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Alternative disinfection methods to chlorine for use in the fresh-cut industry

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Abstract

The use of chlorine as a disinfectant in the fresh-cut produce industry has been identified as a concern mainly due to public health issues. In fact, this chemical, commonly used as hypochlorous acid and hypochlorite, has already been prohibited in some European countries, due to the potential production of toxic by-products, such as chloroform and other trihalomethanes, chloramines and haloacetic acids. The search for alternative methods of disinfection is therefore a current and on-going challenge in both Academia and Industry. Some methods are well described in the literature on the disinfection of food-contact surfaces and process water and also on the decontamination of the produce. These methods are commonly classified as biological (bacteriocins, bacteriophages, enzymes and phytochemicals), chemical (chlorine dioxide, electrolyzed oxidizing water, hydrogen peroxide, ozone, organic acids, etc) and physical (irradiation, filtration, ultrasounds, ultraviolet light, etc). This review provides updated information on the state of the art of the available disinfection strategies alternative to chlorine that can be used in the fresh-cut industry. The use of combined methods to replace and/or reduce the use of chlorine is also reviewed.

Keywords: Chlorine, disinfection, fresh produce, sanitation, surfaces, water.

List of Abbreviations

AcEOW	Acid electrolyzed oxidizing water
AlEOW	Alkaline electrolyzed oxidizing water
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
EOW	Electrolyzed oxidizing water
EPS	Extracellular polymeric substances
FDA	Food and Drug Administration
GRAS	Generally recognized as safe

MF	Microfiltration
MPV	Minimally processed vegetables
MWCO	Molecular weight cut off
NEOW	Neutral electrolyzed oxidizing water
NF	Nanofiltration
PAA	Peracetic acid
QACs	Quaternary ammonium compounds
RO	Reverse osmosis
SS	Stainless steel
UF	Ultrafiltration
US	Ultrasounds
UV	Ultraviolet

1. Introduction

Fresh produce and minimally processed vegetables (MPV) are widely consumed worldwide as they are important natural sources of essential nutrients. For the modern consumer, these products are necessary to maintain a healthy diet, and their fresh and nutritional status is largely recognized. However, despite the increased awareness of food safety issues, the occurrence of foodborne disease outbreaks related to these products is constantly increasing (Gilbert & McBain, 2003; Ölmez & Kretzschmar, 2009; Vitale & Schillaci, 2016) with several pathogenic bacteria associated, such as *Listeria monocytogenes*, *Clostridium botulinum*, *Bacillus cereus*, *Escherichia coli* O157:H7 and *Salmonella* spp. (Olaimat & Holley, 2012; Seiber, 2012; Warriner, Huber, Namvar, Fan, & Dunfield, 2009), as well as viruses (norovirus and hepatitis A) and protozoa (*Cryptosporidium parvum*) (Berger, Sodha, Shaw, Griffin, Pink, Hand, & Frankel, 2010; Yaron & Romling, 2014). Noteworthy, *E. coli* O157:H7 and *Salmonella* spp. are the two microorganisms linked to the largest foodborne outbreaks and consequent human infections (Warriner et al., 2009; Yaron & Romling, 2014).

Contamination of fresh produce can occur through the water, air, soil, insect vectors, equipment or even through the improper handling by the workers (Martinez-Vaz, Fink, Diez-Gonzalez, & Sadowsky, 2014). For instance, microbial adhesion on food-contact surfaces (i.e. equipment including conveyor belts and containers used along the food chain - in harvesting, post-harvesting and packaging (Food and Drug Administration, 1998)) can ultimately lead to the formation of biofilms (Vitale & Schillaci, 2016; Yaron & Romling, 2014) and the subsequent produce contamination. Biofilms are sessile communities of microorganisms that initially attach to a wet solid surface, and subsequently grow producing extracellular polymeric substances (EPS) that keep the cells strongly together and also protect them from external stress conditions (Kumar & Anand, 1998). Biofilms have a negative impact as they can form on the produce and on the food-contact surfaces impairing surface sanitation and produce decontamination (Kumar & Anand, 1998; Martinez-Vaz et al., 2014). More importantly, microbial contamination can also lead to the internalization of pathogens into the produce. For instance, both *E. coli* and *S.* Typhimurium are capable of penetrating the leaves of iceberg lettuce (Golberg, Kroupitski, Belausov, Pinto, & Sela, 2011), while Seo and Frank (1999) demonstrated that *E. coli* O157:H7 can penetrate 20-100 µm below the surface of lettuce leaves. Through chemotaxis processes and flagellar motility, *Salmonella* spp. can also penetrate lettuce leaves (Kroupitski, Golberg, Belausov, Pinto, Swartzberg, Granot, & Sela, 2009). The internalization can occur in the stomata, vasculature, cut edges, intercellular tissues, etc (Erickson, 2012). Consequently, the elimination of such pathogens already internalized in the produce is rather impossible, making the subsequent minimal processing totally ineffective to assure product safety (Erickson, 2012; Ge, Bohrerova, & Lee, 2013).

To increase the shelf life and also enhance the microbial safety of these products, chlorine is commonly applied as hypochlorous acid and hypochlorite in the fresh-cut industry as a disinfectant at concentrations varying between 50 and 200 ppm of free chlorine and for a maximum exposure time of 5 min (Goodburn & Wallace, 2013; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). It was verified that this is the maximum exposure time applied, since other works (Adams, Hartley, & Cox, 1989) microorganisms". The exposure time can also depend on the microorganism (Tirpanalan, Zunabovic, Domig, & Kneifel, 2011). Chlorine is indeed widely used in the food industry (Sagong, Lee, Chang, Heu, Ryu, Choi, & Kang, 2011; Van Haute,

Sampers, Holvoet, & Uyttendaele, 2013) due to its relatively low price, facility to apply and wide spectrum of antimicrobial effectiveness (Ramos, Miller, Brandão, Teixeira, & Silva, 2013). However, this disinfectant shows, under certain circumstances, limited efficiency in reducing microbial loads (Yaron & Romling, 2014), as it can be easily inactivated by organic matter (Parish, Beuchat, Suslow, Harris, Garrett, Farber, & Busta, 2003; Ramos et al., 2013), and its action is highly pH dependent (Ramos et al., 2013). Furthermore, this disinfectant can produce unhealthy by-products including carcinogenic and mutagenic chlorinated compounds, such as chloroform and other trihalomethanes, chloramines and haloacetic acids, when reacting with organic molecules (Bull, Reckhow, Li, Humpage, Joll, & Hrudey, 2011; Legay, Rodriguez, Sérodes, & Levallois, 2010). Also, it is corrosive and has been included in the indicative list of the Directive on Industrial Emissions (IPPC, 2007/0286 (COD)), aiming to reduce harmful industrial emissions across the EU, therefore benefiting the environment and human health (European Comission, 2007). Its use is already prohibited in some European countries (Belgium, Denmark, Germany and The Netherlands) (Bilek & Turantaş, 2013; Fallik, 2014; Ölmez & Kretzschmar, 2009; Ramos et al., 2013).

Although disinfection with chlorine is widespread in the fresh-cut industry, there is a global concern on developing alternative disinfection strategies to minimize its environmental and public health impacts (Gopal, Coventry, Wan, Roginski, & Ajlouni, 2010; Meireles, Machado, Fulgêncio, Mergulhão, Melo, & Simões, 2015). Different methods to reduce and/or replace the use of chlorine were already developed. Those include biological methods, alternative chemical compounds and physical technologies, or even the combination of methods (Bilek & Turantaş, 2013; Fallik, 2014; Gil, Selma, López-Gálvez, & Allende, 2009; Goodburn & Wallace, 2013; Holah, 2014; Ölmez & Kretzschmar, 2009; Otto, Zahn, Rost, Zahn, Jaros, & Rohm, 2011) (Fig. 1). Most of those methods are recognized as environmentally friendly, and do not represent a potential risk to the health and safety of workers and consumers (Fallik, 2014; Holah, 2014; Lado & Yousef, 2002). Some good reviews on those alternative disinfection strategies have already been published (Forsythe & Hayes, 1998; Gil et al., 2009; Gopal et al., 2010; Lado & Yousef, 2002; Ölmez & Kretzschmar, 2009; Ramos et al., 2013; Tirpanalan et al., 2011) and the last one was written in 2013 (Ramos et al., 2013). The purpose of this review is to provide updated information on all those alternative methods (biological, chemical and physical) taking into account each target: produce,

130 food-contact surfaces and water (Table 1). The use of combined methods to replace
131 and/or reduce the use of chlorine is also reviewed.
132

133 **2.1. Biological-based methods**

134 2.1.1. Bacteriocins

135 One possibility to prevent the growth of both spoilage and pathogenic microorganisms
136 is the exploitation of their competition with other microorganisms, typically with
137 beneficial ones (Ramos et al., 2013). Lactic acid bacteria (LAB) are an example of such
138 beneficial microorganisms, having GRAS (Generally Recognized as Safe) status.
139 Furthermore, LAB produce antimicrobial compounds, such as organic acids and
140 bacteriocins, which can be used as antimicrobials (Rodgers, 2001, 2008). A well-known
141 example of such compound is the bacteriocin nisin. This natural preservative is
142 produced by *Lactococcus lactis* and is effective mainly against Gram positive bacteria
143 (Arevalos-Sánchez, Regalado, Martin, Domínguez-Domínguez, & García-Almendárez,
144 2012; Davies & Delves-Broughton, 1999; Hansen, 1994; Magalhães, Mena, Ferreira,
145 Silva, Almeida, Gibbs, & Teixeira, 2014). The Gram negative bacteria are not affected
146 by this peptide due to their outer membrane. Nevertheless, this drawback can be
147 overcome by exposing the cells to chelating agents or osmotic shock that destabilize the
148 outer membrane before the use of nisin (Angiolillo, Conte, & Del Nobile, 2014; Delves-
149 Broughton, 2005). Nisin acts on the cell membrane forming pores that result in cell
150 death (Arevalos-Sánchez et al., 2012; Bari, Ukuku, Kawasaki, Inatsu, Isshiki, &
151 Kawamoto, 2005). The major advantage on the use of nisin as a disinfectant is the fact
152 that it is harmless and is already used as a food preservative (O'Keeffe & Hill, 1999).
153 With regard to fresh produce, Bari et al. (2005) used nisin at 50 ppm for 1 min on mung
154 bean and broccoli and achieved the reduction of *L. monocytogenes* by 2.20 and 4.35 log
155 CFU/g, respectively. The disinfecting efficacy observed with 1 min contact time was
156 higher for broccoli. Allende, Martínez, Selma, Gil, Suárez, and Rodríguez (2007)
157 incorporated nisin and coagulin individually and combined in tryptic soy agar (TSA)
158 plates and obtained reductions of *L. monocytogenes* of 1.0-1.5 log colony forming units
159 (CFU) after 48 hours of storage at 4 ^{0}C. Bacteriocins have also been used to disinfect
160 stainless steel (SS) and glass surfaces. Arevalos-Sánchez et al. (2012) used nisin at
161 6.75×10^{-3} ppm for 5 min at 20 ^{0}C to eliminate *L. monocytogenes* from SS and achieved

a 2.58 log CFU/cm^2 reduction. Applying the same concentration for 20 min in glass surfaces resulted in 1.92 log CFU/cm^2 reduction, which means that glass surfaces are more difficult to clean. The search for new chemicals from the microbial metabolism will certainly provide new disinfectants to be applied in the food industry. In fact, it is estimated that less than 1% of the bacteria are culturable with current methods, which lead to an underestimation of the microbial diversity and a lack of knowledge on the microbial metabolites available in Nature (Lasa, Mira, Camelo-Castillo, Belda-Ferre, & Romalde; Ling, Schneider, Peoples, Spoering, Engels, Conlon, Mueller, Schaberle, Hughes, Epstein, Jones, Lazarides, Steadman, Cohen, Felix, Fetterman, Millett, Nitti, Zullo, Chen, & Lewis, 2015).

2.1.2. Bacteriophages

The use of bacteriophages as preservatives and disinfecting agents is not a recent application, and the interest on these agents has increased throughout the years (Hughes, Sutherland, Clark, & Jones, 1998; Kudva, Jelacic, Tarr, Youderian, & Hovde, 1999; Sharma, Ryu, & Beuchat, 2005; Spricigo, Bardina, Cortés, & Llagostera, 2013). Bacteriophages are viruses that infect bacteria causing their lysis (Simões, Simões, & Vieira, 2010). The main advantages on the use of lytic bacteriophages to destroy unwanted bacteria are their: i) specificity; ii) mode of action (Spricigo et al., 2013); iii) availability (Hughes et al., 1998); and iv) reduced effects on the organoleptic properties of the products (Sharma et al., 2005). Spricigo et al. (2013) used three different lytic bacteriophages (UAB_Phi 20, UAB_Phi78, and UAB_Phi87) to control *S.* Typhimurium and *S.* Enteritidis on lettuce. The treatment was performed for 60 min at room temperature and the reduction achieved was 3.9 and 2.2 log CFU/g for *S.* Typhimurium and *S.* Enteritidis, respectively (Spricigo et al., 2013). Although CFU reduction was observed, the treatment time is too long making this strategy impractical for the fresh-cut industry. Kudva et al. (1999) demonstrated that the combination of these three phages was capable to cause the lysis of *E. coli* O157:H7 at both 4 and 37 ^{0}C. The use of phages was also studied to inactivate *L. monocytogenes* on melons by (Leverentz, Conway, Camp, Janisiewicz, Abuladze, Yang, Saftner, & Sulakvelidze, 2003). These authors obtained a reduction of 2.0–4.6 log CFU per sample (Leverentz et al., 2003). Sillankorva, Oliveira, Vieira, Sutherland, and Azeredo (2004) used a lytic phage (phage ϕ S1) on SS coupons and were able to remove 80% of *P. fluorescens* biofilms. These evidences on the efficacy of bacteriophages to control spoilage and

pathogenic microorganisms are promising. Nevertheless, further research is required to increase the antimicrobial action of bacteriophages and to reduce the contact time.

