resulted in a 2.2 $\log_{10}$-reduction in the number of *L. monocytogenes*. In contrast, chlorine-based-sanitizers were effective in reducing coliform in fresh produce, by showing 2.0 $\log_{10}$-reductions on average (Yeon et al., 2005; Samadi et al., 2009; Tomás-Callejas et al., 2012a). In practical, the antimicrobial activity of chlorine-based-sanitizers remained unaffected by either increasing the levels (i.e. concentration) of these sanitizing agents or increasing the processing

time in which fresh produce were dipped; this kind of sanitizer/disinfectant yielded ≤ 1.0 $\log_{10}$-reductions in the microbial populations no matter what different strains of microorganism and various fresh produce were applied. Processing time for which fresh produce was immersed with chlorine solutions had little relevance to reducing microbial loads contaminated on fresh produce, as well as did the use of higher concentrations of chlorine. Additionally, antimicrobial activity of sanitizers could be underestimated, depending on the presence of organic matters in the product. Mullerat et al. (1995) demonstrated that the presence of protein significantly reduced the antimicrobial activity of chlorine, thereby implying a great magnitude of difficulty in optimizing sanitization practices in response to the appearance of contaminants and pollutants.

Traditionally, commercial chlorine sanitizers have been considered as one of the most widely used sanitizers from the last few decades. Commercial chlorine sanitizers are known to inactivate microorganisms in several ways; (1) the cytoplasmic membrane has been taken into consideration as a possibly main target of chlorines, changing its permeability (Venkobachar et al., 1997); (2) DNA damage caused by chloramine derived from chlorine (Dukan et al., 1996); (3) inhibition of an enzyme that is implicated with the synthesis of the cell wall components. However, it was revealed from the present study that the use of chlorine would be controversial for sanitizing fresh produce due to their low effectiveness specified in Table 1 and simultaneous formation of some toxic residues on the product. It is apparent that commercial chlorine sanitizers are a good servant with respect to that they are economical and easy-to-use (Dalvi et al., 2000); nevertheless, if used indiscriminately, it would result in the formation of carcinogenic halogenated disinfection by-product, such as trihalomethanes and haloacetic acids, high exposures of which to humans has been mainly associated with cancer and adverse reproductive

outcomes (Hua and Reckhow, 2007; Costet et al., 2011).

EFFICACY OF ALTERNATIVE SANITIZING SOLUTIONS

Many sanitizing solutions have been developed as alternatives to conventional chlorine sanitizers, including electrolyzed oxidizing water, ozonated water, hydrogen peroxide, and peroxyacetic acid. In this chapter, decontamination activities and characteristics of these individual alternatives were discussed (Table 2). Electrolyzed oxidizing water (EO) is considered promising as an environmental-friendly decontamination agent (Kim et al., 2000). EO is typically generated by the passage of a salt solution (~1% NaCl) through an osmosis electrochemical cell, where the conversion of chloride ion and water molecules into chlorine oxidants such as Cl_2, HOCl, and ClO^- occurs at the anode (Guentzel et al., 2008). These acidic EO solutions generated on the anode terminal have been used for reducing microbial populations. As shown in Table 2, Desa et al. (2009) examined for ca. 100 ppm EO to reduce the numbers of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* serovar Typhimurium on tomato, resulting in above 3.0 $\log_{10}$-reductions in the initial loads. Treatment of *E. coli* O157:H7 and *L. monocytogenes* in lettuce with ca. 50 ppm EO for 1 min showed reductions in the levels of 2.4 and 2.8 $\log_{10}$ CFU/g, respectively (Park et al., 2011). Likewise, the numbers of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were diminished by 2.0, 2.0, and 2.1 $\log_{10}$ CFU/g after treatment with 300 ppm EO for 5 min, respectively (Yang et al., 2003). Interestingly, pathogenic bacteria inoculated on tomato were reduced more than 3.0 $\log_{10}$-reductions after treatment with EO and chlorine-based-sanitizers, respectively (See Table 1). According to Park et al. (2008), it was concluded that the smooth surface of tomato contributed to rapid dispersal of sanitizing solutions, such as EO, around the surface due to the less retention capacity of moistures on the

surface. Although a clear definition of the absolute 'smooth surface' of fresh fruits and vegetables is very ambiguous among numerous studies (to be exact, it was different, depending on subjective points of view of the authors), this kind of function would remain in effect among the sanitizing agent clearly. Thus, it seemed likely that EO's effectiveness could be higher than that of chlorine-based-sanitizers in that the average $\log_{10}$ reductions of EO sanitizer against several food-borne pathogens were in the levels of ≤ 3.01. It has been also shown that acidic EO showed better inhibitory effects against *E. coli* O157:H7 than sodium hypochlorite when used at the same concentration (Jadeja et al., 2013). According to Liao et al. (2007), water flow rate, concentrations of NaCl and current densities all had an effect on oxidation-reduction potential of EO, which from the anode stream possesses high oxidation-reduction potential (ca. > 1,150 mV), and presence of hypochlorous acid (Kim et al., 2000). EO gave rise to the release of *β*-galactosidase from the *E. coli* O157:H7 cells, causing collapse of the outer and inner membranes. Also, it was reported that EOs could cause the formation of glutathione disulphide-glutathione couples on microbial cells, resulting in the cell death (Liao et al., 2007). At the same time, EO has a disadvantage that, at < pH 3, dissolved Cl_2 gas can be rapidly volatilized into the atmosphere, decreasing the biocidal effectiveness of this solution over time (Len et al., 2000). Then, there is a need to adjust the pH of EO solutions to uniformly achieve better microbial reductions during processing.

Acidified sodium chlorite (ASC) has attracted many interests from the food industry as one of the promising antimicrobial agents. ASC is an approved sanitizer for washing fruits and raw agricultural commodities in concentrations of 500-1,200 ppm by FDA (CDC 2012). When ASCs were exposed to strong acidic conditions, chlorous acid derived from the conversion of chlorite

ion into its acid form, showed a strong oxidative mode of action, which is associated with penetration and breaks of oxidative bonds on cell walls (Kemp et al., 2000; Ukeke et al., 2005; Tomás-Callejas et al., 2012a). Allende et al. (2007) observed above 6.0 $\log_{10}$-reductions when *E. coli* O157:H7 inoculated on carrot was treated with 250 ppm ASC for 30 min. Similarly, reduction levels of *Salmonella* spp. on cucumber and bell pepper were ≥ 5.0 and 4.0 $\log_{10}$ after treatment with 1200 ppm ASC for 2 min, respectively (Yuk et al., 2006). Treatment of *E. coli* O157:H7 inoculated on spinach with 200 ppm ASC for 2 min resulted in a 3.1 $\log_{10}$-reduction (Zhou et al., 2009). However, this sanitizer did not show consistent microbial reductions since the standard deviations shown in Table 2 were much larger. In contrast with those of aforementioned studies, between 0.7 and 2.7 $\log_{10}$-reductions in the number of *E. coli* O157:H7 inoculated on various fresh fruits and vegetables (cabbage, carrot, cilantro, lettuce, and others) were achieved after treatment with 50-500 ppm ASC for several minutes (Cruz et al., 2006; Allende et al., 2009; Nei et al., 2009; Nei et al., 2010; Tomás-Callejas et al., 2012a). Likewise, it has been shown that treatment of *Salmonella* spp. in alfalfa seed and strawberry with ≤ 100 ppm ASC for less than 45 min yielded ≤ 2.0 $\log_{10}$-reductions (Liao, 2009; Issa-Zacharia et al., 2010). These results would be mainly resulted from a wide spectrum of experimental conditions; nevertheless, it was determined that the use of ASC to preserve fresh fruits and vegetables from microbial contaminations would be better than those of conventional chlorine-based-sanitizers. In particular, ASCs are known to effectively inactivate some physiological disorders such as enzymatic browning that shortens deteriorations of the organoleptic qualities of products, dodging consumer's demands (Xiao et al., 2011). Since sodium chlorite and citric acid are the routine components of ASC, its low pH concentrations (pH < 2.5) and oxidative potentials make

it possible to strongly inhibit the activation of polyphenol oxidase which is principally responsible for expressing of the browning (Luo et al., 2011).

Given the approval as an effective sanitizing agent by FDA (FDA, 2015), ozone has been widely used in order to decrease microbial risks in municipal waters, as well as some food such as meat and poultry, showing strong antimicrobial activities against a wide range of pathogenic bacteria. Bialka and Demirci (2007) observed 4.9 and 2.5 $\log_{10}$-reductions when *Salmonella* spp. and *E. coli* O157:H7 inoculated on blueberry were treated with 7.9 ppm ozonated water for 30 min, respectively. Reduction levels of coliform and total mesophilic bacteria on lettuce were 3.2 and 1.6 $\log_{10}$ after treatment with 10 ppm ozonated water for 3 min, respectively (Beltran et al., 2005). Notably, it seemed likely that Gram-negative bacteria including *E. coil* O157:H7 and *S.* Typhimurium were susceptible to this ozone water treatment, while treatment of *E. coli* O157:H7 on lettuce with 10 ppm ozone water for 10 min resulted in a 1.1 $\log_{10}$-reduction (Singh et al., 2002b). Indeed, ozone, at least, in the aqueous phase was more effective in reducing the number of Gram-negative bacteria than that of Gram-positive bacteria, which possess thicker cell wall structures comparatively. In comparison with the results of *Listeria* spp., decreases in the loads of *L. innocua* on lettuce and spinach by 2.07 and 2.06 $\log_{10}$ CFU/g were observed after 12 ppm ozone water for 15 min, respectively (Karaca and Velioglu 2014). In a study conducted by Alexandre et al. (2014), a 2 ppm ozone treatment induced the similar decline in the number of *L. innocua* on pepper. It has been reported that ozone is able to inactivate microorganisms mainly due to its strong oxidation activity; (1) oxidation by molecular ozone, and (2) oxidation by free radicals formed from decomposition of ozone (Hoigne et al., 1985). Especially, ozone inhibited the growth of *E. coli* O157:H7 and *Listeria innocua* either directly as a result of a rupture in the

cell membrane or indirectly as a consequence of the disintegration of the cell wall. Ozone is a sanitizing solution, which was declared to be generally recognized as safe (GRAS) for food contact applications in 1997 (Graham 1997). Ozone would offer several advantages including decreased productions of the residues on the surface of fresh produce due to its instability, rapid evaporations, and then exert better antimicrobial effects over conventional chlorine-based solutions (Khadre et al., 2001). Evidently, the average $\log_{10}$ reductions of ozone against various microbes were higher than that of the chlorine-based-sanitizers, following by placing the third behind EO and ASC. Furthermore, as ozone reaction does not depend on pH levels, there is no need to adjust the pH in wash waters thereafter (Ölmez et al., 2009). This is because that the inactivation kinetic of ozone become known to vary and it depends on the species of microorganism, as well as inoculum size (Kim et al., 2008), indicating that which microorganism was most sensitive to ozone or which type of fresh produce was the most suitable with ozone for microbial reductions would be inconclusive.