2.1.3. Enzymes

Enzymes can attack directly the biofilms interfering with their development process, catalyze the formation of antimicrobials, interfere with quorum sensing events, or even destroy a mature biofilm (Simões et al., 2010; Thallinger, Prasetyo, Nyanhongo, & Guebitz, 2013). Enzymes mainly target the extracellular polymeric matrix which surrounds the biofilm cells and influences the shape of biofilm structure and its resistance to shear forces (Lequette, Boels, Clarisse, & Faille, 2010). Therefore, enzymes can be considered as an alternative method to conventional chemical disinfectants to remove biofilms from produce leaves and/or from abiotic surfaces. Like the bacteriophages the application of enzymes requires prolonged contact times to be effective in biofilm control. Another disadvantages on the use of enzymes for biofilm removal is the fact that the EPS are heterogeneous. Therefore the use of pure enzymes do not guarantee complete biofilm elimination. In fact, they should be used as a mixture or combined with other treatments, particularly antimicrobial agents (Augustin, Ali-Vehmas, & Atroshi, 2004; Lequette et al., 2010). These formulations are mostly applied for the disinfection of food-contact surfaces (Thallinger et al., 2013). Another drawback on the use of enzymes is their relative high cost (Augustin et al., 2004; Simões et al., 2010; Thallinger et al., 2013).

Typical applications of enzymes on biofilm removal are the use of proteases in pipelines and the removal of proteins from contact lenses (Augustin et al., 2004). Some studies have been developed to remove bacterial biofilms found in the food industry particularly on SS surfaces, with the use of proteolytic enzymes (Lequette et al., 2010). Lequette et al. (2010) used a buffer with an anionic surfactant mixed with α-amylase during 30 min and found that this treatment reduced the biofilm of *Bacillus mycoides* on SS surfaces by 2.98 log CFU/cm^2. Augustin et al. (2004) obtained reductions of 4 log CFU/mL after treatment with enzymatic solutions (Pandion, Resinase, Spezyme and Paradigm used individually) for 30 min.

2.1.4. Phytochemicals

Plants have the ability to produce secondary metabolites (phytochemicals) with antimicrobial properties against several microorganisms, including pathogens (Belletti, Lanciotti, Patrignani, & Gardini, 2008; Cowan, 1999). These metabolites are divided into diverse chemical classes, such as alkaloids, essential oils, phenolics, polyphenolics, polyacetylenes, lectins and peptides (Borges, Abreu, Malheiro, Saavedra, & Simõe, 2013; Cowan, 1999); and subclasses: isothiocyanates, terpenoids, thiosulfinates, phenolic acids, simple phenols, terpenoids, polyamines, polyketides, quinones, flavones, flavonoids, flavonols, etc (Cowan, 1999; Newman, Cragg, & Snader, 2000; Simões, Lemos, & Simões, 2012). Many of these molecules have GRAS status and are already widely used in the food industry (Singh, Singh, Bhunia, & Stroshine, 2002b). Given their great variability, the mode of action of phytochemicals is quite diverse. The most common effect involves the increase of the cell membrane permeability leading to the leakage of intracellular compounds (Singh et al., 2002b; Tiwari, Valdramidis, O' Donnell, Muthukumarappan, Bourke, & Cullen, 2009). Such promising phytochemicals are the essential oils, which are mostly used as flavoring agents for foodstuffs and in perfumery; however, they are also used as antimicrobial agents in the food industry (Borges et al., 2013; Cowan, 1999). Carvacrol is the main component of the essential oil of oregano and thyme. This phytochemical was used by Roller and Seedhar (2002) to disinfect kiwi at a concentration of 150 ppm and resulted in 4.6 log CFU/g reduction of the total viable counts. It has also been used on food-contact surfaces. Soni, Oladunjoye, Nannapaneni, Schilling, Silva, Mikel, and Bailey (2013) used concentrations from 0.05 to 0.1% of carvacrol (with an exposure time of 1 hour) and reduced 7 log CFU of *Salmonella* sp. on polystyrene (PS) and SS surfaces. Other authors (Knowles & Roller, 2001) used carvacrol (2 mM) and were able to eliminate 2-3 log CFU of adhered bacteria (listeriae and salmonellae) from SS. Gündüz, Gönül, and Karapınar (2010) used oregano oil to inactivate *S.* Typhimurium on lettuce at three different concentrations (25, 40 and 75 ppm) and for four different contact times (5, 10, 15 and 20 min). The treatments did not exceed a reduction of 1.92 log CFU/g regardless the condition tested (Gündüz et al., 2010). Those authors also found that the efficacy of 75 ppm of oregano oil was comparable to 50 ppm of chlorine in the disinfection of lettuce (Gündüz et al., 2010). Phenolic compounds are the most important and abundant class of phytochemicals (Borges et al., 2013). Cinnamic acid was also used in the study of Roller and Seedhar (2002) with antimicrobial efficacy similar to carvacrol. Even if they are green chemicals, phytochemicals can alter the organoleptic properties of the fresh

produce (Belletti et al., 2008; Kentish & Ashokkumar, 2011; Roller & Seedhar, 2002) and the high cost of some of those products can limit their current use at industrial scale (Roller & Seedhar, 2002). In order to have a practical application in the food industry with higher CFU reductions and lower contact times, the use of phytochemicals has to be further studied on the search for new and more effective products and/or on their potential synergistic effects when combined with other methods.

2.2. Chemical-based methods

2.2.1. Calcium lactate

Calcium is usually used to maintain the firmness of fresh produce during storage (Rico, Martín-Diana, Frías, Henehan, & Barry-Ryan, 2006) since this is able to interact with pectin, maintaining the structure of the cell wall, while lactate has antimicrobial properties (Martín-Diana, Rico, Barry-Ryan, Frias, Mulcahy, & Henehan, 2005a; Martín-Diana, Rico, Frías, Henehan, Mulcahy, Barat, & Barry-Ryan, 2006). Calcium lactate has also the advantage of not giving an off-flavor and bitterness to the products (Martín-Diana, Rico, Barry-Ryan, Frías, Mulcahy, & Henehan, 2005b). However, the number of research studies with this product is scarce. One such study by Martín-Diana et al. (2005b) concluded that using a solution of 3×10^4 ppm of calcium lactate resulted in the same reduction of mesophilic counts in fresh-cut lettuce as a solution of 12×10^4 ppm of sodium hypochlorite, while this treatment was considered acceptable by the consumer.

2.2.2. Chlorine dioxide

The use of chlorine dioxide (ClO_2) in the fresh-cut industry was studied by Tomás-Callejas et al. (2012) and compared with sodium hypochlorite. The authors found that ClO_2: i) has a higher oxidation capacity; ii) does not react with nitrogen or ammonia to form harmful by-products (Rico et al., 2007); iii) has lower reactivity with organic matter; iv) is less corrosive than chlorine (Ölmez & Kretzschmar, 2009); and v) can inhibit enzymatic browning (Chen, Zhu, Zhang, Niu, & Du, 2010). However, the use of ClO_2 also presents some disadvantages: i) its maximum allowed concentration is low (3 ppm) (21CFR173.300, 2014); ii) it is unstable since it is explosive and has to be generated on site (Gómez-López, Rajkovic, Ragaert, Smigic, & Devlieghere, 2009); iii) its antimicrobial efficiency is pH dependent (Ölmez & Kretzschmar, 2009); and iv) it is

readily degraded when exposed to sunlight (Tomás-Callejas, López-Gálvez, Sbodio, Artés, Artés-Hernández, & Suslow, 2012). ClO_2 can be produced by two different ways: the reaction of an acid with sodium chlorite, or the reaction of sodium chlorite with chlorine gas (Ölmez & Kretzschmar, 2009) and as thus this can be obtained in either aqueous or gaseous forms, respectively (Macnish, Leonard, & Nell, 2008). Although it is a disinfectant accepted by FDA (Food and Drug Administration) (21CFR173.300, 2014), its use is still under assessment by the EU in the Regulation No 1062/2014 (EFSA, 2015). The mode of action of ClO_2 is related to its penetration through the cell membrane and the subsequent inhibition of metabolic functions (Joshi, Mahendran, Alagusundaram, Norton, & Tiwari, 2013). López-Gálvez, Allende, Truchado, Martínez-Sánchez, Tudela, Selma, and Gil (2010) found that ClO_2 is as effective as sodium hypochlorite with the advantage of not forming trihalomethanes. Singh, Singh, Bhunia, and Stroshine (2002a) used ClO_2 at 10 ppm for 5 min and obtained 1.2 log CFU/g reduction of *E. coli* O157:H7 on lettuce. In the work of Mahmoud and Linton (2008) treatment with ClO_2 gas significantly reduced selected pathogens and inherent microorganisms on lettuce; however, a negative impact on the visual leaf quality was observed. Chung, Huang, Yu, Shen, and Chen (2011) evaluated the bactericidal efficacy of ClO_2 and sodium hypochlorite solution for six types of fresh-cut vegetables and fruits and found that 100 ppm of ClO_2 solution reduced 3.5-4.0 log CFU per g of lettuce, carrot and tomato which was better than the action of the sodium hypochlorite solution. Using the same concentration of ClO_2, Keskinen, Burke, and Annous (2009) achieved 1.25 log CFU/g reduction of *E. coli* O157:H7 on lettuce. Trinetta, Vaidya, Linton, and Morgan (2011) studied gaseous ClO_2 and found no chemical residues in the fresh products tested (tomatoes, lettuce, cantaloupe, alfalfa sprouts, oranges, apples and strawberries). Kreske, Ryu, Pettigrew, and Beuchat (2006) reduced the biofilms of *B. cereus* in 4.42 log CFU per SS coupon applying 200 µg/mL of ClO_2. This sanitizer has also been used to remove *L. monocytogenes* biofilms by Robbins, Fisher, Moltz, and Martin (2005). These authors used 5% ClO_2 for 10 min and achieved a reduction of 4.14 log CFU/chip. Apparently, ClO_2 is a disinfectant equally effective compared to sodium hypochlorite but at lower concentrations and similar contact times.

2.2.3. Copper compounds

Microorganisms need copper (Cu) at very low concentrations as a micronutrient, mainly used as a cofactor for certain enzymes and metalloproteins. However, at high

concentrations it alters the membrane integrity, inactivates enzymes and produces free radicals causing cell death (Ibrahim, Yang, & Seo, 2008). Copper compounds have been mainly used as fungicides, acting as a mediator of hydroperoxide, inducing cell damage (Costa, 2008). This process is irreversible and affects the respiratory chain, with the consequent loss of viability (Cerioni, Rapisarda, Hilal, Prado, & Rodríguez-Montelongo, 2009). The major limitation on the use of copper is related with its toxicity. Copper concentrations ranging from 0.6 to 2.4 ppm have been reported as 96 h LC50 median lethal concentration values for juvenile *Penaeus monodon* (Chen & Lin, 2001). Copper is usually used in combination with other products such as lactic acid (Gyawali, Ibrahim, Abu Hasfa, Smqadri, & Haik, 2011; Ibrahim et al., 2008), sodium hypochlorite combined with ultrasounds (Rodgers & Ryser, 2004), sodium hypochlorite and hydrogen peroxide (Cerioni, Lazarte Mde, Villegas, Rodriguez-Montelongo, & Volentini, 2013; Cerioni et al., 2009; Cerioni, Volentini, Prado, Rapisarda, & Rodriguez-Montelongo, 2010). These combinations demonstrated to increase significantly the antimicrobial effects compared to the products alone.

2.2.4. Electrolyzed oxidizing water

Electrolyzed oxidizing water (EOW) is a relatively new technology applied in the food industry. EOW, also known as activated water, is formed by the electrodyalisis of a sodium chloride solution in an electrolysis chamber with an anode and a cathode separated by a membrane (Cheng, Dev, Bialka, & Demirci, 2012; Demirci & Bialka, 2010; Deza, Araujo, & Garrido, 2003). To produce EOW, a salt diluted solution and current are passed through the chamber dissociating the solution into two separated streams: acid EOW (AcEOW) and alkaline EOW (AlEOW) (Hricova, Stephan, & Zweifel, 2008; Ongeng, Devlieghere, Debevere, Coosemans, & Ryckeboer, 2006). The acid solution (pH between 2.5 and 3.5) is formed at the anode and it comprises HCl, HOCl, Cl_2, OCl^-, and O_2 and it also has a high oxidation-reduction potential (ORP) - between 1000 and 1200 mV. This solution is antimicrobial and with a mode of action similar to chlorine (DNA mutations, disruption of cell proteins and enzymes). Additionally and due to its acidity, the cell membrane can be disrupted and the action of hypochlorous acid is facilitated (Demirci & Bialka, 2010; Huang, Hung, Hsu, Huang, & Hwang, 2008). The alkaline solution (pH between 10 and 11.5) is produced at the cathode and is composed by hydroxyl ions, which can react with sodium ions forming sodium hydroxide (Cheng et al., 2012; Hricova et al., 2008). This alkaline solution

works as a detergent and has a negative ORP (-800 to -900 mV) (Cheng et al., 2012). The neutral EOW (NEOW) (pH of 7 and an ORP of 700 mV) can be formed by the mixture of these two solutions (Cheng et al., 2012). In fact, the existence of a separating membrane is not mandatory and the anode and cathode solutions can be mixed inside the electrolysis cell. This method can be advantageous as the absence of the membrane avoids the occurrence of fouling, while the combined solution has advantages (Demirci & Bialka, 2010). NEOW is not so aggressive to the food-contact surfaces and is more stable than AcEOW, as chlorine decay occurs at low pH (Abadias, Usall, Oliveira, Alegre, & Viñas, 2008; Cheng et al., 2012; Deza et al., 2003). NEOW can be used to disinfect food-contact surfaces and decontaminate the produce as it does not change the color or the appearance of the produce due to the neutral pH of the solution (Ayebah & Hung, 2005; Rico, Martín-Díana, Barry-Ryan, Frías, Henehan, & Barat, 2008b). This method is environmentally friendly since it only uses salt and water to produce the chemical solution; there are no problems on handling the solution; and when this solution comes in contact with organic matter or when diluted with tap water it becomes water and can be safely discarded (Aday, 2016; Huang et al., 2008). Moreover, its use has already been approved by the FDA at a maximum concentration of 200 ppm (Food and Drug Administration, 2013). According to Sakurai, Nakatsu, Sato, and Sato (2003) it has been used to disinfect digestive endoscopes between patients, being safe for the human body and for the environment. However, it is recommended to be produced in a ventilated place as the generation process leads to the production of Cl_2 and H_2. Furthermore, the equipment used for electrolysis is expensive as it is still not widely distributed and used (Cheng et al., 2012).

In terms of surface disinfection, several works have been performed mainly on SS surfaces. Arevalos-Sánchez, Regalado, Martin, Meas-Vong, Cadena-Moreno, and García-Almendárez (2013) observed that *L. monocytogenes* biofilms on SS were completely inhibited after 3 min of contact time with NEOW at 70 ppm of free chlorine. Kim, Hung, Brackett, and Frank (2001) reduced *L. monocytogenes* biofilms on SS surfaces by 9 log CFU/cm^2 after 5 min of treatment with EOW at 56 ppm of free chlorine. Deza, Araujo, and Garrido (2005) studied the disinfection of both SS and glass surfaces with NEOW at 63 ppm of free chlorine for the reduction of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *L. monocytogenes*, reaching 6 log CFU/cm^2 after 1 min of treatment. EOW was also studied for the decontamination of lettuce. Park, Alexander, Taylor, Costa, and Kang (2008) observed that using AcEOW

(pH of 2.06 and 37.5 ppm of free chlorine) for 1 min reduced 4.45 log CFU/g of *E. coli* O157:H7 on green onions. Keskinen et al. (2009) used AcEOW (pH 2.6) at 50 ppm of free chlorine to reduce *E. coli* O157:H7 on lettuce. After 2 min treatment 1 log CFU/g reduction was achieved (Keskinen et al., 2009). Deza et al. (2003) in their study with tomatoes, found that NEOW was adequate to reduce *E. coli* O157:H7, *S.* Enteritidis and *L. monocytogenes* by 6 log CFU/mL, following 5 min exposure to the disinfectant at 89 ppm of free chlorine, without affecting the organoleptic properties of the fresh product. Guentzel, Liang Lam, Callan, Emmons, and Dunham (2008) obtained similar results (6 log CFU/mL reduction) using lettuce contaminated with *E. coli*, *S.* Typhimurium, *S. aureus*, *L. monocytogenes* and *Enterococcus faecalis*, following 10 min exposure time to NEOW at 20 ppm of free chlorine. Abadias et al. (2008) concluded that the use of NEW (at 50 ppm of free chlorine) was equally effective as decontaminating lettuce with 120 ppm of chlorine solution, obtaining 1-2 log CFU/mL reduction of *E. coli* O157:H7, *Salmonella*, *L. innocua* and *Erwinia carotovora*. Aday (2016) also found that the browning effect caused by 25 ppm of EOW was very low. Therefore, the application of NEW is recommended to reduce the chlorine concentration (Abadias et al., 2008), since the efficiency is higher and the free chlorine content is lower. Further studies are required to characterize the chemical species formed during the generation of NEW, their antimicrobial activity, stability and interaction with organic matter. There are evidences showing that in the presence of organic matter NEOW generates lower amounts of organochlorinated molecules than sodium hypochlorite (Ayebah, Hung, Kim, & Frank, 2006).

2.2.5. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is an oxidizer that can form cytotoxic species. The formation of these cytotoxic species is what assures its antimicrobial properties (Ölmez & Kretzschmar, 2009; Rahman, Jin, & Oh, 2010; Rico et al., 2007) which can be either bactericidal or bacteriostatic (Brul & Coote, 1999; Ölmez & Kretzschmar, 2009), depending on the concentration, pH and temperature (Beuchat, 1998). This disinfectant can be applied on food-contact surfaces (Rico et al., 2007). However, according to Van Haute, Tryland, Veys, and Sampers (2015) the use of H_2O_2 cannot avoid the cross-contamination which can still occur in the vegetables washing water, as its decomposition is fast and the disinfection kinetics is slow. Another disadvantage is the browning effects that H_2O_2 can cause to the vegetables, particularly to lettuce (Beuchat,

1998; Ölmez & Kretzschmar, 2009; Rico et al., 2007). To overtake this aspect this chemical must be added in combination with a suitable anti-browning compound (Ölmez & Kretzschmar, 2009), such as sodium erythorbate (Sapers, Miller, Pilizota, & Kamp, 2001). Although H_2O_2 has a GRAS status, its use in fresh produce decontamination is not allowed by FDA (Ölmez & Kretzschmar, 2009). Huang, Ye, and Chen (2012) used 3×10^4 ppm of H_2O_2 to decontaminate baby spinach leaves for 5 minutes and obtained 1.6 log CFU/g reduction of *E. coli* O157:H7. Huang and Chen (2011) achieved similar log CFU reduction (1.5 log CFU/g) of *E. coli* O157:H7 on the same product, but with a lower concentration of H_2O_2 (2×10^4 ppm). Using a higher concentration of H_2O_2 (5×10^4 ppm) for 2 minutes, Ukuku and Fett (2002) achieved a reduction of 2.0-3.5 log CFU/cm^2 of *L. monocytogenes* from melon surfaces. Despite the fact that the concentrations used are very high it is an environmental friendly disinfectant, as it is quickly decomposes into water and oxygen in the presence of catalase (an enzyme commonly found in plants); furthermore it is colorless and non-corrosive (Fallik, 2014; St. Laurent, de Buzzaccarini, De Clerck, Demeyere, Labeque, Lodewick, & van Langenhove, 2007).

2.2.6. Ozone

Ozone (O_3) is produced as a gas that can be dissolved in water. When it is used in a dissolved form, only a small concentration (1-5 ppm) is needed to exert antimicrobial activity. However, higher concentrations are required when it is used as gas, since the humidity of the air affects its penetration into the cells and the consequent disinfection process (Chauret, 2014; Horvitz & Cantalejo, 2014). It is a strong oxidizer with a high bactericidal potential (Foong-Cunningham, Verkaar, & Swanson, 2012). Furthermore, it spontaneously decomposes to a non-toxic product, O_2 (Atungulu & Pan, 2012; Kim, Yousef, & Khadre, 2003). Nevertheless, its use has some disadvantages: i) is unstable and rapidly decomposes (Chawla, Kasler, Sastry, & Yousef, 2012); ii) can become very toxic (Chauret, 2014) as it can affect the respiratory tract and cause irritation to the eyes and throat (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009); iii) its use is sensitive to the presence of organic matter; iv) has to be generated on site (Chauret, 2014); v) it is not suitable to be used on the produce, as it can affect its physicochemical properties (Foong-Cunningham et al., 2012); and vi) is potentially corrosive to the equipment (Sapers, 2009). However, ozone was already approved by FDA to be used on the food industry (21CFR173.368, 2014). In fact, it has been used as a decontaminant

for the produce and disinfectant for the process water (Foong-Cunningham et al., 2012) and food-contact surfaces (Chauret, 2014). Selma, Beltran, Allende, Chacon-Vera, and Gil (2007) applied aqueous ozone at 5 ppm during 5 min to shredded lettuce, and achieved 1.8 log CFU reduction of *Shigella sonnei*. Vurma, Pandit, Sastry, and Yousef (2009) used ozone (5-10 ppm) in the gaseous form to decontaminate spinach leaves. Those authors obtained 1.8 log CFU/g reduction of *E. coli* O157:H7 (Vurma et al., 2009). Khadre and Yousef (2001) disinfected SS surfaces with aqueous ozone (5.9 ppm) for 1 min and achieved complete elimination of the microflora present (*B. subtilis* and *P. fluorescens*). Rosenblum, Ge, Bohrerova, Yousef, and Lee (2012) treated process water contaminated with *B. subtilis* with 2 ppm ozone for 10 min causing 1.56 log CFU/mL reduction. The antimicrobial effective concentrations of ozone are much lower when compared to sodium hypochlorite. However, the corrosiveness and the low stability have to be considered (Simões & Simões, 2013).

2.2.7. Quaternary ammonium compounds

Quaternary ammonium compounds (QACs) are cationic surfactants (Ramos et al., 2013) usually used at concentrations between 200 and 400 ppm for the disinfection of food-contact surfaces (Chauret, 2014). Their mode of action is promoted through their interference with the lipid bilayer of membranes (Velázquez, Barbini, Escudero, Estrada, & Guzmán, 2009). These disinfectants have little effect on spores (Holah, 2014), but are highly effective against Gram positive bacteria (Chaidez, Lopez, & Castro-del Campo, 2007; Ohta, Kondo, Kawada, Teranaka, & Yoshino, 2008; Ramos et al., 2013). The main advantages of QACs are: i) their stability in solution; ii) long shelf-life; iii) environmentally friendly nature; iv) safe to handle; v) non-corrosive nature (Holah, 2014); vi) are odorless; vii) are effective in a wide range of temperature and pH conditions (Bari & Kawamoto, 2014); and viii) are able to disinfect food-contact surfaces more easily than other disinfectants (Ramos et al., 2013). However, like chlorine, QACs antimicrobial activity is affected by the presence of organic matter (Holah, 2014). Moreover, they have low activity in hard water (Bari & Kawamoto, 2014; Holah, 2014) and are not approved for direct contact with food (Ramos et al., 2013). Benzalkonium chloride, benzethonium chloride and cetylpyridinium chloride are examples of commonly used QACs (Izumi, 2014). Velázquez et al. (2009) showed that benzalkonium chloride (0.1 ppm) damaged lettuce leaves with the appearance of yellow spots on the produce after 7 days of storage. On the other hand, Park, Kim, and Koo

(2013) proved the efficacy of this disinfectant against microorganisms (*B. cereus*, *Staphylococcus aureus* and *E. coli*) isolated from fresh-cut products. Microbial growth was completely inhibited when used at 2 ppm (Park et al., 2013). Wang, Li, and Slavik (2001) used cetylpyridinium chloride at 5×10^3 ppm (much higher concentration than the one used for benzalkonium chloride) to decontaminate broccoli, cauliflower, and radishes and obtained reductions of 3.70, 3.15 and 1.56 log CFU/g for *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7, respectively. Chaidez et al. (2007) used a mixture of n-alkyl dimethyl benzyl ammonium chloride sulfosuccinate dioetil at 100 ppm and urea at 200 ppm and found that *E. coli* was more resistant to the treatment with QACs than *Staphylococcus aureus*.

2.2.8. Sodium bicarbonate

Sodium bicarbonate ($NaHCO_3$) is currently used as food additive, has GRAS status and has a wide acceptance by the consumers and the food industry, as it is non-toxic and it does not cause damage to the fruits and vegetables (Smilanick, Margosan, Mlikota, Usall, & Michael, 1999). Furthermore, it has a low cost and can also be used to disinfect food-contact surfaces such as SS (Malik & Goyal, 2006). $NaHCO_3$ has been used to control green and blue molds on citrus (Palou, Smilanick, Usall, & Viñas, 2001; Smilanick et al., 1999). Palou et al. (2001) evaluated the effects of a treatment with $NaHCO_3$ (2.5 min at room temperature) for the control of blue mold caused by *Penicillium italicum* on citrus. A solution of 1×10^4 ppm was not effective, but solutions of 2×10^4 to 4×10^4 ppm reduced the blue mold by 50% (Palou et al., 2001). Smilanick et al. (1999) found that 1.2×10^6 and 2.6×10^6 ppm were the effective doses necessary to inhibit 50% and 95% of *P. digitatum* spores, respectively. Malik and Goyal (2006) used $NaHCO_3$ at a concentration of 5×10^4 ppm and obtained 99.22% reduction of feline calicivirus on food-contact surfaces within 1 min of exposure time. As it was described for H_2O_2, $NaHCO_3$ has to be applied in higher concentrations than sodium hypochlorite (Smilanick et al., 1999).

2.2.9. Weak Organic Acids

Weak organic acids, natural or chemically synthetized, are commonly used as preservatives in the food industry (Hirshfield, Terzulli, & O'Byrne, 2003; Lianou, Koutsoumanis, & Sofos, 2012). Their application is well accepted by the consumers since most of them are naturally present in foods as ingredients. Many organic acids

522 have GRAS status and are FDA and EC (European Commission) approved. Besides
523 their use as preservatives, they are also used as antioxidants, flavoring agents, acidulants
524 and pH regulators (Carpenter & Broadbent, 2009; Theron & Lues, 2011). Citric, acetic
525 and lactic acids are the most common acids applied in the food industry (Ölmez &
526 Kretzschmar, 2009; Rico et al., 2007). Their mode of action is based on the acidification
527 of the cytoplasm, disruption of proton motive force, osmotic stress and inhibition of
528 macromolecule synthesis (Brul & Coote, 1999; Carpenter & Broadbent, 2009;
529 Hirshfield et al., 2003). Furthermore, they have a quick mode of action against an
530 extensive range of bacteria grown under varying temperatures (Hirshfield et al., 2003;
531 Sagong et al., 2011). Organic acids have advantages towards sodium hypochlorite when
532 used as disinfectants for the fresh-cut industry, as they interaction with organic
533 molecules do not produce toxic or carcinogenic compounds (Lianou et al., 2012). Their
534 possible disadvantage could be the change in the flavor of the product that could
535 influence its sensorial analysis. Furthermore, when organic acids are used to disinfect
536 fresh-produce, the wastewater may present high values of both chemical oxygen
537 demand (COD) and biochemical oxygen demand (BOD) (Ölmez & Kretzschmar, 2009).
538 Other pointed disadvantages are their high cost and the corrosiveness of the processing
539 equipment that they may provoke (Sagong et al., 2011).
540 Citric acid is a preservative and flavoring agent usually applied in the food and
541 pharmaceutical industries (Ölmez & Kretzschmar, 2009). Contrary to the action of the
542 other acids, this acts as a chelating agent of metallic ions present in the medium,
543 preventing microbial proliferation (Gurtler & Mai, 2014). Samara and Koutsoumanis
544 (2009) used citric acid (5×10^3 to 1×10^4 ppm) at 20 ^{0}C for 1 to 5 min to control *L.*
545 *monocytogenes* from lettuce and obtained 1 log CFU/cm^2 reduction. Acetic acid is
546 soluble in lipids and therefore is able to diffuse through the cytoplasmic membrane,
547 affecting the intracellular pH of microorganisms, causing cell death (Lianou et al.,
548 2012). Akbas and Olmez (2007) tested concentrations of 5×10^3 to 1×10^4 ppm of acetic
549 and lactic acids (used in separate) (20 ^{0}C for 2 to 5 min) in order to decontaminate
550 lettuce leafs. They were able to reduce the populations of *E. coli* and *L. monocytogenes*
551 with acetic acid by 2.2 and 1.3 log CFU/g, respectively; lactic acid caused reductions of
552 2.8 and 2.1 log CFU/g, respectively (Akbas & Olmez, 2007). The available research
553 clearly shows that in order to have significantly antimicrobial effects these organic acids
554 have to be used in much higher concentrations than sodium hypochlorite.