In order to reduce microbial contaminations associated with fresh produce, some studies applied peroxyacetic acid (PAA), which is a strong oxidizing agent that has been approved by the FDA as a disinfectant for fruits and vegetables (FDA, 2015). The antimicrobial activity of PAA is largely implicated with the oxidative reaction because a major target for its action is responsible for thiol groups in enzyme and protein, disrupting the permeability of cell membranes and later protein synthesis (Denyer and Stewart 1998). Kim et al. (2006a) observed 4.1 and 3.0 $\log_{10}$-reductions when *Cronobacter sakazakii* inoculated on apple and tomato was treated with 80 ppm PAA for 1 min, respectively. Reduction levels of *E. coli* O157:H7 inoculated on lettuce and spinach were 2.0 and 2.2 $\log_{10}$ after treatment with 500 ppm PAA for 1 min and 80 ppm PAA for

2 min, respectively (López-Gálvez et al., 2009; Zhou et al., 2009). Treatment of mesophilic aerobes with 60 ppm PAA for 2 min resulted in a 1.1 $\log_{10}$-reduction (Bastos et al., 2005). As a result, effectiveness of PAA was dependant on the strains of microorganism in that this sanitizer was more effective in reducing *C. sakazakii* than *E. coli* O157:H7 and *L. monocytogenes* on apple. The average reductions obtained after PAA-treatments were in the levels of $\leq$ 2.01-$\log_{10}$, indicating that PAA showed enhanced antimicrobial activities, as compared with chlorine based sanitizers.

Hydrogen peroxide is recognized as a safe chemical sanitizer though the use of hydrogen peroxide in the food industry is limited only to some products, including milk for inhibiting the growth of spoilage microorganisms, wine for performing the oxidizing-reduction potential, instant tea for bleaching, corn syrup for reducing the amount of SO_2, and others (Venkitanarayanan et al., 1999; CFR, 2014). Several studies have been performed to assess the use of hydrogen peroxide in fresh produce sanitization to decontaminate microorganisms. Hydrogen peroxide has characteristics of a strong oxidant and its capacity to generate cytotoxic oxidizing species such as hydroxyl radicals, which attack essential cell components such as membrane lipids, and DNA by oxidizing of sulphydryl groups and double bonds (Juven, and Pierson, 1996; Heling and Chandler, 1998). Ukuku et al. (2005) observed 3.0 $\log_{10}$-reductions when *E. coli* O157:H7 and *L. monocytogenes* inoculated on melon were treated with 2.5% hydrogen peroxide for 5 min, respectively. Reduction levels of *Salmonella* spp. on cantaloupe were $\leq$2.2 $\log_{10}$ after treatment with 5% hydrogen peroxide for 5 min (Ukuku, 2004). Treatment of *E. coli* O157:H7 inoculated on apple with 1.0% hydrogen peroxide for 15 min resulted in a 2.8 $\log_{10}$-reduction (Sapers et al., 2003). On the other hand, results obtained from the previously

published literatures revealed that it was as effective as chlorine-based-sanitizers, showing microbial reductions of 1.87 $\log_{10}$ on average. Between 0.8 and 1.8 $\log_{10}$-reductions in the number of *E. coli* O157:H7, *L. monocytogenes*, *S.* Typhimurium, and the total aerobic bacteria were achieved, indicating that hydrogen peroxide appeared to be only minimally effective for the decontamination of fresh produce (Sapers et al., 2001; Sapers et al., 2003; Yeon et al., 2005).

Chlorine dioxide (ClO_2) has attracted increased interest over the past 10 years as an alternative to commercial chlorine sanitizers for the fresh produce industry (Tomás-Callejas et al., 2012b) due to its several advantages, including effectiveness over a broad range of pH (Parish et al., 2003), lower reactivity with organic matter (Gordon and Rosenblatt, 2005), and high effectiveness at low concentrations (Huang et al., 1997). Herein, Wu and Kim (2007) observed a 2.4 $\log_{10}$-reduction when *L. monocytogenes* on blueberry was treated with 10 ppm ClO_2 for 10 min. Treatment of *Pseudomonas aeruginosa* on blueberry with 10 ppm ClO_2 for 10 min resulted in a 2.2 $\log_{10}$-reduction. Although these studies reported microbial reductions of $\geq$ 2.0-$\log_{10}$ between 1.1 and 1.7 $\log_{10}$-reductions in the population of *S.* Typhimurium, *L. monocytogenes*, and *E. coli* O157:H7 inoculated on various fresh produce after ClO_2-treatments with the concentrations of 100-10 ppm, regardless of the type of fresh produce and organisms (Singh et al., 2003; Choi and Lee, 2008; Keskinen et al., 2009; Kim et al., 2009). It seemed likely that sanitizers derived from commercial chlorines showed better inhibitory effects on the growth of Gram-negative microorganisms than Gram-positive ones. Virto et al. (2005) reported that *L. monocytogenes* and *Bacillus subtilis* were more resistant to chlorine than *Y. enterocolitica* and *E. coli*, supporting this finding.

Consumers have become more critical of the use of chemically synthesized sanitizers since

they used to get access to health-associating information on the food-borne hazards caused by development of toxic residues in the product. Accordingly, various studies have been conducted to demonstrate the effects of natural antimicrobials, which are harmless to human body, including organic acids, and herb essential oils. For a long time, these substances have also attracted many interests for reducing/eliminating pathogenic and spoilage microorganisms. Organic acids are natural substances either ubiquitously presented in fruits and vegetables or being extracted from microorganisms as a result of fermentation (Eswaranandam et al., 2006). Commonly, the antimicrobial activity of organic acids is elucidated by diffusing undissociated acids across the microbial membrane (Kong et al., 2001; Ricke, 2003; Mani-López et al., 2012). Under a certain situation that the external pH is less than that of the bacteria cell, the polarity of the molecules on the membrane is decreasing, causing the penetration of acids in the protonated form across the lipid membrane and into the cytoplasm. Once internalized, it dissociates into anions and protons, provoking the cells to export the excessive protons from the cytoplasm. Then, the depletion of energy for maintaining the intracellular pH homeostatically causes the cell death at the end. The antimicrobial activity of organic acids was listed in Table 3. Huang and Chen (2011) observed a 2.7 $\log_{10}$-reduction when *E. coli* O157:H7 inoculated on spinach was treated with 1% citric acid for 5 min. Reduction levels of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* were not only between 2.0 and 2.6 $\log_{10}$ on broccoli sprout, but ≤ 3.00 $\log_{10}$ on clover sprout after treatment with 0.5% acids for 5 min (Kim et al., 2009a; Kim et al., 2009b). Probably, organic acids would be effective in decontaminating sprout products as far as they resulted in the microbial reduction of ≤ 1.3 $\log_{10}$ on spinach, lettuce, and escarole from the other studies (Allende et al., 2008; Huang et al., 2011; Sagong et al., 2011). In an exceptional case, a

2.0 $\log_{10}$-reduction was achieved, followed by treatment of *E. coli* inoculated on lettuce with 0.05% lactic acid for 1 min (López-Gálvez et al., 2009).

Effectiveness of essential oils such as thyme, carvacrol, cineole, and oregano in inhibiting the growth of spoilage and pathogenic bacteria was listed in Table 3. Notably, more than 3.0 $\log_{10}$-reductions in *Shigella flexneri*, *Shigella sonnei*, *Salmonella* spp., and *Aeromonas hydrophila* on lettuce, carrot, a mixture of various fresh produce, and tomato were reported from several cases (Bagamboula et al., 2004; Gündüz et al., 2010; Eattson et al., 2011; de Sousa et al., 2012). Among them, *Shigella* spp. on lettuce showed microbial reductions of ≥ 5.0 $\log_{10}$ after 0.1% thyme oil for 24 hrs. These cases had the inoculation of Gram-negative bacteria on the surface in common, thereby implying that essential oils would be difficult to permeate into the thick peptidoglycan wall of Gram-positive organisms relatively. Apart from these results, the number of *S.* Typhimurium and *E. coli* O157:H7 on apple and carrot were decreased, showing by ≤ 1.4 $\log_{10}$-reductions after 5 ppm thyme oil for more than 10 min (Tornuk et al., 2011). Gutierrez et al. (2009) also reported that immersing lettuce inoculated with *Enterobacteria* and *Pseudomonas* spp. in 250 ppm cineole oil for 2 min resulted in 1.2 $\log_{10}$-reductions, respectively. Averagely, microbial reductions resulted from the use of essential oils were in the levels of 2.07 $\log_{10}$ in the most cases. A significant difference between organic acids and essential oils for decontaminating these microorganisms was not demonstrated, but these sanitizing methods did not surpass the effects of chorine-based-sanitizers. Furthermore, some natural antimicrobials have important problems, including flavor abuses and large difficulties in their dissolution for which an emulsifier or solvent such as ethanol, methanol, and Tween 80 were required. Although a majority of essential oils extracted from herbs and spices are classified as GRAS (Burt 2004),

their utilization in foods has not been available in the food industry, since effective doses showing promising antimicrobial activities may exceed organoleptically acceptable levels to the consumers (Raybaudi-massilia et al., 2006).

EFFECTS OF GASEOUS CHLORINE DIOXIDE

Gaseous ClO_2 has attracted many interests from fields of science due to its strong and enhanced antimicrobial activity. ClO_2 gas is commonly applied either a batch or a continuous module to sanitize fresh produce (Han et al., 2004). With the usual batch treatment, a sachet was put inside the chamber containing fresh fruits and vegetables and the ClO_2 gas began to be released gradually from the sachet. On the other hand, continuous method relies on the continuous injection of ClO_2 gas through a tube linked between gas generator and chamber. It is difficult to maintain a constant gas concentration during operation of the former process, whereas the latter method is capable of providing a supply uniformly. Selection of either the batch or the continuous method can be an influencing factor on microbial survivals and growth, as well as generation techniques of ClO_2 gas were also considered as one of the important factors involving in the antibacterial activity of this sanitizing method for cleaning fresh produce. From the previous literatures, ClO_2 gas was traditionally generated by having a chemical reaction between secondary chemical (i.e. chlorine, hydrochloric acid, and others) and sodium chloride (Mahmoud and Linton, 2008; Trinetta et al., 2010; Bhagat et al., 2011). These studies reacted 2-4% chlorine gas with sodium chlorite, generating ClO_2 gas, and exposed the gas to 8-10% sodium chlorite to collect the residual chlorine gas subsequently, while ClO_2 gas can be generated by membrane electrode assembly (MEA) method by means of oxidation-reduction reactions occurred on each anode and cathode located in the conductivity membrane (Kim and Mun 2012). The electrolytic