There are other organic acids that can also be used in the food industry to control microbial growth, such as peracetic acid (PAA), which is usually applied as a disinfecting agent of food-contact surfaces under lower concentrations that the other mentioned organic acids (da Silva Fernandes, Kabuki, & Kuaye, 2015). This acid combines the active oxygen characteristics of peroxide within an acetic acid molecule. It is sporicidal and very efficient due to its high oxidizing potential (Martín-Espada, D'Ors, Bartolomé, Pereira, & Sánchez-Fortún, 2014; Sudhaus, Nagengast, Pina-Pérez, Martínez, & Klein, 2014). It is believed that PAA acts by disrupting the chemiosmotic function of the cytoplasmic membrane (Kitis, 2004). In a study of Vandekinderen, Devlieghere, De Meulenaer, Ragaert, and Van Camp (2009) a treatment of fresh-cut iceberg lettuce with 120 ppm of PAA reduced the native microbial load by 1.2 log CFU/g without affecting the sensorial or nutritional quality of the product. Ge et al. (2013) achieved 0.99 log CFU/g reduction of *S.* Typhimurium on lettuce by applying PAA at 40 ppm for 5 min. Park, Choi, Park, Park, Chung, Ryu, and Kang (2011) studied the decontaminating effects of propionic, acetic, lactic, malic, and citric acid (1×10^4 ppm, 10 min) against *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on organic fresh lettuce obtaining reductions of 0.93-1.52 (propionic), 1.13-1.74 (acetic), 1.87-2.54 (lactic), 2.32-2.98 (malic) and 1.85-2.86 (citric) log CFU/g. These authors suggested that organic acids are relevant for decontamination of fresh produce.

2.3. Physical-based methods

2.3.1. Ionizing irradiation

Ionizing irradiation, such as x-rays, gamma-rays and electron beams, produces ions and electrically charged atoms and molecules. The mode of action of all these forms of ionizing radiation is similar: they act on water molecules forming free radicals that destroy or inhibit microorganisms (Ramos et al., 2013). Despite that this method is quite effective in microbial growth control, FDA only approves the use of a maximum level of 1.0 kGy to decontaminate vegetables. Thus, if the produce is treated with doses higher than 1.0 kGy it cannot be designated as “fresh” (21CFR101.95, 2014). Furthermore, this physical method should better be used in combination with a chemical method, as it only reduces the microbial load to facilitate further chemical disinfection (Doona, Feeherry, Feng, Grove, Krishnamurthy, Lee, & Kustin, 2015). The main advantages of the ionizing radiation are the very low energy requirements and the

587 reduced heating of the food (Ramos et al., 2013). This method has not yet been adopted
588 in the fresh produce industry mainly because: i) further research is needed to determine
589 the necessary doses for different products (Goodburn & Wallace, 2013); ii) the
590 consumer still have a strong negative perception of irradiated foods (Goodburn &
591 Wallace, 2013; Ramos et al., 2013); iii) the quality of the fresh produce can be affected,
592 especially at high doses (Ramos et al., 2013).

593 2.3.2. Membrane filtration

594 Membrane separation can be used to treat the process water, to avoid cross-
595 contamination of the produce (Allende, Selma, Lopez-Galvez, Villaescusa, & Gil, 2008;
596 Gil et al., 2009). This procedure involves the flow of water through a
597 semipermeable membrane and the consequent retention of the undesired
598 contaminants on the membrane. Microfiltration (MF), ultrafiltration (UF),
599 nanofiltration (NF) and reverse osmosis (RO), are membrane unit operations that can
600 be applied in the food industry to treat the process water (Cassano & Basile, 2013).
601 MF is a dead-end filtration, where the feed flows vertical to the membrane and all the
602 solids become retained on the membrane. In MF the membrane pore size is
603 between 0.05 and 10 μm (Berk, 2009; Salehi, 2014), the molecular weight cut off
604 (MWCO) is 200 kDa (Singh, 2015) and the pressure used is below 0.2 MPa (Salehi,
605 2014). This membrane process is usually applied for the retention of microorganisms
606 (Salehi, 2014). UF membranes have a pore size of 0.001-0.05 μm, a MWCO between
607 1 and 300 kDa (Singh, 2015) and a pressure of 0.2-0.5 MPa. UF is usually used to
608 separate proteins and other high molecular weight organic compounds (Salehi, 2014;
609 Singh, 2015). Regarding NF, the membrane pore size is lower than 2 nm (Salehi,
610 2014), the MWCO is between 100 and 1000 Da (Singh, 2015) and the pressure is
611 of 0.5-1.5 MPa (Salehi, 2014). Normally NF is used for water softening and the
612 removal of salts (Singh, 2015). In RO the membrane has a pore size of 0.6 nm
613 (Singh, 2015), with a MWCO of 100 Da (Salehi, 2014) and the pressure used is
614 between 1.5 and 10 MPa (Berk, 2009; Salehi, 2014; Singh, 2015). The main
615 disadvantage of these methods is the high investment costs associated with the
616 implementation of a membrane separation unit (Casani, Rouhany, & Knøchel,
617 2005). Moreover, the costs (acquisition, energy and maintenance) of membrane
618 technology and the limited life span, due to the high pressure drop caused by biofilms,
reduces their wide use in the food industry (Melo & Flemming, 2010).

2.3.3. Steam jet-injection

Steam jet-injection is a heat treatment that destroys microorganisms and inactivates enzymes that might be responsible for produce spoilage (Rico, Martín-Díana, Barry-Ryan, Frías, Henehan, & Barat, 2008a). The heat time exposure is usually short (≈10 seconds). Consequently, the impairment of organoleptic properties is reduced (Martín-Diana, Rico, Barry-Ryan, Frías, Henehan, & Barat, 2007). However, the heat exposure still promotes the loss or reduction of the bioavailability of some nutrients (Rico et al., 2008a). Moreover, the high power consumption associated to the steam jet formation and also the high temperature of the effluents generated have to be considered (Martín-Diana et al., 2007; Rico et al., 2008a). Rico et al. (2008a) reported that 10 seconds was the ideal time to obtain a satisfactory mesophilic load reduction for fresh-cut lettuce. In fact, the authors observed a significant loss of vitamin C and carotenoids for longer treatments. Martín-Diana et al. (2007) concluded that the mesophilic load reduction of fresh-cut lettuce was the same when using steam jet-injection and chlorine at 120 ppm.

2.3.4. Temperature

Control of temperature is a key point in microbial growth control. Either refrigeration or heating of water can be applied to control or reduce microbial load, respectively. Furthermore, the air temperature can also be reduced to delay microbial proliferation. However, as previously stated for irradiation, this physical method should be used as complement, as it is not usually effective alone to ensure the desired microbiological safety of product. Low temperatures can delay food spoilage, but also mask the latent state of the pathogens (Parish et al., 2003). For a product to be considered fresh, the "high" temperatures used have a defined threshold (≈40 – 60 ^{0}C, 1-5 min) (21CFR101.95, 2014; Fallik, 2014; Parish et al., 2003). Moreover, the high temperatures can induce damages of the produce tissues favoring microbial entrance and consequent spoilage (Parish et al., 2003).

2.3.5. Ultrasounds

Ultrasounds (US) are sonic waves at high amplitude, above human-hearing threshold (Otto et al., 2011; Paniwnyk, 2014) that form cavitation bubbles (Seymour, Burfoot, Smith, Cox, & Lockwood, 2002). These bubbles collapse generating the mechanical energy responsible for the disinfecting action (detachment) and the chemical energy responsible for the free radicals formation (destruction) (Bermúdez-Aguirre, Mobbs, &

651 Barbosa-Cánovas, 2011; Sagong et al., 2011; Seymour et al., 2002), increasing the
652 permeability of cell membranes (Bilek & Turantaş, 2013). By this collapse, hot spots
653 are formed (high temperatures and pressure) and free radicals are released, causing
654 DNA modifications in the cells (São José, Andrade, Ramos, Vanetti, Stringheta, &
655 Chaves, 2014). In the food industry, US are used at low frequencies, in the range of 20-
656 100 kHz (Paniwnyk, 2014; Sagong et al., 2011), and require the presence of a fluid for
657 transmission. The high-intensity treatments necessary to inactivate the microorganisms
658 can be a drawback as these can affect the organoleptic properties of the produce
659 (Seymour et al., 2002). Microbial resistance to US varies according to the cell shape
660 (coccus are more resistant), size (smaller cells are more resistant), Gram nature (Gram
661 positive bacteria are more resistant) and cellular metabolism (aerobic microorganisms
662 are more resistant) (Chemat, Zill e, & Khan, 2011; Paniwnyk, 2014).

663 Comparing this technique with the other methods (chlorine, copper compounds,
664 ionizing irradiation), the main advantages are the safety associated with the sound
665 waves and also the fact that it is environmentally friendly (Kentish & Ashokkumar,
666 2011). The UK Health Protection Agency (HPA) recommends an exposure limit for the
667 general public to airborne ultrasound sound pressure levels (SPL) of 70 dB (at 20 kHz),
668 and 100 dB (at 25 kHz and above) (AGNIR, 2010). This method is effective at an
669 optimum temperature of 60 °C, which is definitely a disadvantage when working with
670 fresh produce (high temperatures can change the food properties). Seymour et al. (2002)
671 used water at temperatures of 5 °C or 20 °C in the treatment of iceberg lettuce with US
672 (5 min) and they concluded that both temperatures had no influence on the organoleptic
673 properties of the fresh food. Therefore, the highest temperature is more favorable as the
674 effects of US on iceberg lettuce decontamination is increased. The water hardness and
675 the amount of dissolved gases have to be taken into account due to the fact that their
676 variability can reduce the cavitation process. Given that US should be applied for a
677 short period of time, they do not affect the appearance of the produce (Sagong et al.,
678 2011). Additionally, US can also be used in combination with other disinfectants, such
679 as chlorine, improving thus the efficacy of both methods (São José et al., 2014).

680 This physical method has been extensively studied in the food industry for produce
681 decontamination and water disinfection. Seymour et al. (2002) reported 1.5 log CFU/g
682 reduction of *S.* Typhimurium on cut iceberg lettuce at 32-40 kHz for 10 min. These
683 authors tested the effects of different frequencies (25, 32 and 70 kHz) and found no

684 statistical significant differences (Seymour et al., 2002). Birmpa, Sfika, and Vantarakis
685 (2013) achieved 2.30, 5.72 and 1.88 log CFU/g reduction of *E. coli*, *S.* Enteritidis, *L.*
686 *innocua*, respectively, on lettuce at 37 kHz for 30 min. Kim, Feng, Kushad, and Fan
687 (2006) obtained a lower log CFU/g reduction (1.08) of *E. coli* O157:H7 on broccoli
688 seeds using the same treatment time and a higher frequency (40 kHz, at 23 ^{0}C for 30
689 min). The disinfection of the washing water is also important and Elizaquível, Sánchez,
690 Selma, and Aznar (2012) found a 4.4 log CFU/mL reduction of *E. coli* O157:H7 in the
691 washing water using US at 20 kHz for 53 min, which is obviously a high exposure time
692 and therefore not appropriate for the fresh-cut industry.

693 2.3.6. Ultraviolet light

694 Ultraviolet (UV) light is an electromagnetic radiation with wavelengths ranging
695 between 100 and 400 nm. It is subdivided in four groups: UV-A, UV-B, UV-C and
696 vacuum UV (Gray, 2014). The UV-C (190-280 nm) light (Artés et al., 2009) is used as
697 antimicrobial as this induces DNA damages, leading to cell death (Birmpa et al., 2013).
698 However, at lower doses the microorganisms can remain alive, due to their DNA repair
699 mechanisms (Shama, 2014). Insomuch the appropriate precautionary measures are
700 taken, it is a non-toxic, safe and environmentally friendly treatment (Otto et al., 2011),
701 however, its prolonged use can alter the organoleptic properties of the food (Demirci &
702 Krishnamurthy, 2010). UV light can be used for disinfection by either applying a
703 continuous mode (UV lamps) (Gray, 2014), or pulsed UV light (Condón, Álvarez, &
704 Gayán, 2014). UV lamps have a tube with a gas (xenon or krypton), mercury and also
705 have an electrode at each side of the tube. When electrical current is passed, the
706 mercury atoms become excited and UV light is produced when the atoms return to their
707 basal state (Gray, 2014). The advantages of UV lamps are their high efficiency
708 (depending on the dose and exposure time) and the reduced process times (Birmpa et
709 al., 2013). When compared to UV lamps, the mode in pulsed UV light is not continuous
710 (the pulse rate is 1 to 20 pulses per second), therefore the energy is multiplied (100 to
711 1100 nm) being more efficient (Demirci & Krishnamurthy, 2010). Another advantage is
712 the fact that pulsed UV light can be a mercury free alternative. The main drawback is
713 the temperature increase, which can damage the produce (Condón et al., 2014; Demirci
714 & Krishnamurthy, 2010).