apparatus presents its shape like a length of a 15-20 cm cylinder, where is separated by upper section, middle layer, and lower section. When sodium chlorite in water is injected into the porous oxidation-resistant conductivity membrane located in the middle layer through an inlet hole, chemical reactions occur, specifically the oxidation of sodium chlorite and water at the anode terminal in the upper section and reduction of a hydrogen ion at the cathode terminal in the lower section. The detail formula procedure is following as: (1) $NaClO_2 \rightarrow Na^+ + ClO_2 + e$; (2) $H_2O \rightarrow 2H^+ + 1/2O_2 + 2e$; (3) $H^+ + e \rightarrow 1/2H_2$. The ClO_2 gas generated by membrane electrolysis is emitted through an outlet connected in upper section into a chamber for treatment. In our preliminary studies, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on fresh fruits and vegetables, including tomato, apple, lettuce, and a bundle of sesame leaf, were treated with ClO_2 gas generated by MEA at the levels previously reported in a study conducted by Trinetta et al. (2010) for more than two hours, and then it resulted in < 2.0 $\log_{10}$ CFU/g reductions in the numbers of these pathogens, thereby implying that the actual concentrations of ClO_2 gas may be different, depending on the generation method to be employed. There were numerous reports that demonstrated the effectiveness of ClO_2 gas as a strong alternative agent for sanitizing fresh produce (Table 2). Du et al. (2003) reported a 7.0 $\log_{10}$-reduction when *E. coli* O157:H7 inoculated on apple was treated with 15 ppm ClO_2 gas for 6 min. Reduction levels of *E. coli* O157:H7 inoculated on green pepper were 6.5 $\log_{10}$ CFU/g after treatment with 1.2 ppm ClO_2 gas for 30 min (Han et al., 2000a; Han et al., 2000b). Treatment of *L. monocytogenes* inoculated on green pepper with 3 ppm ClO_2 gas for 30 min resulted in more than 6.0 $\log_{10}$-reductions (Han et al., 2001b). Other publications (Dalvi et al., 2000; Han et al., 2001a; Singh et al., 2002a; Du et al., 2003; Lee et al., 2004; Kaye et al., 2005a; Yuk et al., 2006; Mahmoud et al.,

2008; Mahmoud and Linton, 2008; Mahovic et al., 2009; Bhagat et al., 2010; Bhagat et al., 2011) have observed microbial reductions of ≥ 5.0 $\log_{10}$ after treatment of fresh produce with ClO_2 gas. Commonly, such a high level of microbial reduction was reported in the cases where the fruits and vegetables had smooth or less smooth surfaces, including tomato, apple, lettuce, orange, and others. In contrast, pathogenic bacteria inoculated on mushroom, cucumber, strawberry, cantaloupe, and others with the relatively rough surface showed the decreasing microbial reductions of a range of 1.0-4.6 $\log_{10}$ (Selby et al., 2004; Kaye et al., 2005a; Mahmoud et al., 2004; Popa et al., 2007; Mahmoud et al., 2008; Matsufuji et al., 2009). In support of these findings, a study conducted by Matsufuji et al. (2009), found less than 1.0 $\log_{10}$-reductions in the number of *E. coli* K-2 on paprika and cucumber after 10 ppm ClO_2 gas treatment for 30 min. These differences could be caused by (1) the different volume/size of a chamber containing samples to be treated, (2) the weight of samples, and (3) varying levels of relative humidity in a chamber. Especially, it was reported that varying levels of relative humidity could affect significantly the experimental result. In a chamber with the relative humidity of $\leq$ 70%, significant differences in the levels of *E. coli* O157:H7 and *Salmonella* spp. on fresh produce were not reported before and after treatment with ClO_2 gas (Kaye et al., 2005b). This situation was in accordance with our previous study, showing reductions of ca. ≤ 1.0 $\log_{10}$ CFU/g in the number of *E. coli* O157:H7, *S.* Typhimurium, *L. monocytogenes*, and *Staph. aureus* on cabbage, lettuce, and sesame leaf after varying levels of ClO_2 gas treatments for 30 min at $\leq$ 80% relative humidity (data not shown). These results indicated that effects of ClO_2 gas could be greatly different, depending on the levels of relative humidity. With regard to the various types of microorganisms used in the previous studies, ClO_2 gas appears to be more effective in

inactivating pathogenic bacteria on fresh produce than the other sanitizing methods aforementioned, showing the microbial reduction of more than 4.0 $\log_{10}$ CFU/g on average. Most importantly, its sanitizing effects were independent upon the Gram-types of organisms because treating *L. monocytogenes* on cantaloupe, apple, strawberry, lettuce, green pepper, and tomato with 1-50 ppm ClO_2 gas for several minutes also resulted in 4.3, 4.2, 4.7, 4.3, $\geq$ 6.0, and $\geq$ 5.0 $\log_{10}$-reductions, respectively. Interestingly, ClO_2 in gaseous phases exhibited stronger antimicrobial activities than those of the liquefied ClO_2. This is because that ClO_2 gas appears to be related with the easier access of this agent close to microorganisms localized internally. Once fresh fruits and vegetables are treated with the ClO_2 gas, it would have an effect on the microbial internalization because ClO_2 gas is capable of permeating into the interior of fresh products, passing through tiny pores formed on surfaces or other channels.

EFFICACY OF PHYSICAL SANITIZING METHODS

Table 5 showed a summary of the results originated from the previous published literatures with regard to physical sanitizing methods used to reduce or eliminate pathogenic microorganisms on fresh produce. As is well known, sanitizing the products with physical methods exhibits enhanced microbial reductions, assuring an extended shelf-life of a product. Such a technique available in the food industry can include hydrostatic pressure (HP), ultraviolet (UV), irradiation, ultrasound, modified atmosphere packaging and others. Herein, this study focused on HP, UV, and ultrasound, so called sonication, because these physical methods have been widely studied for a lengthy period of time so that a more objective point of view to the study of science would be arranged from a lot of sources of the corresponding information. HP is able to inactivate the initial load of food-borne microorganisms, most likely due to protein

denaturation and cell injury. Decreases in the membrane fluidity, which result from a line through the process of a liquid crystalline phase and gel phase, cause the solubilization, the leakage of intracellular contents, and then a shrink in cell volume, leading to the cell death. Compared to other modes of the antimicrobial action, HP showed a promising effect for sanitizing fresh products (Table 5). When *E. coli* O157:H7 on alfalfa seed was treated with 600 MPa for 2 min, it resulted in ≤ 6.0 $\log_{10}$-reductions in the population (Neetoo et al., 2009a). Not only treating *E. coli* O157:H7 and *Salmonella* spp. on green onion with 500 MPa HP for 2 min led to the microbial reduction in levels of ≥ 5.0 $\lg o_{10}$, but also a 6.6-$\log_{10}$ reduction in *Salmonella* spp. on pepper observed, following by 450 MPa HP for 2 min (Neetoo et al., 2011; Neetoo and Chen, 2012). Neetoo et al. (2008) reported a 5.7 $\log_{10}$-reduction when *E. coli* O157:H7 inoculated on alfalfa seed was treated with 600 MPa of HP for 6 min. With the exception of a study conducted by Ariefdjoham et al. (2004), most cases exhibited at least more than 3.0 $\log_{10}$ reductions in the microbial number. For instances, a reduction level of *Salmonella* spp. inoculated on alfalfa seed was a 4.5 $\log_{10}$ CFU/g after treatment with 600 MPa of HP for 25 min (Neetoo et al., 2010). It has been also reported that aerobic mesophilic bacteria on cabbage were susceptible to HP treatment with 300 MPa HP for 10 min, thereafter resulting in 4.2 $\log_{10}$-reductinos (Peñas et al., 2010). As shown in Table 5, HP yielded microbial reductions of 3.9 $\log_{10}$ CFU/g on average and it became effective in decontaminating fresh produce from microbiological risks. However, its practical operation in the food industry still confronted several problems that several studies imposed a mild/severe quality loss, wherein the use of HP would be associated with an organoleptic change and nutritional loss in food materials (Martín et al., 2002). According to a study conducted by Jung et al. (2003), HP deteriorated the quality of

beef meat, showing significant changes in its redness, contents of metmyoglobin, and total colors. Then this research indicated that the increasing magnitude of HP would result in larger differences in the food qualities. After HP operation, degradation of astaxanthin in smoked salmon, changes in fish tissues, and discoloration in fruits and vegetables were determined by Bermúdez-Aguirre and Barbosa-C ánovas (2011).

The beneficial characteristics of UV were described clearly in various publications (Ge et al., 2013; Guan et al., 2013; Jahid et al., 2014; George et al., 2015). Briefly, UV is a nonthermal and nonionizing radiation, which possesses germicidal/antimicrobial activities at a wavelength between 200-280 nm. Usually, it has been used for decontaminating water, fruits, and root vegetables (Morgan, 1989; Culter and Zimmerman, 2011). With these reasons, UV was known for its prominent antimicrobial activities against a variety of microorganisms. Birmpa et al. (2013) reported that treating *Salmonella* spp. on lettuce with 7 J/cm^2 UV for 30 min was successful in reducing the microbial load, showing by a < 4.0 $\log_{10}$-reduction. The number of *E. coli* O157:H7 and *Salmonella* spp. on raspberry were decreased by 3.9 and 3.4 $\log_{10}$-reductions, respectively (Bialka and Demirci, 2008). Such a bacterial reduction of 3.0 $\log_{10}$ was seen in a study of Yaun et al. (2004), following by the treatment of *E. coli* O157:H7 and *Salmonella* spp. on lettuce with UV at 24 mW/cm^2 for 10 sec. On the other hand, several studies have reported that UV treatment did not result in successful sanitizing effects of contaminated strawberry, lettuce, and clover sprout. These may arise from that UV cannot penetrate into the deep interior of fresh produce (Morgan1989). According to Guan et al. (2012), antimicrobial activity of this technique was different, depending on the dose of UV between 2.45 and 3.15 kJ/m^2. It was revealed that UV at 2.70 kJ/m^2 showed significantly higher microbial reductions on fresh products because UV

appears to inactivate microorganisms by the formation of dimmers in RNA and DNA, which results in the inhibition of DNA replications (Sommers and Cooke, 2009). In our preliminary study, UV was also effective for decontaminating the food-borne pathogens, including *C. sakazakii*, *E. coli* O157:H7, *L. monocytogenes*, and *S.* Typhimurium, inoculated on the food-contacting-surfaces of stainless steel and polyvinylchloride (data not shown). Probably, UV would exhibit a beneficial effect for preventing the cross contamination between fresh products and culinary apparatus.