715 The method of disinfection by using UV light is usually applied for wastewater

716 treatment (Hunter & Townsend, 2010), while there are few applications in the food
717 industry. As examples, Birmpa et al. (2013) used UV (254 nm) to reduce the microbial
718 load on lettuce. The treatment reduced 1.75, 1.27, 1.39 and 1.21 log CFU/g the
719 populations of *E. coli*, *L. innocua*, *S.* Enteritidis and *Staphylococcus aureus*,
720 respectively (Birmpa et al., 2013). Bermúdez-Aguirre and Barbosa-Cánovas (2013)
721 used UV light (253.7 nm) to decontaminate lettuce, obtaining 1.7 log CFU/g
722 inactivation of *E. coli* within 1 h of treatment, which is a long exposure time for the log
723 CFU reduction observed. Ge et al. (Ge et al., 2013) achieved 2.28 log CFU/g reduction
724 of *S.* Typhimurium on lettuce by applying UV-C treatment for 5 min with an irradiation
725 fluency of 450 mJ/cm^2.

726

727 ## 3. Combination of disinfection methods

728 Most of the biological, chemical and physical methods which were previously described
729 have reduced effectiveness in microbial growth control when applied alone. Therefore,
730 these methods have to be combined in order to increase their antimicrobial efficacy.
731 Moreover, when combined with chlorine they will help reducing the use of chlorine to
732 achieve the desired antimicrobial effect. Combinations of physical-chemical (Gabriel,
733 2015), chemical-chemical (Singh et al., 2002b), chemical-biological (Arevalos-Sánchez
734 et al., 2012) and biological-biological (Lequette et al., 2010) methods have already been
735 successfully described. The main aim of these combinations is to achieve a more
736 effective disinfection process. In fact, the combination of diverse methods allows an
737 wider antimicrobial action (Goodburn & Wallace, 2013).

738 Ionizing irradiation is a method to decontaminate fresh produce that is mandatorily
739 combined with a chemical method. Foley, Dufour, Rodriguez, Caporaso, and Prakash
740 (2002) combined this method with chlorine and proved that applying 0.55 kGy of
741 gamma-rays together with 200 ppm of chlorine on shredded iceberg lettuce were able to
742 reduce the population of *E. coli* O157:H7 by 5.4 log CFU/g. However, they used a high
743 concentration of chlorine which is not the purpose of combining the two methods.
744 Huang and Chen (2011) combined a physical process (heating at 50 ^{0}C) with H_2O_2
745 (2×10^4 ppm) to decontaminate baby spinach. When the physical-chemical combination
746 was applied, the reduction of *E. coli* O157:H7 was 2.2 log CFU/g, and when H_2O_2 was

used alone, the reduction was significantly lower (Huang & Chen, 2011). Delaquis, Fukumoto, Toivonen, and Cliff (2004) also combined high temperature with chlorine at 100 ppm. The authors found that the heating of the fresh-cut iceberg lettuce at 50 ^{0}C for 1 min resulted in 1.5 log CFU/g reductions of the total microbial population, while the combination with chlorine caused an additional 0.5 log CFU/g reduction (Delaquis et al., 2004). Nevertheless, the use of high temperatures can affect the organoleptic properties of the produce (Parish et al., 2003). Seymour et al. (2002) combined US (32-40 kHz) with 25 ppm chlorine in a 10 min treatment to eliminate *S.* Typhimurium from fresh cut lettuce. These authors achieved 2.7 log CFU/g reduction, which was higher than the reduction obtained with chlorine (1.7 log CFU/g) or US (1.5 log CFU/g) alone. Sagong et al. (2011) reported the combination of US (40 kHz) with lactic acid (2%) to decontaminate organic lettuces for 5 min at 20 ^{0}C. They observed 2.75, 2.71 and 2.50 log CFU/g reductions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively (Sagong et al., 2011). The combination of UV (15 W UV-C lamp) and US (frequencies switched between 28, 45 and 100 kHz at 1 millisecond time intervals) was also assessed by Gabriel (2015). This combination decreased the time necessary to obtain the same effect (5 log CFU/mL reduction of *E. coli*) when both technologies were applied alone (Gabriel, 2015). Rico et al. (2006) combined calcium lactate (1.5×10^4 ppm, at 50 ^{0}C) with ozone (1 ppm) to extend the shelf life of lettuce, observing a reduced enzymatic browning. The efficiency of ClO_2 (20-40 ppm) was also improved in combination with US (170 kHz), reducing *Salmonella* and *E. coli* O157:H7 on lettuce by 2.6 and 1.8 log CFU/g (Huang, Xu, Walker, West, Zhang, & Weese, 2006). Ibrahim et al. (2008) combined 5 ppm ozone with 40 ppm ClO_2 for 5 min to decontaminate turnip greens and the total bacterial count was reduced by 2.17 log CFU/g.

The combination of copper with lactic acid was considered antimicrobial synergistic by Ibrahim et al. (2008). Gyawali et al. (2011) observed 3.93 log CFU/cm^2 reduction of *E. coli* O157:H7 on lettuce surface combining 40 ppm copper and 2×10^3 ppm lactic acid. Rahman et al. (2010) studied cabbage decontamination combining AlEOW (100 ppm) with citric acid (1×10^4 ppm) at 50 ^{0}C, reducing *L. monocytogenes* and *E. coli* O157:H7 by 3.99 and 4.19 log CFU/g, respectively. Citric acid (1×10^4 ppm) was combined with ozonated water (3 ppm) for 1 min to decontaminate lettuce (Yuk, Yoo, Yoon, Moon, Marshall, & Oh, 2006). When 5 ppm ozone was used for 5 min it caused 1.09 and 0.94 log CFU/g reduction of the populations of *E. coli* O157:H7 and *L. monocytogenes*,

780 *respectively. However, when this was combined with citric acid higher reductions were*
781 *observed: 2.31 and 1.84 log CFU/g for E. coli O157:H7 and L.*
782 *monocytogenes, respectively (Yuk et al., 2006). Combining sodium bicarbonate with*
783 *H_2O_2 proved to be more efficient than using sodium bicarbonate alone. Malik and*
784 *Goyal (2006) reduced 99.68% the population of feline calcivirus by combining*
785 *2×10^4 ppm of sodium bicarbonate with 2×10^4 ppm of H_2O_2 for 10 min. Van*
786 *Haute, López-Gálvez, Gómez-López, Eriksson, Devlieghere, Allende, and Sampers*
787 *(2015) combined peracetic acid (20 ppm) and lactic acid (4000 ppm) to treat the*
788 *wash water of fresh-cut leafy vegetables. The authors proved that the use of this*
789 *treatment is better than the use of chlorine, however, higher disinfectant*
790 *concentrations are needed because the inactivation of E. coli O157 is slower,*
791 *when compared to the chlorine kinetics. Enzymes have also been used in combination*
792 *with US in order to remove E. coli biofilms from SS surfaces (Oulahal-Lagsir,*
793 *Martial-Gros, Bonneau, & Blum, 2003). When US (40 kHz) were used alone, 30%*
794 *of biofilm mass removal was achieved. However, when US were used in combination*
795 *with enzymes (trypsin) biofilm removal reached 76% (Oulahal-Lagsir et al.,*
796 *2003). The combination of UV and ozone was also applied by Selma, Allende,*
797 *López-Gálvez, Conesa, and Gil (2008) achieving 6.6 log CFU/mL microbial*
798 *reduction in escarole wash water. Ge et al. (2013) studied the combination of UV-C*
799 *with chlorine and UV-C with PAA. When they combined UV-C (irradiation*
800 *fluency of 900 mJ/cm^2, 10 min) with chlorine (200 ppm, 10 min) 2.40 log CFU/g*
801 *reduction of S. Typhimurium on lettuce was achieved; when combining UV-C*
802 *(irradiation fluency of 900 mJ/cm^2, 10 min) with PAA (80 ppm, 10 min) 2.52 log*
803 *CFU/g was obtained. The treatments applied alone caused 2.29, 0.99 and 0.95 log*
804 *CFU/g reduction for UV-C (irradiation fluency of 900 mJ/cm^2, 10 min), chlorine and*
805 *PAA, respectively (Ge et al., 2013). From all the previous examples, it becomes rather*
806 *clear that the combination of methods is a promising effort to replace and/or reduce the*
use of chlorine.

807

4. Conclusions and future perspectives

808

809 *The environmental and public health concerns on the use of chlorine are constantly*
810 *increasing, with some European countries having already prohibited its use (Bilek &*
811 *Turantaş, 2013; Fallik, 2014; Ölmez & Kretzschmar, 2009; Ramos et al., 2013).*
Chlorine is considered pollutant due to its ability to form mutagenic and carcinogenic

compounds when reacting with organic matter (Kumar & Anand, 1998; Ölmez & Kretzschmar, 2009) and it has also been included in the indicative list of the Directive on Industrial Emissions (IPPC, 2007/0286 (COD)) (European Comission, 2007). Several new strategies aiming to completely avoid or reduce the use of chlorine have already been studied and were described in this review: greener alternatives based on the use of biological compounds - bacteriocins, bacteriophages, enzymes and phytochemicals; the use of alternative chemicals - ClO_2, electrolyzed oxidizing water, H_2O_2, ozone, organic acids, etc.; or even physical methods - irradiation, filtration, US, UV light, etc; and also the combination of strategies. It is worth to be noted that the disinfection of the water from the produce washing process should be studied in more detail since its use without proper microbiological quality can lead to cross-contamination and microbial accumulation, impairing the decontamination of the produce, disinfection of food-contact surfaces and water. Water disinfected properly can be re-used in the process, reducing water consumption. Furthermore, biofilm formation and pathogens internalization should be also seriously considered, as both can reduce the action of disinfectants. Despite the advantages of the alternative methods which were here described, much research is still needed for the discovery and development of new strategies and for their effective use at industrial scale.

5. Search methodology

The databases used on the search of this work were the Scopus webpage, as well as books and encyclopedias in the authors' possession. The search lasted 3 years in the area of disinfection of fresh-cut vegetables. The keywords used were fresh-cut vegetables industry, minimally processed vegetables, disinfection, sanitation, decontamination, chlorine, alternatives, biological, physical, chemical, electrolyzed oxidizing water, quaternary ammonium compounds, ultrasounds, ultraviolet, hydrogen peroxide, ozone, sodium bicarbonate, enzymes, phytochemicals, bacteriocins, organic acids, peracetic acid, irradiation, copper, bacteriophages, steam jet injection.

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References

21CFR101.95. 101.95, Food labelling "Fresh", "freshly frozen", "fresh frozen", "frozen fresh" C.F.R. Specific requirements for descriptive claims that are neither nutrient content claims nor health claims (2014).

21CFR173.300. 173.300 Chlorine dioxides C.F.R. Secondary direct food additive for human consumption (2014).

21CFR173.368. 173.368 Ozone C.F.R. Secondary direct food additives permitted in food for human consumption (2014).

Abadias, M., Usall, J., Oliveira, M., Alegre, I., & Viñas, I. (2008). Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. *International Journal of Food Microbiology, 123*(1–2), 151-158.

Adams, M. R., Hartley, A. D., & Cox, L. J. (1989). Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiology, 6*(2), 69-77.

Aday, M. S. (2016). Application of electrolyzed water for improving postharvest quality of mushroom. *LWT - Food Science and Technology, 68*, 44-51.

AGNIR. (2010). Health Effects of Exposure to Ultrasound and Infrasound (pp. 167-170). United Kingdom: Health Protection Agency.

Akbas, M. Y., & Olmez, H. (2007). Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. *Letters in Applied Microbiology, 44*(6), 619-624.

Allende, A., Martínez, B., Selma, V., Gil, M. I., Suárez, J. E., & Rodríguez, A. (2007). Growth and bacteriocin production by lactic acid bacteria in vegetable broth and their effectiveness at reducing *Listeria monocytogenes* in vitro and in fresh-cut lettuce. *Food Microbiology, 24*(7–8), 759-766.

Allende, A., Selma, M. V., Lopez-Galvez, F., Villaescusa, R., & Gil, M. I. (2008). Impact of wash water quality on sensory and microbial quality, including *Escherichia coli* cross-contamination, of fresh-cut escarole. *Journal of Food Protection, 71*(12), 2514-2518.

Angiolillo, L., Conte, A., & Del Nobile, M. A. (2014). Food additives: Natural preservatives. In Y. Motarjemi (Ed.), *Encyclopedia of Food Safety* (pp. 474-476). Waltham: Academic Press.

Arevalos-Sánchez, M., Regalado, C., Martin, S. E., Domínguez-Domínguez, J., & García-Almendárez, B. E. (2012). Effect of neutral electrolyzed water and nisin on *Listeria monocytogenes* biofilms, and on listeriolysin O activity. *Food Control, 24*(1–2), 116-122.

Arevalos-Sánchez, M., Regalado, C., Martin, S. E., Meas-Vong, Y., Cadena-Moreno, E., & García-Almendárez, B. E. (2013). Effect of neutral electrolyzed water on lux-tagged *Listeria monocytogenes* EGDe biofilms adhered to stainless steel and visualization with destructive and non-destructive microscopy techniques. *Food Control, 34*(2), 472-477.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., & Artés-Hernández, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology, 51*(3), 287-296.

Atungulu, G. G., & Pan, Z. (2012). Microbial decontamination of nuts and spices. In A. Demirci & M. O. Ngadi (Eds.), *Microbial Decontamination in the Food Industry* (pp. 125-162): Woodhead Publishing.

Augustin, M., Ali-Vehmas, T., & Atroshi, F. (2004). Assessment of enzymatic cleaning agents and disinfectants against bacterial biofilms. *Journal of Pharmacy & Pharmaceutical Sciences, 7*(1), 55-64.

Ayebah, B., & Hung, Y.-C. (2005). Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *Journal of Food Process Engineering, 28*(3), 247-264.

Ayebah, B., Hung, Y. C., Kim, C., & Frank, J. F. (2006). Efficacy of electrolyzed water in the inactivation of planktonic and biofilm *Listeria monocytogenes* in the presence of organic matter. *Journal of Food Protection, 69*(9), 2143-2150.

Bari, M. L., & Kawamoto, S. (2014). Process hygiene - Types of sterilant. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 216-225). Oxford: Academic Press.

Bari, M. L., Ukuku, D. O., Kawasaki, T., Inatsu, Y., Isshiki, K., & Kawamoto, S. (2005). Combined efficacy of nisin and pediocin with sodium lactate, citric acid, phytic acid, and potassium sorbate and EDTA in reducing the *Listeria monocytogenes* population of inoculated fresh-cut produce. *Journal of Food Protection, 68*(7), 1381-1387.