Commonly, ultrasound (US) sanitization has been applicable for the surface decontamination of fresh produce. Once operated, US at a controlled frequency ranging from 20 to 100 kHz is required for a liquid medium for power transmission (Roberts 1991). Then, multitudinous bubbles, micrometers in size, arise and begin to implode within just a few seconds as a consequence of a cavitation phenomenon (a series of compression, expansion, and collapse) through inner pressure changes, resulting in the generation of high temperature and pressure that are closely associated with the antimicrobial activity (Scherba et al., 1991; Raso et al., 1998; Hua and Thompson, 2000; Piyasena et al., 2003). According to a study conducted by Seymour et al. (2002), the authors observed a 1.6 $\log_{10}$ reduction when *S.* Typhimurium inoculated on lettuce was treated with 32-40 kHz of US for 10 min. In addition, the reduction of *S.* Typhimurium inoculated on tomato was in the levels of <1.0 $\log_{10}$ after US at 45 kHz for 10 min (José and Vanetti, 2012). Similarly, several studies (Scouten and Beuchat, 2002; Kim et al., 2006b) reported that US exhibited less than 1.0 $\log_{10}$ reductions for reducing the number of microorganisms on fresh fruits and vegetables, thereby indicating that the efficacy of US, alone, for decontaminating fresh products was lower than that of chlorine-based-sanitizers.

KEY LIMITATIONS FOR DETERIORATING THE CLEANING EFFECTS OF FRESH PRODUCE

Sanitizing methods that designed to eliminate/reduce a range of pathogenic bacteria should display desirable levels of inactivation efficacies in a field of practice. Nevertheless, chlorine-based-sanitizers described in here were far distinct from giving reliable performances to the consumers. Understanding the underlying problems behind the low sanitizing efficacy for disinfecting fresh fruits and vegetables may lead to meaningful improvements in the food industry for developing novel and effective alternatives. The type of products and the localization of microorganisms either on the surface or in the interior, can cause sanitizers to differ greatly in their ability to decontaminate fresh fruits and vegetables (Kreske et al., 2006; Allende et al., 2007). Numerous studies have shown that the use of chlorine-based-solutions at the concentrations permitted by the FDA was not enough to remove the population of microorganisms effectively. Many of the sanitizing strategies investigated exerted limited efficacies in inactivating pathogenic bacteria contaminated on fresh produce. One of the answers causing for this lack of efficacy could be elucidated by the means of the adhesion of pathogens to natural opening cavities, including pores, stoma, cut, and cuts in where aqueous sanitizers are not accessible (Wang et al., 2008). While the use of sanitizing agents might be sufficient in eliminating the bacterial contamination which occurs only on the surface, a transfer and/or attachment of pathogenic bacteria into the cores of products can pose severe risks of human diseases insofar as almost of the routinely used sanitizers are focusing on achieving hygienic practices only at the surface of fresh produce. As shown Figure 1B, scanning electron microscopy photographs of the surface of an apple after washing with 100 ppm sodium

hypochlorite showed the dispersal of surviving aggregates of *E. coli* O157:H7. In particular, a small cluster of *E. coli* O157:H7 cells was engulfed and remained attached to the stoma, although a majority of these cells were detached or were killed during the cleaning procedure (Figure 1b), implying that stoma located on the surface of fresh fruits and vegetables can act as a protective place (a shelter) where sanitizers cannot easily penetrate. Additionally, free bacteria and other microorganisms within water droplets on the plant body can be entered into the palisade parenchyma zone through stoma located on the upper and lower epidermises, posing a risk for the internalization of the invasive pathogens (Figure 1A). Furthermore, the stem of products, as well as some scars on the outside can present a surface by which bacteria are able to penetrate the inward side of fruits and vegetables (USFDA 2015). According to a investigation by Ge et al. (2013), green onions injected with *S*. Typhimurium for the infiltration-adhesion into the cores yielded 0.06-0.17 $\log_{10}$ CFU/g reductions in the population after 100-200 ppm chlorine for 5-10 min. No significant differences were not observed in the numbers of *E. coli* O157:H7 internalized into lettuce and spinach after treatment with 300 ppm sodium hypochlorite for 3 min, as compared with those that did not suffer from any treatment (Neimira, 2007). As the ability of many sanitizing agents described in this study to penetrate through the interior spaces of fresh produce would be regarded as being ineffective, effectiveness of each sanitizing method could be decreased if there would be the potential internalization of pathogenic bacteria into the intracellular ultrastructures, which would minimize the interaction between microorganisms and antimicrobials.

Bacterial internalization would be different, depending on (1) temperature and (2) preferred adhesion of the pathogens on the contacting surface. Brodini et al. (2007) examined for the

transfer of *Salmonella* from the surface to the inferior tissue of the mangoes by pressure differentials encounter in and outside; particularly, mangoes supplied had been immersed in a thermal bath containing a crystal violet dye at 46°C for 90 min and then transferred to a water tank (21°C) inoculated with *Salmonella* spp. for 30 min. As a consequence of this process, it has been shown that various spots including a rind, a stem, and a blossom-end of the mangoes were dyed optically, resulting in the internalization of *Salmonella* spp. in the levels of ≥ 3 $\log_{10}$ on the 3rd day of the storage at an ambient temperature. According to a report by USFDA (2015), oranges and grapefruits, which had been held at 21°C before the initiation of a trial, were submerged in water (4°C) containing a dye for 10 min, showing clear uptakes of the dye into the intact of products. These phenomena could be possibly explicated by the contraction of air-spaces in different sizes, followed by pressure differentials. Otherwise, if a temperature of a sanitizing solution is lower than that of a fresh produce contaminated with the food-borne pathogens at the moment of the processing it would cause the internal localization of such an organism through an accompanying flow of the washing water into the interior structures and tissues. Since it has been evident that proliferation of pathogens is strongly involved in the formation of an initial attachment on surface, individual hydrophobicity of surface and microorganism would affect the attachment and internalization either directly or indirectly. From instances, *E. coli* O157:H7 showing a hydrophilic property has a preferential affinity with the cut-surfaces of apple and lettuce (Hassan and Frank 2004). Patel and Sharma (2010) have shown that the numbers of *Salmonella enterica* serotype Tennessee attached to the cut-surfaces of lettuce and cabbage were in the levels of 4.68 and 3.01 $\log_{10}$ CFU/cm^2, respectively, whereas a small piece of *S.* Tennessee ranging from 1.83 to 3.19 $\log_{10}$ CFU/cm^2 were determined on the

surface of lettuce and cabbage under the same condition. In this context, cuticle waxy layers covered around the surface of product would be regarded as being hydrophobic, implying that *E. coli* O157:H7 and *Salmonella* spp. would attach preferentially to the hydrophilic cut-edges of fresh produce (Ells and Hansen, 2006; Boyer et al., 2007). With respect to this, fresh produce tends to be cut into a small bite-size piece with kitchen utensils before consumption, the preferential attachment of food-borne pathogens via the cross section could cause a severe bacterial infection to the consumers because the cut-surface will serve as a rapid route from the stem where the bacterial contamination is likely to be already internalized. On the other hand, there was a case of *S.* Typhimurium that did not show the preferential adhesion neither on mangoes with the hydrophilic surface nor tomatoes with the hydrophibic property. In this research to give insight about the interrelation between the surface roughness and hydrophobicity of the product, the authors found that no significant differences in the free energy for the adhesion of *S.* Typhimurium were observed, showing by 3.33 and 4.78 mJ/m^2 on mangoes and tomatoes, respectively ($p>0.05$). Mahdhi et al. (2012) examined that increasing hydrophobicty of cell surface would be in proportion as the increasing adhesive ability of microorganisms. Then, these results indicated that hydrophobic/-philic interactions of the products will not always be consistent with the increasing attachment of pathogens. Even then, the capacity of pathogenic bacteria to attach preferably to a certain surface is not involved in the requisite internalization. Rather, it seemed likely that a number of factors such as the different growth phase of microorganisms, a curli production, and the presence/absence of certain surfactants (Hassan and Frank, 2003) would be attributed to the hydrophobicity interaction between the organisms and the surface of fresh produce. Especially, it has been reported that hydrophobicity of *E. coli*

O157:H7 in the stationary phase declined greatly from 19.26-31.87 to 7.91-16.38 over a prolonged incubation period (Patel et al., 2011). Several studies (Ryu et al., 2004; Ryu et al., 2005) investigated the interface between the hydrophobicity and curli, suggesting that higher hydrophobicity with the increasing production of a curli could prevent cells of *E. coli* O157:H7 from being attached to the hydrophilic surface. A hydrophobic surfactant such as Span 85 reduced the level of *E. coli* O157:H7 attached to the hydrophobic lettuce leaf surface significantly, as compared with those attached to cut-surfaces (Fernandes et al., 2014). In addition, it has been proved that biofilms of *L. monocytogenes* produced at 37°C exhibited a strong hydrophobicity two times as higher as that developed at 4°C, indicating that temperature difference at the moment of initiating biofilms formation could be of much importance for the bacterial adhesion (Bonaventura et al., 2008). Making a conclusion about the clear correlation between hydrophobicity/-philic property and adhesion of pathogens on the intact/cut-surfaces of fresh fruits and vegetables is not yet possible now; thus, continuous studies should be further conducted with the goal of introducing the effective control measurement of food-borne outbreaks associated with fresh produce.

Biofilms can be one of the key factors implicated in the limited effect of sanitizer methods against various microorganisms. Biofilms are the communities of microorganisms that are attached to a surface and they possess a complex matrix of an extracellular exopolysaccharide (EPS) material that envelopes microorganisms located on the surface. A wide range of bacteria such as *E. coli* O157:H7, *L. monocytogenes, Staph. aureus, S.* Typhimurium, *Cronobacter* spp., and *Pseudomonas* spp. were proven to be capable of adhering, and then producing biofilms. Pathogenic biofilms provide these bacteria, as well as numerous other spoilage microorganisms,

with a defense mechanism against environmental challenges such as desiccation, sanitizers, and antimicrobial agents (Junkins and Doyle, 1992; Jeong and Frank, 1994; Somers et al., 1994; Brown et al., 1995; Dewanti and Wong, 1995; Jones and Bradshaw, 1996; Mah and O'Toole, 2001; Rayner et al., 2004; Kim and Wei, 2006; Lapidot et al., 2006; Ölmez and Temur, 2010). The internalization of pathogens in fresh produce can deteriorate the antimicrobial activity of the sanitization methods consecutively due to the development of biofilms. It has been reported that biofilms could occur not only on cuticle layer but also the inside of vascular system by attached bacteria (Fig 1A). As the substructure constituting the body of a plant is not simple, bacteria either entrapped/hidden by several inner structures or localized inward might be allowed to survive after cleaning strategies, many concerns should be drawn to prevent an aftermath of food-borne outbreaks. Bae et al. (2012) examined for the effects of a chlorine-based-solution and an alcohol sanitizer against *E. coli* O157:H7 and *Staph. aureus* with or without biofilms, and then these bacteria that had been enveloped in a mass of biofilms before treatment showed better survivals than those without biofilms, allowing these bacteria embedded in biofilms to be less likely removed through common cleaning practices. In a study conducted by Ölmez and Temur (2010), while microbial reductions of *L. monocytogenes* and *E. coli* O157:H7 inoculated on lettuce were in the levels of ≤ 4.00 $\log_{10}$ CFU/g after individual treatment with 2 ppm ozone, 100 ppm chlorine, and organic acids when they had been stored at 10°C for 6 hrs beforehand, these pathogens in biofilms that produced at 10°C for 24-48 h showed ≤ 2.50 $\log_{10}$ CFU/g reductions after the same sanitizing treatments. From our preliminary studies (data not shown), treatment of *E. coli* O157:H7 in biofilms that produced at 100% relative humidity and 25°C for 5 days with 100 ppm chlorine-based-sanitizer were highly effective, showing by bacterial reductions of 7.06