Belletti, N., Lanciotti, R., Patrignani, F., & Gardini, F. (2008). Antimicrobial efficacy of citron essential oil on spoilage and pathogenic microorganisms in fruit-based salads. *Journal of Food Science, 73*(7), 1750-3841.

Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., & Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology, 12*(9), 2385-2397.

Berk, Z. (2009). Membrane processes. In Z. Berk (Ed.), *Food Process Engineering and Technology* (pp. 233-257). San Diego: Academic Press.

Bermúdez-Aguirre, D., & Barbosa-Cánovas, G. V. (2013). Disinfection of selected vegetables under nonthermal treatments: Chlorine, acid citric, ultraviolet light and ozone. *Food Control, 29*(1), 82-90.

Bermúdez-Aguirre, D., Mobbs, T., & Barbosa-Cánovas, G. (2011). Ultrasound applications in food processing. In H. Feng, G. Barbosa-Canovas & J. Weiss (Eds.), *Ultrasound Technologies for Food and Bioprocessing* (pp. 65-105): Springer New York.

Beuchat, L. (1998). *Surface Decontamination Of Fruits And Vegetables Eaten Raw: A Review*: OMS.

Bilek, S. E., & Turantaş, F. (2013). Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. *International Journal of Food Microbiology, 166*(1), 155-162.

Birmpa, A., Sfika, V., & Vantarakis, A. (2013). Ultraviolet light and ultrasound as non-thermal treatments for the inactivation of microorganisms in fresh ready-to-eat foods. *International Journal of Food Microbiology, 167*(1), 96-102.

Borges, A., Abreu, A., Malheiro, J., Saavedra, M. J., & Simõe, M. (2013). Biofilm prevention and control by dietary phytochemicals. In A. Méndez-Vilas (Ed.), *Microbial Pathogens and Strategies for Combating them: Science, Technology and Education* (Vol. 1, pp. 32-41): Formatex Research Center.

Brul, S., & Coote, P. (1999). Preservative agents in foods: mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology, 50*(1–2), 1-17.

Bull, R. J., Reckhow, D. A., Li, X., Humpage, A. R., Joll, C., & Hrudey, S. E. (2011). Potential carcinogenic hazards of non-regulated disinfection by-products: Haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology, 286*(1–3), 1-19.

Carpenter, C. E., & Broadbent, J. R. (2009). External concentration of organic acid anions and pH: key independent variables for studying how organic acids inhibit growth of bacteria in mildly acidic foods. *Journal of Food Science, 74*(1), R12-15.

Casani, S., Rouhany, M., & Knøchel, S. (2005). A discussion paper on challenges and limitations to water reuse and hygiene in the food industry. *Water Research, 39*(6), 1134-1146.

Cassano, A., & Basile, A. (2013). Integrating different membrane operations and combining membranes with conventional separation techniques in industrial processes. In A. Basile (Ed.), *Handbook of Membrane Reactors* (Vol. 2, pp. 296-343): Woodhead Publishing.

Cerioni, L., Lazarte Mde, L., Villegas, J. M., Rodriguez-Montelongo, L., & Volentini, S. I. (2013). Inhibition of *Penicillium expansum* by an oxidative treatment. *Food Microbiology, 33*(2), 298-301.

Cerioni, L., Rapisarda, V. A., Hilal, M., Prado, F. E., & Rodríguez-Montelongo, L. (2009). Synergistic antifungal activity of sodium hypochlorite, hydrogen peroxide, and cupric sulfate against *Penicillium digitatum*. *Journal of Food Protection, 72*(8), 1660-1665.

Cerioni, L., Volentini, S. I., Prado, F. E., Rapisarda, V. A., & Rodriguez-Montelongo, L. (2010). Cellular damage induced by a sequential oxidative treatment on *Penicillium digitatum*. *Journal of Applied Microbiology, 109*(4), 1441-1449.

Chaidez, C., Lopez, J., & Castro-del Campo, N. (2007). Quaternary ammonium compounds: an alternative disinfection method for fresh produce wash water. *Journal of Water and Health, 5*(2), 329-333.

Chauret, C. P. (2014). Sanitization. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 360-364). Oxford: Academic Press.

Chawla, A. S., Kasler, D. R., Sastry, S. K., & Yousef, A. E. (2012). Microbial decontamination of food using ozone. In A. Demirci & M. O. Ngadi (Eds.), *Microbial Decontamination in the Food Industry* (pp. 495-532): Woodhead Publishing.

Chemat, F., Zill e, H., & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry, 18*(4), 813-835.

Chen, J.-C., & Lin, C.-H. (2001). Toxicity of copper sulfate for survival, growth, molting and feeding of juveniles of the tiger shrimp, *Penaeus monodon*. *Aquaculture, 192*(1), 55-65.

Chen, Z., Zhu, C., Zhang, Y., Niu, D., & Du, J. (2010). Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology, 58*(3), 232-238.

Cheng, K. C., Dev, S. R. S., Bialka, K. L., & Demirci, A. (2012). Electrolyzed oxidizing water for microbial decontamination of food. In A. Demirci & M. O. Ngadi (Eds.), *Microbial Decontamination in the Food Industry* (pp. 563-591): Woodhead Publishing.

Chung, C.-C., Huang, T.-C., Yu, C.-H., Shen, F.-Y., & Chen, H.-H. (2011). Bactericidal effects of fresh-cut vegetables and fruits after subsequent washing with chlorine dioxide. *International Proceedings of Chemical, Biological & Environmental Engineering, 9*, 107-112.

Condón, S., Álvarez, I., & Gayán, E. (2014). Non-thermal processing - pulsed UV light. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 974-981). Oxford: Academic Press.

Costa, L. G. (2008). Toxic effects of pesticides. In C. D. Klaassen (Ed.), *Casarett & Doull's Toxicology: The Basic Science Of Poisons* (7th ed., pp. 905-930): McGraw-Hill.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564-582.

da Silva Fernandes, M., Kabuki, D. Y., & Kuaye, A. Y. (2015). Behavior of *Listeria monocytogenes* in a multi-species biofilm with *Enterococcus faecalis* and *Enterococcus faecium* and control through sanitation procedures. *International Journal of Food Microbiology, 200*(0), 5-12.

Davies, E. A., & Delves-Broughton, J. (1999). Bacteriocins - Nisin. In C. A. Batt & P. D. Patel (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 191-198). Oxford: Elsevier.

Delaquis, P. J., Fukumoto, L. R., Toivonen, P. M. A., & Cliff, M. A. (2004). Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biology and Technology, 31*(1), 81-91.

Delves-Broughton, J. (2005). Nisin as a food preservative. *Food Australia, 57*(12), 525-532.

Demirci, A., & Bialka, K. L. (2010). Electrolyzed oxidizing water *Nonthermal Processing Technologies for Food* (pp. 366-376): Wiley-Blackwell.

Demirci, A., & Krishnamurthy, K. (2010). Pulsed ultraviolet light *Nonthermal Processing Technologies for Food* (pp. 249-261): Wiley-Blackwell.

Deza, M. A., Araujo, M., & Garrido, M. J. (2003). Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water. *Letters in Applied Microbiology, 37*(6), 482-487.

Deza, M. A., Araujo, M., & Garrido, M. J. (2005). Inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

on stainless steel and glass surfaces by neutral electrolysed water. *Letters in Applied Microbiology, 40*(5), 341-346.

Doona, C. J., Feeherry, F. E., Feng, H., Grove, S., Krishnamurthy, K., Lee, A., & Kustin, K. (2015). Combining sanitizers and nonthermal processing technologies to improve fresh-cut produce safety. In S. D. Pillai & S. Shayanfar (Eds.), *Electron Beam Pasteurization and Complementary Food Processing Technologies* (pp. 95-125): Woodhead Publishing.

EFSA. (2015). EFSA Panel on Contaminants in the Food Chain (CONTAM). Risks for public health related to the presence of chlorate in food. *EFSA Journal, 13*(6), 4135-4238.

Elizaquível, P., Sánchez, G., Selma, M. V., & Aznar, R. (2012). Application of propidium monoazide-qPCR to evaluate the ultrasonic inactivation of *Escherichia coli* O157:H7 in fresh-cut vegetable wash water. *Food Microbiology, 30*(1), 316-320.

Erickson, M. C. (2012). Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology, 3*, 283-310.

European Comission. 2007/0286(COD) C.F.R. Proposal for a directive of the European Parliament and of the council on industrial emissions (Integrated pollution prevention and control) (2007).

Fallik, E. (2014). Microbial quality and safety of fresh produce. In W. J. Florkowski, R. L. Shewfelt, B. Brueckner & S. E. Prussia (Eds.), *Postharvest Handling* (3rd ed., pp. 313-339). San Diego: Academic Press.

Foley, D. M., Dufour, A., Rodriguez, L., Caporaso, F., & Prakash, A. (2002). Reduction of *Escherichia coli* 0157:H7 in shredded iceberg lettuce by chlorination and gamma irradiation. *Radiation Physics and Chemistry, 63*(3–6), 391-396.

Food and Drug Administration. (1998). Guidance for industry: Guide to minimize microbial food safety hazards for fresh fruits and vegetables Retrieved 28th May, 2015, from http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm064574.htm#i

Food and Drug Administration. (2013). Food additive status list Retrieved 28th December, 2015, from http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm091048.htm

Foong-Cunningham, S., Verkaar, E. L. C., & Swanson, K. (2012). Microbial decontamination of fresh produce. In A. Demirci & M. O. Ngadi (Eds.), *Microbial Decontamination in the Food Industry. Novel Methods and Applications* (pp. 3-29): Woodhead Publishing.

Forsythe, S. J., & Hayes, P. R. (1998). Cleaning and disinfection: methods *Food Hygiene, Microbiology and HACCP* (pp. 327-363): Springer US.

Gabriel, A. A. (2015). Combinations of selected physical and chemical hurdles to inactivate *Escherichia coli* O157:H7 in apple and orange juices. *Food Control, 50*, 722-728.

Ge, C., Bohrerova, Z., & Lee, J. (2013). Inactivation of internalized *Salmonella* Typhimurium in lettuce and green onion using ultraviolet C irradiation and chemical sanitizers. *Journal of Applied Microbiology, 114*(5), 1415-1424.

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology, 134*(1–2), 37-45.

Gilbert, P., & McBain, A. J. (2003). Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance. *Clinical Microbiology Reviews, 16*(2), 189-208.

Golberg, D., Kroupitski, Y., Belausov, E., Pinto, R., & Sela, S. (2011). *Salmonella* Typhimurium internalization is variable in leafy vegetables and fresh herbs. *International Journal of Food Microbiology, 145*(1), 250-257.

Gómez-López, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: A review. *Trends in Food Science & Technology, 20*(1), 17-26.

Goodburn, C., & Wallace, C. A. (2013). The microbiological efficacy of decontamination methodologies for fresh produce: A review. *Food Control, 32*(2), 418-427.

Gopal, A., Coventry, J., Wan, J., Roginski, H., & Ajlouni, S. (2010). Alternative disinfection techniques to extend the shelf life of minimally processed iceberg lettuce. *Food Microbiology, 27*(2), 210-219.

Gray, N. F. (2014). Ultraviolet disinfection. In S. L. Percival, R. M. Chalmers, M. Embrey, P. Hunter, J. Sellwood & P. Wyn-Jones (Eds.), *Microbiology of Waterborne Diseases* (2nd ed., pp. 617-630). London: Academic Press.

Guentzel, J. L., Liang Lam, K., Callan, M. A., Emmons, S. A., & Dunham, V. L. (2008). Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. *Food Microbiology, 25*(1), 36-41.

Gündüz, G. T., Gönül, Ş. A., & Karapınar, M. (2010). Efficacy of oregano oil in the inactivation of *Salmonella typhimurium* on lettuce. *Food Control, 21*(4), 513-517.

Gurtler, J. B., & Mai, T. L. (2014). Preservatives - traditional preservatives - organic acids. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 119-130). Oxford: Academic Press.

Gyawali, R., Ibrahim, S. A., Abu Hasfa, S. H., Smqadri, S. Q., & Haik, Y. (2011). Antimicrobial activity of copper alone and in combination with lactic acid against *Escherichia coli* O157:H7 in laboratory medium and on the surface of lettuce and tomatoes. *Journal of Pathogens, 650968*(10), 23.

Hansen, J. N. (1994). Nisin as a model food preservative. *Critical Reviews in Food Science and Nutrition, 34*(1), 69-93.

Hirshfield, I. N., Terzulli, S., & O'Byrne, C. (2003). Weak organic acids: a panoply of effects on bacteria. *Science Progress, 86*(Pt 4), 245-269.

Holah, J. T. (2014). Cleaning and disinfection practices in food processing. In H. L. M. Lelieveld, J. T. Holah & D. Napper (Eds.), *Hygiene in Food Processing* (2nd ed., pp. 259-304): Woodhead Publishing.

Horvitz, S., & Cantalejo, M. J. (2014). Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Reviews in Food Science and Nutrition, 54*(3), 312-339.

Hricova, D., Stephan, R., & Zweifel, C. (2008). Electrolyzed water and its application in the food industry. [Review]. *Journal of Food Protection, 71*(9), 1934-1947.

Huang, T.-S., Xu, C., Walker, K., West, P., Zhang, S., & Weese, J. (2006). Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. *Journal of Food Science, 71*(4), M134-M139.

Huang, Y.-R., Hung, Y.-C., Hsu, S.-Y., Huang, Y.-W., & Hwang, D.-F. (2008). Application of electrolyzed water in the food industry. *Food Control, 19*(4), 329-345.

Huang, Y., & Chen, H. (2011). Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157:H7 on baby spinach. *Food Control, 22*(8), 1178-1183.

Huang, Y., Ye, M., & Chen, H. (2012). Efficacy of washing with hydrogen peroxide followed by aerosolized antimicrobials as a novel sanitizing process to inactivate *Escherichia coli* O157:H7 on baby spinach. *International Journal of Food Microbiology, 153*(3), 306-313.