and 6.32 $\log_{10}$ CFU/g on apple, respectively. Mah and O'Toole (2001) clearly determined that cells grown in biofilms were far distinct from planktonic cells in that they had an increased resistance to antimicrobial agents. It has been also revealed that *Staph. aureus* has a strong ability to attach preferentially to and produce more biofilms on hydrophobic substrates (Pagedar et al., 2010). Similarly, Auger et al. (2009) demonstrated that diarrheal isolates of *B. cereus* showing the highest hydrophobicity index (LnK) of 4.52 were positively correlated with the strong biofilms development capacity though these correlations were greatly different, depending on the strains and origins of *B. cereus*. There was a point of view addressing that the facilitation of the adhesion, mediated by the interactive hydrophobicity, between the bacterial cell and product surface could play an important role in the integrity of biofilms (Baillie and Douglas 1998). From these results, it seemed plausible that formation of biofilms either directly by bacteria attached to the surface or indirectly by the resident-flora on fresh fruits and vegetables could, possibly, play a key role for increasing the survivals of pathogens. Therefore, pathogenic bacteria survived in biofilms on food contacting surfaces would cause the cross-contamination of the fresh produce. Continuous efforts should be paid to control unexpected expressions of virulence of biofilms.

One of the interesting issues currently available in the food industry is the effect of preservative agents and environmental challenges against the survivals of food-borne pathogens. A number of fresh produce such as cucumber, apple, lettuce, cabbage, garlic, onion, and others have been consumed in a form of fermented foods after minimal processing as these have several advantages over unhygienic ingestions of the materials without any processing due to the preservation activity by some metabolites produced after the fermentation; however, this kind of

minimally processed food still harbors a potential risk of causing human infections as a result of the bacterial contamination. In most cases, fermented foods inhibit the growth of harmful microorganisms either as the result of the addition of salt alone or in combination with acidic organic acid metabolites. As shown in Table 4, it can be proven that various organic acids were contributed to reducing the numbers of pathogenic bacteria in fresh produce. Despite these results, it has been suggested that survivals of pathogens in foods would be enhanced in the presence of salts and organic acids together. Such an antagonistic effect of salt combined with organic acids were reported in several studies (Lee and Kang, 2009; Yoon et al., 2014; Bae and Lee, 2015). Higher levels of *S. flexneri* were observed in cucumber puree containing 0.5% acetic acid or 0.25% lactic acid combined with 3% salt for 7 days of storage. Compared with results obtained after treatments of various organic acids alone, not only *E. coli* O157:H7, but also *S.* Typhimurium survived better in pickles with 3% salt in a combination with acetic acid, lactic acid, and propionic acid, respectively. Casey and Condon (2002) also demonstrated that the use of acetic acid or formic acid combined with 4% salt resulted in the antagonistic effect on the survivals of *E. coli* O157:H45. Among different types of acidulants, the protective effect toward microbial cells were facilitated by the use of 3-4% salts in the combination with acetic acid, lactic acid, propionic acid, and formic acid, respectively. Constructionally, all of these organic acids contain a single carboxyl (-COOH) group. It can be surmised that such organic acids would lower the amount of H^+ in undissociated forms, decreasing the energy consumption of microbial cells, or change components of cell membranes, preventing the insertion of undissociated H^+ into the intracellular space. Until now, a clear mechanism governing the antagonistic activity of salt in combination with organic acid has not elucidated. Thus, further studies should be required for

resolving this phenomenon to inhibit the growth of potentially food-borne pathogens as the combinational treatment is greatly responsible for preserving minimally processed fresh produce.

CONCLUSIONS

A wide variety of opportunities could exist for the contamination of fresh fruits and vegetables to occur by contacting with pathogenic bacteria through numerous channels, including the type of fresh produce, attributes of the microbial strains, surface properties (both hosts and bacteria). A range of sanitizing methods are widely employed in an attempt to eliminate the microbial populations on fresh commodities for achieving good hygienic practices in the food industry. An overview of these approaches was presented in this manuscript to demonstrate the exact efficacies of each sanitizing method, ranging from chlorine-based-sanitizers to ASW, ozonated water, essential oil, HP, and ClO_2 in aqueous and gaseous phases, and others. As a result, in most cases, a varying sort of aqueous sanitizers/disinfects displayed ≤ 3.0 $\log_{10}$-reductions in the number of microorganisms inoculated on a number of fresh fruits and vegetables. In particular, a traditionally used chlorine-based-solutions were not effective in reducing microbial loads on surfaces, showing ≤ 1.1 $\log_{10}$-reductions on average, even though they were used in the concentration of up to 600 ppm for several minutes. Amongst the antimicrobial agents shown in Table 2-3, EO water appeared to be consistent and very effective, achieving microbial reductions of ≥ 3.0 $\log_{10}$. In contrast, HP, UV, and gaseous chlorine dioxide treatments showed ≥ 6.0-$\log_{10}$ reductions in various studies (Table 3-4). However, the use of these sanitizing methods sometimes had negative influences on the appearance of fresh fruits and vegetables, resulting in the change/loss of natural flavors off from various fruits and vegetables due to applications of the excessive doses that can cause organoleptic damages in the tissue of

products. Otherwise, average microbial reductions of ClO_2 gas, HP, EO water, ASC, ozone, UV, herb oil, PAA, hydrogen peroxide, organic acid, ClO2, and chlorine-based-sanitizer were 4.07, 3.94, 3.01, 2.54, 2.33, 2.17, 2.07, 2.04, 1.87, 1.74, 1.49, and 1.12-$\log_{10}$, respectively.

Until now, numerous researches have made an attempt to increase the sustainability of microbial reductions and/or to satisfy consumer's acceptability of the disinfection/sanitization methods. In a study conducted by Ramos et al. (2013), the authors reviewed the applied methodologies to assuring food quality and safety, including conventional chemical disinfectants and emerging physical sanitization methods, suggesting that the degree of inactivation (efficacy of these cleaning strategies) were very dependent on the sort of fresh fruits and vegetables, and pathogenic bacteria to be employed. Thereafter, the authors indicated that currently developed methodologies for cleaning fresh commodities showed mild antimicrobial activities, whereas these methods are still limited to meet both the increasing sanitization effect and consumer acceptability simultaneously. Goodburn and Wallace (2013) also studied newly emerging technologies such as electrostatic spray, silver, pulsed light, and biological controls for decontaminating fresh fruits and vegetables, and then concluded that these methods would be able to become a prominent alternative in the food industry, showing < 4.3 $\log_{10}$-reductions in the microbial population. Nevertheless, the authors did not take an adequate consideration that effectiveness of the individual technologies would fluctuate variably according to the surface property of fresh commodities and the cellular physiology of localized pathogens. Clearly, it becomes evident that limited sanitization efficiencies could be available in that stoma, puncture, and scar in apple, mango, and others can provide the pathogenic bacteria with a shelter to evade sanitizing agents during the processing. Additionally, biofilms production could also exhibit

improved microbial resistances against various sanitizing agents, followed by localization of the resident or survived organisms on foods. Then, decreasing antimicrobial activities would be different, depending on the type of fresh fruits and vegetables, and microorganisms. Given that precise classifications of the surface property as hydrophobic\-philic would be possible, it might be helpful to deduce the meaningful relationship, leading to a better view of how best to decontaminate fresh fruits and vegetables most effectively, but clear correlations have not been revealed from previously published studies. Importantly, the decreased efficacy of various sanitizing methods might arise as a consequence of a combination of the aforementioned factors. Accordingly, it is still early in this study to determine which is the optimal approach for decontaminating fresh fruits and vegetables from the microbial contamination. Nevertheless, continuous attention has to be paid for altering the existing sanitization methods urgently. In particular, the synergistic effects would deserve a favorable response from the food industry, by combing two or more separate decontamination methods. As shown in a case of the gaseous chlorine dioxide, conversion of the aqueous disinfectants into aerosolization would be beneficial for eliminating the food-borne pathogens located in punctures that these sanitizing agents in the aqueous phase are unaccessible. Aerosolization facilitates the diffusion of gaseous disinfectants through the inner space, and then would have an advantage over the conventional chlorine-based sanitizers for decontaminating the internalized pathogens. Until now, food-borne outbreaks associated with fresh produce have occurred continuously and then, equivalent sanitizing methods have been also developed. Despite these efforts, there are many problems in the use/choice of appropriate sanitizing methods to decontaminate fresh produce efficiently because of the several disadvantages over harmful resides formation and organoleptic damages in the

product, as well as that current consumers do not favor chemically synthetic compounds. It is essential to understand microbial behaviors better to develop effective alternatives that can meet all of these requirements. The present study will contribute to providing with the primary information associated with the current sanitization/disinfection strategy.

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Table 1 Effectiveness of the chlorine-based-sanitizers for cleaning fresh fruits and vegetables

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
	600 ppm	3 min	*Escherichia coli* O157:H7	1.3	Spinach	Niemira et al., 2010
	600 ppm	3 min	*Escherichia coli* O157:H7	1.8	Lettuce	Niemira et al., 2010
	300 ppm	15 min	Coliform	2.8	Mixed vegetables	Samadi et al., 2009
	300 ppm	3 min	*Escherichia coli* O157:H7	<1.0	Spinach	Niemira, 2007
	300 ppm	3 min	*Escherichia coli* O157:H7	<1.0	Lettuce	Niemira, 2007
	200 ppm	30 min	*Salmonella* spp.	0.3	Spinach	Neal et al., 2012
	200 ppm	30 min	*Escherichia coli* O157:H7	1.0	Spinach	Neal et al., 2012
	200 ppm	3 min	*Salmonella* spp.	0.5	Spinach	Hadjok et al., 2008
	200 ppm	3 min	*Salmonella* spp.	1.9	Lettuce	Hadjok et al.
	200 ppm	3 min	*Salmonella* spp.	0.3	Broccoli	Hadjok et al.
	200 ppm	3 min	*Salmonella* spp.	0.3	Onion	Hadjok et al.
	200 ppm	3 min	*Salmonella* spp.	0.3	Tomato	Hadjok et al.
	200 ppm	3 min	*Escherichia coli* O157:H7	0.5	Spinach	Hadjok et al.
	200 ppm	2 min	*Salmonella* spp.	<2.0	Bell pepper	Yuk et al., 2006a
	200 ppm	< 1 min	*Salmonella* Baildon	0.4	Lettuce	Weissinger et al., 2000
	200 ppm	< 1 min	*Salmonella* Baildon	0.5	Tomato	Weissinger et al., 2000
Commercial -chlorine	150 ppm	1 min	Coliform	1.0	Buckwheat sprout	Lee et al., 2009
	150 ppm	1 min	*Escherichia coli*	1.9	Buckwheat sprout	Lee et al., 2009
	150 ppm	1 min	*Sphingomonas* spp.	0.5	Buckwheat sprout	Lee et al., 2009
	100 ppm	10 min	*Salmonella* Typhimurium	0.8	Sesame leaf	Yeon et al., 2005
	100 ppm	10 min	*Listeria monocytogenes*	0.7	Sesame leaf	Yeon et al., 2005
	100 ppm	10 min	*Salmonella* Typhimurium	0.9	Cabbage	Choi et al., 2008
	100 ppm	10 min	*Listeria monocytogenes*	0.7	Cabbage	Choi et al., 2008
	100 ppm	10 min	Coliform	2.1	Sesame leaf	Yeon et al., 2005
	100 ppm	10 min	*Escherichia coli*	1.1	Sesame leaf	Yeon et al., 2005
	100 ppm	5 min	*Escherichia coli*	1.0-1.5	Lettuce	Francis and O'Beirne, 2002
	100 ppm	5 min	Coliform	2.2	Escarole	Tomás-Callejas et al., 2012a
	100 ppm	5 min	*Escherichia coli* O157:H7	1.9	Spinach	Lee and Bae, 2008
	100 ppm	5 min	*Escherichia coli* O157:H7	1.3	Spinach	Nei et al., 2009
	100 ppm	5 min	*Listeria innocua*	0.5-1.0	Lettuce	Francis and O'Beirne, 2002
	100 ppm	5 min	*Listeria monocytogenes*	2.2	Spinach	Rahman et al., 2010
	100 ppm	1 min	*Escherichia coli* O157:H7	0.7	Lettuce	López-Gálvez et al., 2010
Average log_{10}-reduction (*1.12*)						

Table 2 Efficacy of various aqueous-phase disinfectants/sanitizers for cleaning fresh fruits and vegetables

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
Electrolyzed oxidizing water	300 ppm	5 min	*Escherichia coli* O157:H7	2.0	Lettuce	Yang et al., 2003
	300 ppm	5 min	*Salmonella* Typhimurium	2.0	Lettuce	Yang et al., 2003
	300 ppm	5 min	*Listeria monocytogenes*	2.1	Lettuce	Yang et al., 2003
	≤100 ppm	30 sec	*Escherichia coli* O157:H7	4.4	Tomato	Deza et al., 2003
	≤100 ppm	30 sec	*Salmonella* Enteritidis	3.7	Tomato	Deza et al., 2003
	≤100 ppm	30 sec	*Listeria monocytogenes*	4.7	Tomato	Deza et al., 2003
	≤50 ppm	1 min	*Escherichia coli* O157:H7	2.8	Lettuce	Park et al., 2001
	≤50 ppm	1 min	*Listeria monocytogenes*	2.4	Lettuce	Park et al., 2001
			Average log_{10}-reduction (*3.01*)			
Acidified sodium chlorite	1,200 ppm	2 min	*Salmonella* spp.	>4.0	Bell pepper	Yuk et al., 2006a
	1,200 ppm	2 min	*Salmonella* spp.	>5.0	Cucumber	Yuk et al., 2006a
	500 ppm	15 min	*Escherichia coli* O157:H7	2.7	Cabbage	Inatsu et al., 2005
	500 ppm	1 min	*Escherichia coli* O157:H7	1.9	Tatsoi leaves	Tomás-Callejas et al., 2012a
	250 ppm	30 min	*Escherichia coli* O157:H7	>6.0	Carrot	Allende et al., 2007
	250 ppm	1 min	*Escherichia coli* O157:H7	2.0	Cilantro	Allende et al., 2009
	200 ppm	120 min	*Escherichia coli* O157:H7	1.8	Mung bean seed	Nei et al., 2010
	200 ppm	2 min	*Escherichia coli* O157:H7	3.1	Spinach	Zhou et al., 2009
	100 ppm	45 min	*Salmonella* spp.	1.3	Alfalfa seed	Liao, 2009
	100 ppm	5 min	*Escherichia coli* O157:H7	1.3	Spinach	Nei et al., 2009
	50 ppm	2 min	*Escherichia coli* O157:H7	0.7	Lettuce	Keskinen et al., 2009
	20 ppm	5 min	*Salmonella* spp.	>2.0	Strawberry	Issa-Zacharia et al., 2010
			Average log_{10}-reduction (*2.54*)			
Ozone water	12 ppm	15 min	*Listeria innocua*	2.1	Lettuce	Karaca and Velioglu, 2014
	12 ppm	15 min	*Listeria innocua*	2.1	Spinach	Karaca and Velioglu, 2014
	10 ppm	10 min	*Escherichia coli* O157:H7	1.1	Lettuce	Singh et al., 2002a
	10 ppm	3 min	Coliform	3.2	Lettuce	Beltran et al., 2005
	10 ppm	3 min	Total mesophilic bacteria	1.6	Lettuce	Beltran et al., 2005
	8 ppm	32 min	*Escherichia coli O157*:H7	2.5	Blueberry	Bialka et al., 2007
	8 ppm	32 min	*Salmonella* spp.	4.9	Blueberry	Bialka et al., 2007
	5 ppm	5 min	Aerobic mesophilic bacteria	1.4	Lettuce	Koseki et al., 2006
	2 ppm	3 min	*Listeria innocua*	2.8	Pepper	Alexandre et al., 2011
	0.2 ppm	5 min	Total bacterial count	1.7	Celery	Zhang et al., 2005
			Average log_{10}-reduction (2.33)			
Sanitizer	**Concentration**	**Time**	**Microorganism**	**Log_{10} reduction**	**Produce**	**Reference**
Peroxyacetic acid	500 ppm	1 min	*Escherichia coli* O157:H7	2.0	Lettuce	López-Gálvez et al., 2009
	80 ppm	2 min	*Escherichia coli* O157:H7	2.2	Spinach	Zhou et al., 2009
	80 ppm	1 min	*Cronobacter sakazakii*	4.1	Apple	Kim et al., 2006a
	80 ppm	1 min	*Cronobacter sakazakii*	3.0	Tomato	Kim et al., 2006a
	80 ppm	1 min	*Cronobacter sakazakii*	2.5	Lettuce	Kim et al., 2006a
	80 ppm	1 min	*Escherichia coli* O157:H7	<1.5	Apple	Abadias et al., 2011
	80 ppm	1 min	*Salmonella* spp.	<1.0	Apple	Abadias et al., 2011
	80 ppm	1 min	*Listeria monocytogenes*	<1.0	Apple	Abadias et al., 2011
			Average log_{10}-reduction (*2.04*)			
Hydrogen peroxide	5.0%	5 min	*Salmonella* spp.	2.2	Cantaloupe	Ukuku, 2004
	5.0%	1 min	Total plate count	1.8	Cantaloupe	Sapers et al., 2001
	2.5%	5 min	*Escherichia coli* O157:H7	3.0	Melon	Ukuku et al., 2005
	2.5%	5 min	*Listeria monocytogenes*	3.0	Melon	Ukuku et al., 2005
	2.0%	10 min	Total aerobic bacteria	1.5	Sesame leaf	Yeon et al., 2005
	2.0%	10 min	Coliform	1.7	Sesame leaf	Yeon et al., 2005
	2.0%	10 min	*Escherichia coli*	0.8	Sesame leaf	Yeon et al., 2005

	2.0%	10 min	*Salmonella* Typhimurium	0.9	Sesame leaf	Yeon et al., 2005
	2.0%	10 min	*Listeria monocytogenes*	1.0	Sesame leaf	Yeon et al., 2005
	1.0%	15 min	*Escherichia coli* O157:H7	2.8	Apple	Sapers et al., 2003
			Average log_{10}-reduction (*1.87*)			
Chlorine dioxide	200 ppm	2 min	*Escherichia coli* O157:H7	1.5	Lettuce	Keskinen et al., 2009
	100 ppm	10 min	*Salmonella* Typhimurium	1.1	Lettuce	Choi and Lee, 2008
	100 ppm	10 min	*Listeria monocytogenes*	1.1	Lettuce	Choi and Lee
	100 ppm	10 min	*Salmonella* Typhimurium	1.2	Cabbage	Choi and Lee
	100 ppm	10 min	*Listeria monocytogenes*	1.7	Cabbage	Choi and Lee
	100 ppm	10 min	*Escherichia coli* O157:H7	1.2	Lettuce	Choi and Lee
	100 ppm	10 min	*Escherichia coli* O157:H7	1.5	Cabbage	Choi and Lee
	50 ppm	5 min	*Escherichia coli* O157:H7	1.1	Alfalfa seed	Singh et al., 2003
	50 ppm	5 min	*Escherichia coli* O157:H7	1.7	Broccoli sprout	Kim et al., 2009a
	50 ppm	5 min	*Salmonella* Typhimurium	1.5	Broccoli sprout	Kim et al., 2009a
	50 ppm	5 min	*Listeria monocytogenes*	1.2	Broccoli sprout	Kim et al., 2009a
	10 ppm	10 min	*Listeria monocytogenes*	2.4	Blueberry	Wu and Kim, 2007
	10 ppm	10 min	*Pseudomonas aeruginosa*	2.2	Blueberry	Wu and Kim, 2007
			Average log_{10}-reduction (*1.49*)			

Table 3 Effectiveness of naturally occurring antimicrobials for cleaning fresh fruits and vegetables

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
Citric acid	1.0%	5 min	*Escherichia coli* O157:H7	2.7	Baby spinach	Huang et al., 2011
	0.5%	10 min	*Escherichia coli* O157:H7	1.3	Lettuce	Huang et al.
	0.5%	10 min	*Salmonella* Typhimurium	0.9	Lettuce	Huang et al.
	0.5%	10 min	*Listeria monocytogenes*	0.8	Lettuce	Sagong et al., 2011
Fumaric acid	0.5%	5 min	*Escherichia coli* O157:H7	2.2	Broccoli sprout	Kim et al., 2009a
	0.5%	5 min	*Salmonella* Typhimurium	2.0	Broccoli sprout	Kim et al., 2009a
	0.5%	5 min	*Listeria monocytogenes*	2.6	Broccoli sprout	Kim et al., 2009a
	0.5%	5 min	*Escherichia coli* O157:H7	<3.0	Clover sprout	Kim et al., 2009b
	0.5%	5 min	*Salmonella* Typhimurium	<3.0	Clover sprout	Kim et al., 2009b
	0.5%	5 min	*Listeria monocytogenes*	<2.0	Clover sprout	Kim et al., 2009b
Lactic acid	0.5%	10 min	*Escherichia coli* O157:H7	1.1	Lettuce	Sagong et al., 2011
	0.5%	10 min	*Listeria monocytogenes*	1.5	Lettuce	Sagong et al., 2011
	0.5%	10 min	*Salmonella* Typhimurium	1.2	Lettuce	Sagong et al., 2011
	0.5%	1 min	*Escherichia coli*	2.0	Lettuce	López-Gálvez et al., 2009
	0.002%	3 min	Yeast and Mold	1.8	Escarole	Allende et al., 2008
Malic acid	0.5%	10 min	*Escherichia coli* O157:H7	1.1	Lettuce	Sagong et al., 2011
	0.5%	10 min	*Salmonella* Typhimurium	1.1	Lettuce	Sagong et al., 2011
	0.5%	10 min	*Listeria monocytogenes*	1.0	Lettuce	Sagong et al., 2011
Average log_{10}-reduction (*1.74*)						