Hughes, K. A., Sutherland, I. W., Clark, J., & Jones, M. V. (1998). Bacteriophage and associated polysaccharide depolymerases - novel tools for study of bacterial biofilms. [Research Support, Non-U S Gov't]. *Journal of Applied Microbiology, 85*(3), 583-590.

Hunter, G. L., & Townsend, B. R. (2010). Ultraviolet light. In D. M. Desiderio & N. M. M. Nibbering (Eds.), *White's Handbook of Chlorination and Alternative Disinfectants* (5th ed.): Wiley.

Ibrahim, S. A., Yang, H., & Seo, C. W. (2008). Antimicrobial activity of lactic acid and copper on growth of *Salmonella* and *Escherichia coli* O157:H7 in laboratory medium and carrot juice. *Food Chemistry, 109*(1), 137-143.

Izumi, H. (2014). Preservatives - overall approach to hygienic processing. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 158-165). Oxford: Academic Press.

Joshi, K., Mahendran, R., Alagusundaram, K., Norton, T., & Tiwari, B. K. (2013). Novel disinfectants for fresh produce. *Trends in Food Science & Technology, 34*(1), 54-61.

Kentish, S., & Ashokkumar, M. (2011). The physical and chemical effects of ultrasound. In H. Feng, G. Barbosa-Canovas & J. Weiss (Eds.), *Ultrasound Technologies for Food and Bioprocessing* (pp. 1-12): Springer New York.

Keskinen, L. A., Burke, A., & Annous, B. A. (2009). Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157:H7 from lettuce leaves. *International Journal of Food Microbiology, 132*(2–3), 134-140.

Khadre, & Yousef, A. E. (2001). Sporicidal action of ozone and hydrogen peroxide: a comparative study. *International Journal of Food Microbiology, 71*(2-3), 131-138.

Kim, C., Hung, Y.-C., Brackett, R. E., & Frank, J. F. (2001). Inactivation of *Listeria monocytogenes* biofilms by electrolyzed oxidizing water. *Journal of Food Processing and Preservation, 25*(2), 91-100.

Kim, H. J., Feng, H., Kushad, M. M., & Fan, X. (2006). Effects of ultrasound, irradiation, and acidic electrolyzed water on germination of alfalfa and broccoli seeds and *Escherichia coli* O157:H7. *Journal of Food Science, 71*(6), M168-M173.

Kim, J.-G., Yousef, A. E., & Khadre, M. A. (2003). Ozone and its current and future application in the food industry. In S. Taylor (Ed.), *Advances in Food and Nutrition Research* (Vol. Volume 45, pp. 167-218): Academic Press.

Kitis, M. (2004). Disinfection of wastewater with peracetic acid: A review. *Environment International, 30*(1), 47-55.

Knowles, J., & Roller, S. (2001). Efficacy of chitosan, carvacrol, and a hydrogen peroxide-based biocide against foodborne microorganisms in suspension and adhered to stainless steel. *Journal of Food Protection, 64*(10), 1542-1548.

Kreske, A. C., Ryu, J. H., Pettigrew, C. A., & Beuchat, L. R. (2006). Lethality of chlorine, chlorine dioxide, and a commercial produce sanitizer to *Bacillus cereus*

and *Pseudomonas* in a liquid detergent, on stainless steel, and in biofilm. *Journal of Food Protection, 69*(11), 2621-2634.
Kroupitski, Y., Golberg, D., Belausov, E., Pinto, R., Swartzberg, D., Granot, D., & Sela, S. (2009). Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Applied and Environmental Microbiology, 75*(19), 6076-6086.
Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P., & Hovde, C. J. (1999). Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. *Applied and Environmental Microbiology, 65*(9), 3767-3773.
Kumar, C. G., & Anand, S. K. (1998). Significance of microbial biofilms in food industry: A review. *International Journal of Food Microbiology, 42*(1–2), 9-27.
Lado, B. H., & Yousef, A. E. (2002). Alternative food-preservation technologies: Efficacy and mechanisms. *Microbes and Infection, 4*(4), 433-440.
Lasa, A., Mira, A., Camelo-Castillo, A., Belda-Ferre, P., & Romalde, J. L. Analysis of the scallop microbiota by means of 16S rRNA gene pyrosequencing. [Abstract]. *Frontiers in Marine Science*.
Legay, C., Rodriguez, M. J., Sérodes, J. B., & Levallois, P. (2010). Estimation of chlorination by-products presence in drinking water in epidemiological studies on adverse reproductive outcomes: A review. *Science of The Total Environment, 408*(3), 456-472.
Lequette, Y., Boels, G., Clarisse, M., & Faille, C. (2010). Using enzymes to remove biofilms of bacterial isolates sampled in the food-industry. *Biofouling, 26*(4), 421-431.
Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., Saftner, R., & Sulakvelidze, A. (2003). Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied and Environmental Microbiology, 69*(8), 4519-4526.
Lianou, A., Koutsoumanis, K. P., & Sofos, J. N. (2012). Organic acids and other chemical treatments for microbial decontamination of food. In A. Demirci & M. O. Ngadi (Eds.), *Microbial Decontamination in the Food Industry* (pp. 592-664): Woodhead Publishing.
Ling, L. L., Schneider, T., Peoples, A. J., Spoering, A. L., Engels, I., Conlon, B. P., Mueller, A., Schaberle, T. F., Hughes, D. E., Epstein, S., Jones, M., Lazarides, L., Steadman, V. A., Cohen, D. R., Felix, C. R., Fetterman, K. A., Millett, W. P., Nitti, A. G., Zullo, A. M., Chen, C., & Lewis, K. (2015). A new antibiotic kills pathogens without detectable resistance. *Nature, 517*(7535), 455-459.
López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J. A., Selma, M. V., & Gil, M. I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology, 55*(1), 53-60.
Macnish, A. J., Leonard, R. T., & Nell, T. A. (2008). Treatment with chlorine dioxide extends the vase life of selected cut flowers. *Postharvest Biology and Technology, 50*(2–3), 197-207.
Magalhães, R., Mena, C., Ferreira, V., Silva, J., Almeida, G., Gibbs, P., & Teixeira, P. (2014). Bacteria: *Listeria monocytogenes*. In Y. Motarjemi (Ed.), *Encyclopedia of Food Safety* (pp. 450-461). Waltham: Academic Press.
Mahmoud, B. S. M., & Linton, R. H. (2008). Inactivation kinetics of inoculated *Escherichia coli* O157:H7 and *Salmonella enterica* on lettuce by chlorine dioxide gas. *Food Microbiology, 25*(2), 244-252.

Malik, Y. S., & Goyal, S. M. (2006). Virucidal efficacy of sodium bicarbonate on a food contact surface against feline calicivirus, a norovirus surrogate. *International Journal of Food Microbiology, 109*(1–2), 160-163.

Martín-Diana, A. B., Rico, D., Barry-Ryan, C., Frías, J. M., Henehan, G., & Barat, J. M. (2007). Efficacy of steamer jet-injection as alternative to chlorine in fresh-cut lettuce. *Postharvest Biology and Technology, 45*(1), 97-107.

Martín-Diana, A. B., Rico, D., Barry-Ryan, C., Frias, J. M., Mulcahy, J., & Henehan, G. T. M. (2005a). Calcium lactate washing treatments for salad-cut Iceberg lettuce: Effect of temperature and concentration on quality retention parameters. *Food Research International, 38*(7), 729-740.

Martín-Diana, A. B., Rico, D., Barry-Ryan, C., Frías, J. M., Mulcahy, J., & Henehan, G. T. M. (2005b). Comparison of calcium lactate with chlorine as a washing treatment for fresh-cut lettuce and carrots: quality and nutritional parameters. *Journal of the Science of Food and Agriculture, 85*(13), 2260-2268.

Martín-Diana, A. B., Rico, D., Frías, J., Henehan, G. T. M., Mulcahy, J., Barat, J. M., & Barry-Ryan, C. (2006). Effect of calcium lactate and heat-shock on texture in fresh-cut lettuce during storage. *Journal of Food Engineering, 77*(4), 1069-1077.

Martín-Espada, M. C., D'Ors, A., Bartolomé, M. C., Pereira, M., & Sánchez-Fortún, S. (2014). Peracetic acid disinfectant efficacy against *Pseudomonas aeruginosa* biofilms on polystyrene surfaces and comparison between methods to measure it. *LWT - Food Science and Technology, 56*(1), 58-61.

Martinez-Vaz, B. M., Fink, R. C., Diez-Gonzalez, F., & Sadowsky, M. J. (2014). Enteric pathogen-plant interactions: molecular connections leading to colonization and growth and implications for food safety. *Microbes and Environments, 29*(2), 123-135.

Meireles, A., Machado, I., Fulgêncio, R., Mergulhão, F., Melo, L., & Simões, M. (2015). Efficacy of antimicrobial combinations to reduce the use of sodium hypochlorite in the control of planktonic and sessile *Escherichia coli*. *Biochemical Engineering Journal, 104*, 115-122.

Melo, L. F., & Flemming, H. C. (2010). Mechanistic aspects of heat exchanger and membrane biofouling and prevention. In Z. Amjad (Ed.), *The Science and Technology of Industrial Water Treatment* (pp. 365-380). Florida, USA: CRC Press, Talyor and Francis Group.

Newman, D. J., Cragg, G. M., & Snader, K. M. (2000). The influence of natural products upon drug discovery. *Natural Product Reports, 17*(3), 215-234.

O'Keeffe, T., & Hill, C. (1999). Bacteriocins - potential in food preservation. In C. A. Batt & P. D. Patel (Eds.), *Encyclopedia of Food Microbiology* (pp. 183-191). Oxford: Elsevier.

Ohta, Y., Kondo, Y., Kawada, K., Teranaka, T., & Yoshino, N. (2008). Synthesis and antibacterial activity of quaternary ammonium salt-type antibacterial agents with a phosphate group. *Journal of Oleo Science, 57*(8), 445-452.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. [Review]. *Food Microbiology, 32*(1), 1-19.

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology, 42*(3), 686-693.

Ongeng, D., Devlieghere, F., Debevere, J., Coosemans, J., & Ryckeboer, J. (2006). The efficacy of electrolysed oxidising water for inactivating spoilage microorganisms in process water and on minimally processed vegetables. *International Journal of Food Microbiology, 109*(3), 187-197.

Otto, C., Zahn, S., Rost, F., Zahn, P., Jaros, D., & Rohm, H. (2011). Physical methods for cleaning and disinfection of surfaces. *Food Engineering Reviews, 3*(3-4), 171-188.

Oulahal-Lagsir, N., Martial-Gros, A., Bonneau, M., & Blum, L. J. (2003). "*Escherichia coli*-milk" biofilm removal from stainless steel surfaces: synergism between ultrasonic waves and enzymes. [Comparative Study]. *Biofouling, 19*(3), 159-168.

Palou, L., Smilanick, J. L., Usall, J., & Viñas, I. (2001). Control of postharvest blue and green molds of oranges by hot water, sodium carbonate, and sodium bicarbonate. *Plant Disease, 85*(4), 371-376.

Paniwnyk, L. (2014). Application of ultrasound. In D.-W. Sun (Ed.), *Emerging Technologies for Food Processing* (2nd ed., pp. 271-291). San Diego: Academic Press.

Parish, M. E., Beuchat, L. R., Suslow, T. V., Harris, L. J., Garrett, E. H., Farber, J. N., & Busta, F. F. (2003). Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2*, 161-173.

Park, E. J., Alexander, E., Taylor, G. A., Costa, R., & Kang, D. H. (2008). Fate of foodborne pathogens on green onions and tomatoes by electrolysed water. *Letters in Applied Microbiology, 46*(5), 519-525.

Park, K. M., Kim, H. J., & Koo, M. S. (2013). Susceptibility of foodborne pathogens isolated from fresh-cut products and organic vegetable to organic acids and sanitizers. *Journal of Food Hygiene and Safety, 28*(3), 227-233.

Park, S. H., Choi, M. R., Park, J. W., Park, K. H., Chung, M. S., Ryu, S., & Kang, D. H. (2011). Use of organic acids to inactivate *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on organic fresh apples and lettuce. *Journal of Food Science, 76*(6), 1750-3841.

Rahman, S. M., Jin, Y. G., & Oh, D. H. (2010). Combined effects of alkaline electrolyzed water and citric acid with mild heat to control microorganisms on cabbage. [Research Support, Non-U S Gov't]. *Journal of Food Science, 75*(2), 1750-3841.

Ramos, B., Miller, F. A., Brandão, T. R. S., Teixeira, P., & Silva, C. L. M. (2013). Fresh fruits and vegetables - An overview on applied methodologies to improve its quality and safety. *Innovative Food Science & Emerging Technologies, 20*(0), 1-15.

Rico, D., Martin-Diana, A. B., Barat, J., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: A review. *Trends in Food Science & Technology, 18*(7), 373-386.

Rico, D., Martín-Díana, A. B., Barry-Ryan, C., Frías, J. M., Henehan, G., & Barat, J. M. (2008a). Optimisation of steamer jet-injection to extend the shelflife of fresh-cut lettuce. *Postharvest Biology and Technology, 48*, 431-442.

Rico, D., Martín-Díana, A. B., Barry-Ryan, C., Frías, J. M., Henehan, G., & Barat, J. M. (2008b). Use of neutral electrolysed water (EW) for quality maintenance and shelf-life extension of minimally processed lettuce. *Innovative Food Science & Emerging Technologies, 9*(1), 37-48.

Rico, D., Martín-Diana, A. B., Frías, J. M., Henehan, G. T. M., & Barry-Ryan, C. (2006). Effect of ozone and calcium lactate treatments on browning and texture properties of fresh-cut lettuce. *Journal of the Science of Food and Agriculture, 86*(13), 2179-2188.

Robbins, J. B., Fisher, C. W., Moltz, A. G., & Martin, S. E. (2005). Elimination of *Listeria monocytogenes* biofilms by ozone, chlorine, and hydrogen peroxide. *Journal of Food Protection, 68*(3), 494-498.

Rodgers, S. (2001). Preserving non-fermented refrigerated foods with microbial cultures - A review. *Trends in Food Science & Technology, 12*(8), 276-284.

Rodgers, S. (2008). Novel applications of live bacteria in food services: Probiotics and protective cultures. *Trends in Food Science & Technology, 19*(4), 188-197.