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
Thyme oil	0.5%	2 min	Aerobic plate count	2.0	Bell pepper	Uyttendaele et al., 2004
	0.5%	2 min	*Enterobacteriaceae*	<1.0	Bell pepper	Uyttendaele et al., 2004
	0.5%	2 min	*Aeromonas caviae*	<2.0	Bell pepper	Uyttendaele et al., 2004
	0.1%	24 h	*Shigella sonnei*	5.0	Lettuce	Bagamboula et al., 2004
	5 ppm	20 min	*Salmonella* Typhimurium	1.0	Apple	Tornuk et al., 2011
	5 ppm	20 min	*Salmonella* Typhimurium	1.0	Carrot	Tornuk et al.
	5 ppm	20 min	*Escherichia coli* O157:H7	1.4	Apple	Tornuk et al.
	5 ppm	20 min	*Escherichia coli* O157:H7	1.0	Carrot	Tornuk et al.
	5 ppm	5 min	*Escherichia coli* O157:H7	2.3	Alfalfa seed	Singh et al., 2003
Carvacrol	0.25%	1 min	*Salmonella enterica* serovar spp.	>5.0	Alfalfa seed	Eattson et al., 2011
	0.1%	24 h	*Shigella sonnei*	5.0	Lettuce	Bagamboula et al., 2004
	0.6 ppm	5 min	*Listeria monocytogenes*	<2.5	Mixed vegetables	de Sousa et al., 2012
	0.6 ppm	5 min	*Pseudomonas fluorescens*	<2.0	Mixed vegetables	de Sousa et al., 2012
	0.6 ppm	5 min	*Aeromonas hydrophila*	<3.0	Mixed vegetables	de Sousa et al., 2012
Cineole oil	20 ppm	5 min	*Listeria monocytogenes*	<1.0	Mixed vegetables	de Sousa, et al., 2012
	20 ppm	5 min	*Pseudomonas fluorescens*	<1.0	Mixed vegetables	de Sousa, et al., 2012
	20 ppm	5 min	*Aeromonas hydrophila*	<1.5	Mixed vegetables	de Sousa, et al., 2012
Oregano oil	250 ppm	2 min	*Enterobacteria*	1.2	Lettuce	Gutierrez et al., 2009
	250 ppm	2 min	*Pseudomonas* spp.	1.2	Lettuce	Gutierrez et al., 2009
	250 ppm	2 min	Lactic acid bacteria	1.1	Carrot	Gutierrez et al.
	250 ppm	2 min	*Pseudomonas* spp.	0.7	Carrot	Gutierrez et al.
	100 ppm	5 min	*Salmonella* Typhimurium	3.7	Tomato	Gündüz et al., 2010
Average log_{10}-reduction (*2.07*)						

Table 4 Effects of chlorine dioxide in gaseous phase for cleaning fresh fruits and vegetables

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
Gaseous - chlorine dioxide	100 ppm	60 min	*Salmonella* spp.	>5.0	Tomato	Yuk et al., 2006b
	100 ppm	15 min	*Salmonella* spp.	2.0	Mushroom	Selby et al., 2004
	100 ppm	15 min	*Escherichia coli* O157:H7	4.2	Mushroom	Selby et al., 2004
	50 ppm	10 min	*Listeria monocytogenes*	4.3	Cantaloupe	Mahmoud et al., 2008
	50 ppm	10 min	*Escherichia coli* O157:H7	4.6	Cantaloupe	Mahmoud et al., 2008
	50 ppm	6 min	*Salmonella* Poona	5.0	Cantaloupe	Mahmoud et al., 2008
	15 ppm	6 min	*Escherichia coli* O157:H7	7.0	Apple	Du et al., 2003
	10 ppm	30 min	*Escherichia coli* K-2	<1.0	Paprika	Matsufuji et al., 2009
	10 ppm	30 min	*Escherichia coli* K-2	<1.0	Cucumber	Matsufuji et al.
	10 ppm	30 min	*Escherichia coli* K-2	>2.0	Celery	Matsufuji et al.
	10 ppm	30 min	*Escherichia coli* K-2	>2.0	Onion	Matsufuji et al.
	10 ppm	30 min	*Listeria monocytogenes*	4.2	Apple	Du et al., 2002
	10 ppm	30 min	*Salmonella* spp.	3.0	Blueberry	Kaye et al., 2005
	10 ppm	30 min	*Salmonella* spp.	2.3	Strawberry	Kaye et al.
	10 ppm	30 min	*Salmonella* spp.	0.5	Raspberry	Kaye et al.
	10 ppm	10 min	*Escherichia coli* O157:H7	>5.0	Apple	Kaye et al.
	10 ppm	2 min	*Salmonella* spp.	3.9	Tomato	Trinetta et al., 2010
	5 ppm	19 min	*Salmonella* spp.	5.0	Lettuce	Mahmoud et al., 2008
	5 ppm	15 min	*Escherichia coli* O157:H7	5.0	Lettuce	Mahmoud et al., 2008
	5 ppm	10 min	*Listeria monocytogenes*	4.7	Strawberry	Mahmoud et al., 2007
	5 ppm	10 min	*Salmonella* Typhimurium	4.3	Strawberry	Mahmoud et al., 2007
	5 ppm	10 min	*Salmonella* spp.	2.8	Lettuce	Mahmoud and Linton, 2008
	5 ppm	10 min	*Listeria monocytogenes*	4.3	Cantaloupe	Mahmoud et al., 2008
	5 ppm	10 min	*Escherichia coli* O157:H7	4.6	Strawberry	Mahmoud et al., 2007
	5 ppm	10 min	*Escherichia coli* O157:H7	3.9	Lettuce	Mahmoud and Linton, 2008
	5 ppm	10 min	*Escherichia coli* O157:H7	4.6	Cantaloupe	Mahmoud et al., 2008
	5 ppm	6 min	*Salmonella* Poona	5.0	Cantaloupe	Mahmoud et al., 2008
	4 ppm	720 min	*Listeria monocytogenes*	3.9	Blueberry	Popa et al., 2007
	4 ppm	720 min	*Salmonella* Typhimurium	3.6	Blueberry	Popa et al., 2007
	4 ppm	720 min	*Escherichia coli* O157:H7	4.3	Blueberry	Popa et al., 2007
	4 ppm	720 min	*Listeria monocytogenes*	4.3	Apple	Du et al., 2002
	4 ppm	30 min	*Escherichia coli* O157:H7	1.6	Lettuce	Kaye et al., 2005b

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
Gaseous - chlorine dioxide	4 ppm	6 min	*Escherichia coli* O157:H7	5.6	Carrot	Kaye et al., 2005b
	4 ppm	6 min	*Listeria monocytogenes*	4.3	Lettuce	Lee et al., 2004
	4 ppm	6 min	*Salmonella* Typhimurium	5.0	Lettuce	Lee et al., 2004
	4 ppm	6 min	*Escherichia coli* O157:H7	3.4	Lettuce	Lee et al., 2004
	4 ppm	6 min	*Salmonella* spp.	4.4	Cabbage	Kaye et al., 2005b
	4 ppm	6 min	*Salmonella* spp.	5.2	Carrot	Kaye et al.
	4 ppm	6 min	*Salmonella* spp.	1.6	Lettuce	Kaye et al.
	4 ppm	6 min	*Escherichia coli* O157:H7	3.1	Cabbage	Kaye et al.
	3 ppm	30 min	*Listeria monocytogenes*	>6.0	Green pepper	Han et al., 2001b
	2 ppm	30 min	Total aerobic plate	1.5	Leek	Vandekinderen et al., 2009
	1 ppm	30 min	*Escherichia coli* O157:H7	6.5	Green pepper	Han et al., 2000a
	1 ppm	30 min	*Escherichia coli* O157:H7	6.5	Green pepper	Han et al., 2000b
	1 ppm	10 min	*Escherichia coli* O157:H7	6.1	Lettuce	Singh et al., 2002a
	1 ppm	10 min	*Escherichia coli* O157:H7	5.1	Carrot	Singh et al., 2002a
	<1 ppm	120 min	*Salmonella* Typhimurium	>5.0	Tomato	Mahovic et al., 2009
	<1 ppm	30 min	*Salmonella* spp.	5.0	Orange	Bhagat et al., 2011
	<1 ppm	30 min	*Escherichia coli* O157:H7	5.2	Green pepper	Han et al., 2001a
	<1 ppm	30 min	*Escherichia coli* O157:H7	3.0	Green pepper	Han et al., 2000b

	<1 ppm	10 min	*Salmonella* spp.	>5.0	Tomato	Bhagat et al., 2010
	<1 ppm	10 min	*Listeria monocytogenes*	>5.0	Tomato	Bhagat et al., 2010
	<1 ppm	10 min	*Salmonella* Typhimurium	4.3	Strawberry	Bhagat et al., 2010
	<1 ppm	10 min	*Salmonella* spp.	2.8	Lettuce	Mahmoud and Linton, 2008
	<1 ppm	10 min	*Listeria monocytogenes*	4.3	Cantaloupe	Mahmoud et al., 2008
	<1 ppm	10 min	*Escherichia coli* O157:H7	4.6	Strawberry	Mahmoud et al., 2007
	<1 ppm	10 min	*Escherichia coli* O157:H7	3.9	Lettuce	Mahmoud and Linton, 2008
	<1 ppm	10 min	*Escherichia coli* O157:H7	4.6	Cantaloupe	Mahmoud et al., 2008
Average log_{10}-reduction (*4.07*)						

Table 5 Effectiveness of several physical sanitizing technologies for cleaning fresh fruits and vegetables

Sanitizer	Concentration	Time	Microorganism	Log_{10} reduction	Produce	Reference
Hydrostatic-pressure	650 MPa	6 min	*Escherichia coli* O157:H7	5.7	Alfalfa seed	Neetoo et al., 2008
	600 MPa	25 min	*Salmonella* spp.	4.5	Alfalfa seed	Neetoo et al., 2010
	600 MPa	10 min	*Escherichia coli* O157:H7	3.7	Alfalfa seed	Neetoo et al., 2009b
	600 MPa	2 min	*Escherichia coli* O157:H7	<6.0	Alfalfa seed	Neetoo et al., 2009a
	500 MPa	2 min	*Escherichia coli* O157:H7	>5.0	Green onion	Neetoo et al., 2011
	500 MPa	2 min	*Salmonella* spp.	>5.0	Green onion	Neetoo et al., 2011
	475 MPa	8 min	*Escherichia coli* O157:H7	2.0	Alfalfa seed	Ariefdjoham et al., 2004
	475 MPa	8 min	*Listeria monocytogenes*	1.1	Alfalfa seed	Ariefdjoham et al., 2004
	450 MPa	2 min	*Salmonella* spp.	6.6	Pepper	Neetoo and Chen, 2012
	350 MPa	2 min	*Salmonella* Braenderup	3.5	Tomato	Maitland et al., 2011
	300 MPa	10 min	Aerobic mesophilic bacteria	4.2	Cabbage	Peñas et al., 2010
	300 MPa	10 min	Lactic acid bacteria	4.2	Cabbage	Peñas et al., 2010
	250 MPa	2 min	*Escherichia coli* O157:H7	1.3	Strawberry puree	Huang et al., 2013
	250 MPa	2 min	*Salmonella* spp.	2.4	Strawberry puree	Huang et al., 2013
			Average log-reductions (*3.94*)			
Ultraviolet	1 kJ/m^2	3 min	*Escherichia coli* O157:H7	1.0	Clover sprout	Kim et al., 2009b
	1 kJ/m^2	3 min	*Salmonella* Typhimurium	1.1	Clover sprout	Kim et al., 2009b
	1 kJ/m^2	3 min	*Listeria monocytogenes*	0.9	Clover sprout	Kim et al., 2009b
	0.45 kJ/m^2	15 sec.	*Escherichia coli* O157:H7	<1.0	Mushroom	Guan et al., 2013
	150 mJ/cm^2	10 min	*Salmonella* Typhimurium	2.1	Lettuce	Ge et al., 2013
	150 mJ/cm^2	10 min	*Salmonella* Typhimurium	1.5	Green onion	Ge et al., 2013
	59-72 J/cm^2	1 min	*Escherichia coli* O157:H7	3.9	Raspberries	Bialka et al., 2008
	59-72 J/cm^2	1 min	*Salmonella* spp.	3.4	Raspberries	Bialka et al., 2008
	23-34 J/cm^2	1 min	*Escherichia coli* O157:H7	2.1	Strawberries	Bialka et al., 2008
	23-34 J/cm^2	1 min	*Salmonella* spp.	2.8	Strawberries	Bialka et al., 2008
	7 J/cm^2	30 min	*Escherichia coli*	<1.0	Lettuce	Birmpa et al., 2013
	7 J/cm^2	30 min	*Listeria innocua*	<1.0	Lettuce	Birmpa et al., 2013
	7 J/cm^2	30 min	*Salmonella* Enteritidis	<4.0	Lettuce	Birmpa et al., 2013
	7 J/cm^2	30 min	*Staphylococcus aureus*	<1.0	Lettuce	Birmpa et al., 2013
	24 mW/cm^2	<10 s	*Escherichia coli* O157:H7	<3.0	Lettuce	Yaun et al., 2004
	24 mW/cm^2	<10 s	*Salmonella* spp.	<3.0	Lettuce	Yaun et al., 2004
			Average log-reduction (*2.17*)			
	Concentration	**Time**	**Microorganism**	**Log_{10} reduction**	**Produce**	**Reference**
Ultrasound	40 kHz	30 min	*Escherichia coli* O157:H7	0.3	Alfalfa seed	Kim et al., 2006b
	40 kHz	5 min	*Bacillus cereus* spores	<1.5	Carrot	Sagong et al., 2013
	40 kHz	5 min	*Bacillus cereus* spores	<2.0	Cucumber	Sagong et al., 2013
	40 kHz	5 min	*Bacillus cereus* spores	<1.5	Lettuce	Sagong et al., 2013
	40 kHz	1 min	Total aerobic bacteria	0.4	Lettuce	Forghani et al., 2013
	38-40 kHz	5 min	*Salmonella enterica* serovar spp.	0.7	Alfalfa seed	Scouten et al., 2002
	38-40 kHz	5 min	*Escherichia coli* O157:H7	0.6	Alfalfa seed	Scouten et al., 2002
	32-40 kHz	10 min	*Salmonella* Typhimurium	1.6	Lettuce	Seymour et al., 2002
	37 kHz (1,200W)	100 min	*Cronobacter sakazakii*	0.6	Lettuce	Park et al., 2016
	37 kHz	30 min	*Escherichia coli*	1.8	Lettuce	Birmpa et al., 2013
	37 kHz	30 min	*Listeria innocua*	1.3	Lettuce	Birmpa et al., 2013
	37 kHz	30 min	*Salmonella* Enteritidis	1.4	Lettuce	Birmpa et al., 2013
	37 kHz	30 min	*Staphylococcus aureus*	1.2	Lettuce	Birmpa et al., 2013
	26 kHz (200W)	5-25 min	*Salmonella* spp.	1.5-2.5	Lettuce	Millan-Sango et al., 2016
	26 kHz (200W)	5 min	*Escherichia coli* O157:H7	2.7	Lettuce	Millan-Sango et al., 2015

	20 kHz	10 min	*Escherichia coli* O157:H7	0.3	Lettuce	Yoon et al., 2013
	20 kHz	10 min	*Listeria monocytogenes*	0.4	Lettuce	Yoon et al., 2013
	20 kHz	10 min	*Salmonella* Typhimurium	0.5	Lettuce	Yoon et al., 2013
	20 kHz	10 min	*Escherichia coli* O157:H7	1.5	Apple	Yoon et al., 2013
	20 kHz	10 min	*Listeria monocytogenes*	2.7	Apple	Yoon et al., 2013
	20 kHz	10 min	*Salmonella* Typhimurium	2.2	Apple	Yoon et al., 2013
Average log-reduction (*1.31*)						

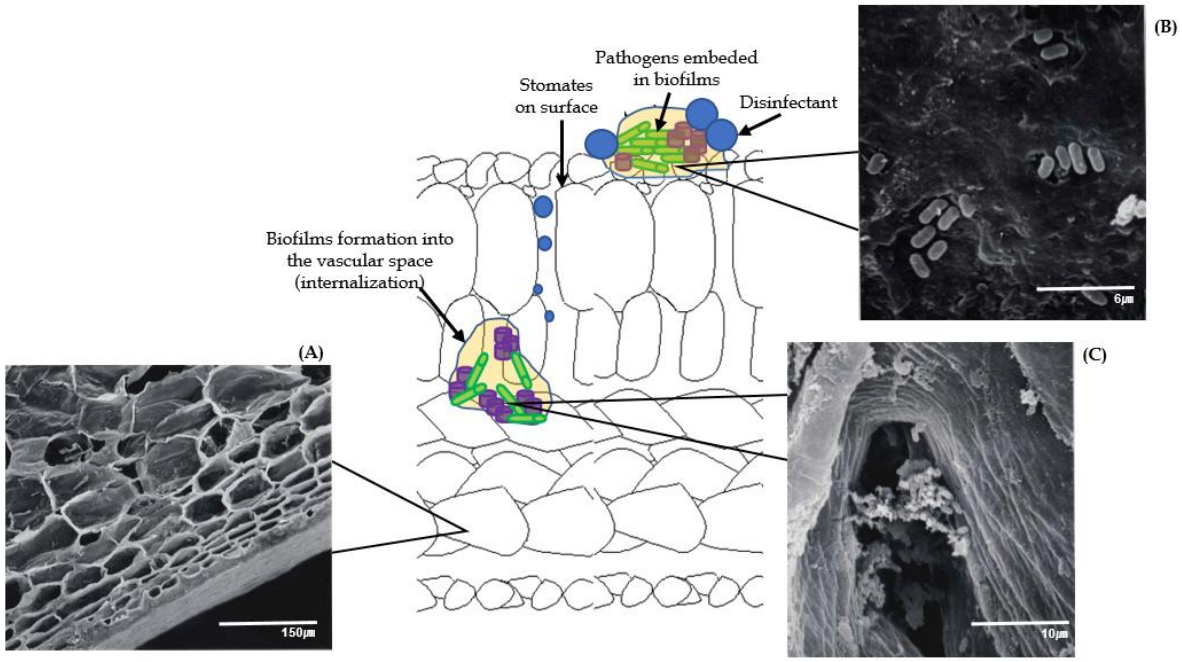

Figure 1 A schematic diagram of the plant body structure contaminated with microorganisms [this picture was rearranged, based on a study conducted by Linton et al., (2006)]. (A), a scanning electron microscopy image of the surface of a gala apple; (B), stomates contaminated with *E. coli* O157:H7; (C) an aggregate of *E. coli* O157:H7 internalized into a stoma of apple.

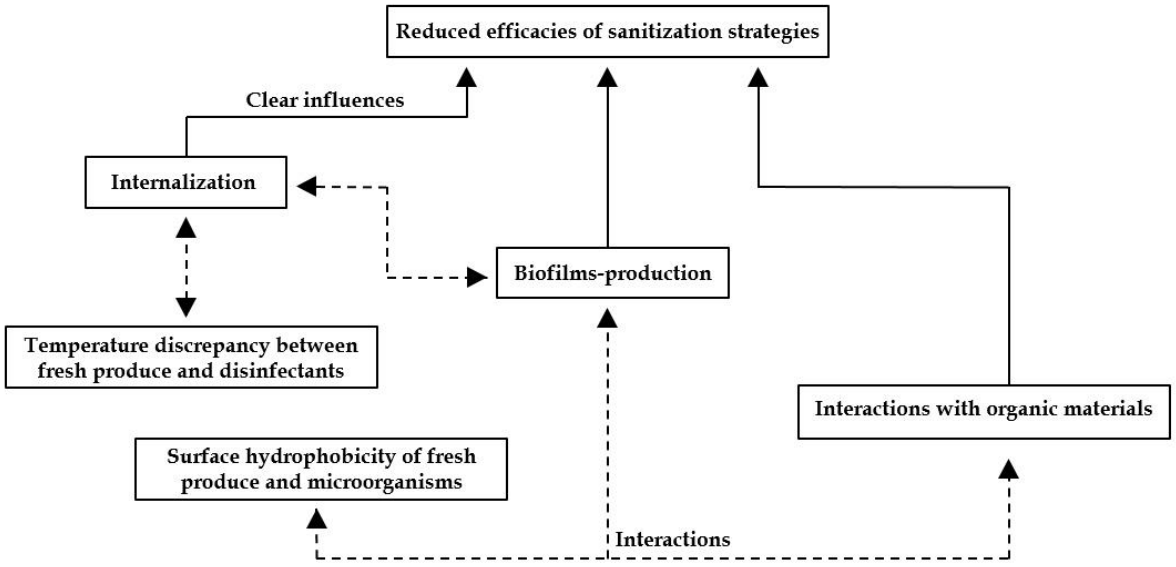

Figure 2 Leading figures in deteriorating the decontamination activity of sanitization methods.