Rodgers, S. L., & Ryser, E. T. (2004). Reduction of microbial pathogens during apple cider production using sodium hypochlorite, copper ion, and sonication. *Journal of Food Protection, 67*(4), 767-771.

Roller, S., & Seedhar, P. (2002). Carvacrol and cinnamic acid inhibit microbial growth in fresh-cut melon and kiwifruit at 4 º and 8 ºC. *Letters in Applied Microbiology, 35*(5), 390-394.

Rosenblum, J., Ge, C., Bohrerova, Z., Yousef, A., & Lee, J. (2012). Ozonation as a clean technology for fresh produce industry and environment: sanitizer efficiency and wastewater quality. *Journal of Applied Microbiology, 113*(4), 837-845.

Sagong, H.-G., Lee, S.-Y., Chang, P.-S., Heu, S., Ryu, S., Choi, Y.-J., & Kang, D.-H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology, 145*(1), 287-292.

Sakurai, Y., Nakatsu, M., Sato, Y., & Sato, K. (2003). Endoscope contamination from HBV- and HCV-positive patients and evaluation of a cleaning/disinfecting method using strongly acidic electrolyzed water. *Digestive Endoscopy, 15*(1), 19-24.

Salehi, F. (2014). Current and future applications for nanofiltration technology in the food processing. *Food and Bioproducts Processing, 92*(2), 161-177.

Samara, A., & Koutsoumanis, K. P. (2009). Effect of treating lettuce surfaces with acidulants on the behaviour of *Listeria monocytogenes* during storage at 5 and 20 °C and subsequent exposure to simulated gastric fluid. *International Journal of Food Microbiology, 129*(1), 1-7.

São José, J. F. B. d., Andrade, N. J. d., Ramos, A. M., Vanetti, M. C. D., Stringheta, P. C., & Chaves, J. B. P. (2014). Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control, 45*(0), 36-50.

Sapers, G. M. (2009). Disinfection of contaminated produce with conventional washing and sanitizing technology. In G. M. Sapers, E. B. Solomon & K. R. Matthews (Eds.), *The Produce Contamination Problem* (pp. 393-424). San Diego: Academic Press.

Sapers, G. M., Miller, R. L., Pilizota, V., & Kamp, F. (2001). Shelf-life extension of fresh mushrooms (*Agaricus bisporus*) by application of hydrogen peroxide and browning inhibitors. *Journal of Food Science, 66*(2), 362-366.

Seiber, J. N. (2012). Modern Issues in Food Safety ? A Perspective. *Journal of Integrative Agriculture, 11*(1), 9-13.

Selma, M. V., Allende, A., López-Gálvez, F., Conesa, M. A., & Gil, M. I. (2008). Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology, 25*(6), 809-814.

Selma, M. V., Beltran, D., Allende, A., Chacon-Vera, E., & Gil, M. I. (2007). Elimination by ozone of *Shigella sonnei* in shredded lettuce and water. [Research Support, Non-U S Gov't]. *Food Microbiology, 24*(5), 492-499.

Seo, K. H., & Frank, J. F. (1999). Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. *Journal of Food Protection, 62*(1), 3-9.

Seymour, I. J., Burfoot, D., Smith, R. L., Cox, L. A., & Lockwood, A. (2002). Ultrasound decontamination of minimally processed fruits and vegetables. *International Journal of Food Science & Technology, 37*(5), 547-557.

Shama, G. (2014). Ultraviolet light. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 665-671). Oxford: Academic Press.

Sharma, M., Ryu, J. H., & Beuchat, L. R. (2005). Inactivation of *Escherichia coli* O157:H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage. *Journal of Applied Microbiology, 99*(3), 449-459.

Sillankorva, S., Oliveira, R., Vieira, M. J., Sutherland, I., & Azeredo, J. (2004). Bacteriophage Φ S1 infection of *Pseudomonas fluorescens* planktonic cells versus biofilms. *Biofouling, 20*(3), 133-138.

Simões, L. C., & Simões, M. (2013). Biofilms in drinking water: problems and solutions. *RSC Advances, 3*(8), 2520-2533.

Simões, M., Lemos, M., & Simões, L. C. (2012). Phytochemicals against drug-resistant microbes. In A. K. Patra (Ed.), *Dietary Phytochemicals and Microbes*: Springer.

Simões, M., Simões, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT - Food Science and Technology, 43*(4), 573-583.

Singh, N., Singh, R. K., Bhunia, A. K., & Stroshine, R. L. (2002a). Effect of inoculation and washing methods on the efficacy of different sanitizers against *Escherichia coli* O157:H7 on lettuce. *Food Microbiology, 19*(2–3), 183-193.

Singh, N., Singh, R. K., Bhunia, A. K., & Stroshine, R. L. (2002b). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *LWT - Food Science and Technology, 35*(8), 720-729.

Singh, R. (2015). Introduction to membrane technology. In R. Singh (Ed.), *Membrane Technology and Engineering for Water Purification (Second Edition)* (pp. 1-80). Oxford: Butterworth-Heinemann.

Smilanick, J. L., Margosan, D. A., Mlikota, F., Usall, J., & Michael, I. F. (1999). Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. *Plant Disease, 83*(2), 139-145.

Soni, K. A., Oladunjoye, A., Nannapaneni, R., Schilling, M. W., Silva, J. L., Mikel, B., & Bailey, R. H. (2013). Inhibition and inactivation of *Salmonella* Typhimurium biofilms from polystyrene and stainless steel surfaces by essential oils and phenolic constituent carvacrol. *Journal of Food Protection, 76*(2), 205-212.

Spricigo, D. A., Bardina, C., Cortés, P., & Llagostera, M. (2013). Use of a bacteriophage cocktail to control *Salmonella* in food and the food industry. *International Journal of Food Microbiology, 165*(2), 169-174.

St. Laurent, J. B., de Buzzaccarini, F., De Clerck, K., Demeyere, H., Labeque, R., Lodewick, R., & van Langenhove, L. (2007). B.1.I - Laundry cleaning of textiles. In I. Johansson & P. Somasundaran (Eds.), *Handbook for Cleaning/Decontamination of Surfaces* (pp. 57-102). Amsterdam: Elsevier Science B.V.

Sudhaus, N., Nagengast, H., Pina-Pérez, M. C., Martínez, A., & Klein, G. (2014). Effectiveness of a peracetic acid-based disinfectant against spores of *Bacillus cereus* under different environmental conditions. *Food Control, 39*(0), 1-7.

Thallinger, B., Prasetyo, E. N., Nyanhongo, G. S., & Guebitz, G. M. (2013). Antimicrobial enzymes: an emerging strategy to fight microbes and microbial biofilms. *Biotechnology Journal, 8*(1), 97-109.

Theron, M. M., & Lues, J. R. (2011). *Organic Acids And Food Preservation*: CRC Press.

Tirpanalan, O., Zunabovic, M., Domig, K., & Kneifel, W. (2011). Mini review: antimicrobial strategies in the production of fresh-cut lettuce products. In A. Méndez-Vilas (Ed.), *Science against microbial pathogens: communicating current research and technological advances* (Vol. 1, pp. 176-188). Badajoz, Spain: Formatex Research Center.

Tiwari, B. K., Valdramidis, V. P., O' Donnell, C. P., Muthukumarappan, K., Bourke, P., & Cullen, P. J. (2009). Application of natural antimicrobials for food preservation. *Journal of Agricultural and Food Chemistry, 57*(14), 5987-6000.

Tomás-Callejas, A., López-Gálvez, F., Sbodio, A., Artés, F., Artés-Hernández, F., & Suslow, T. V. (2012). Chlorine dioxide and chlorine effectiveness to prevent *Escherichia coli* O157:H7 and *Salmonella* cross-contamination on fresh-cut red chard. *Food Control, 23*(2), 325-332.

Trinetta, V., Vaidya, N., Linton, R., & Morgan, M. (2011). Evaluation of chlorine dioxide gas residues on selected food produce. *Journal of Food Science, 76*(1), T11-15.

Ukuku, D. O., & Fett, W. (2002). Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection, 65*(6), 924-930.

Van Haute, S., López-Gálvez, F., Gómez-López, V. M., Eriksson, M., Devlieghere, F., Allende, A., & Sampers, I. (2015). Methodology for modeling the disinfection efficiency of fresh-cut leafy vegetables wash water applied on peracetic acid combined with lactic acid. *International Journal of Food Microbiology, 208*, 102-113.

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaele, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology, 79*(9), 2850-2861.

Van Haute, S., Tryland, I., Veys, A., & Sampers, I. (2015). Wash water disinfection of a full-scale leafy vegetables washing process with hydrogen peroxide and the use of a commercial metal ion mixture to improve disinfection efficiency. *Food Control, 50*(0), 173-183.

Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., & Van Camp, J. (2009). Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiology, 26*(8), 882-888.

Velázquez, L. d. C., Barbini, N. B., Escudero, M. E., Estrada, C. L., & Guzmán, A. M. S. d. (2009). Evaluation of chlorine, benzalkonium chloride and lactic acid as sanitizers for reducing *Escherichia coli* O157:H7 and *Yersinia enterocolitica* on fresh vegetables. *Food Control, 20*(3), 262-268.

Vitale, M., & Schillaci, D. (2016). Food Processing and Foodborne Illness *Reference Module in Food Science*: Elsevier.

Vurma, M., Pandit, R. B., Sastry, S. K., & Yousef, A. E. (2009). Inactivation of *Escherichia coli* O157:H7 and natural microbiota on spinach leaves using

gaseous ozone during vacuum cooling and simulated transportation. [Research Support, Non-U S Gov't]. *Journal of Food Protection, 72*(7), 1538-1546.

Wang, H., Li, Y., & Slavik, M. F. (2001). Efficacy of cetylpyridinium chloride in immersion treatment for reducing populations of pathogenic bacteria on fresh-cut vegetables. *Journal of Food Protection, 64*(12), 2071-2074.

Warriner, K., Huber, A., Namvar, A., Fan, W., & Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. In L. T. Steve (Ed.), *Advances in Food and Nutrition Research* (Vol. Volume 57, pp. 155-208): Academic Press.

Yaron, S., & Romling, U. (2014). Biofilm formation by enteric pathogens and its role in plant colonization and persistence. *Microbial Biotechnology, 7*(6), 496-516.

Yuk, H.-G., Yoo, M.-Y., Yoon, J.-W., Moon, K.-D., Marshall, D. L., & Oh, D.-H. (2006). Effect of combined ozone and organic acid treatment for control of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce. *Journal of Food Science, 71*(3), M83-M87.

Figure Captions

Figure 1 – Schematic overview on the advantages and disadvantages of chlorine and the alternative methods of disinfection and/or decontamination (biological, physical, chemical and their combination).

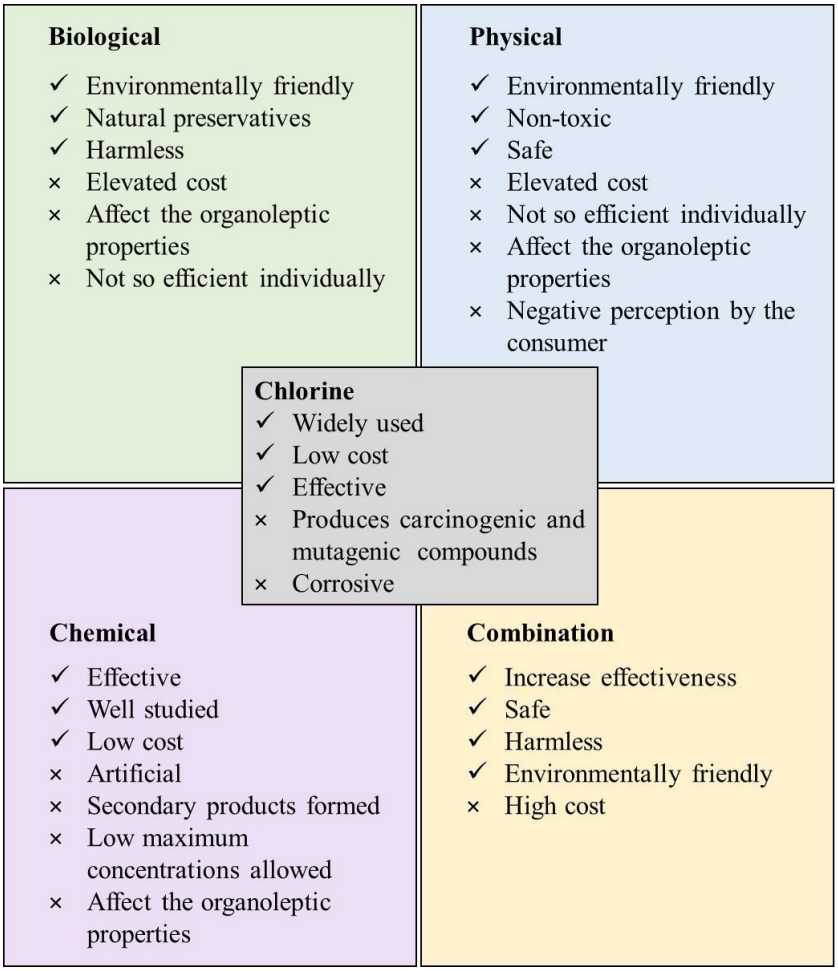
Biological
✓ Environmentally friendly
✓ Natural preservatives
✓ Harmless
× Elevated cost
× Affect the organoleptic properties
× Not so efficient individually
Physical
✓ Environmentally friendly
✓ Non-toxic
✓ Safe
× Elevated cost
× Not so efficient individually
× Affect the organoleptic properties
× Negative perception by the consumer
Chlorine
✓ Widely used
✓ Low cost
✓ Effective
× Produces carcinogenic and mutagenic compounds
× Corrosive
Chemical
✓ Effective
✓ Well studied
✓ Low cost
× Artificial
× Secondary products formed
× Low maximum concentrations allowed
× Affect the organoleptic properties
Combination
✓ Increase effectiveness
✓ Safe
✓ Harmless
✓ Environmentally friendly
× High cost

Figure 1

1 Table 1 - Alternative disinfection methods used in the fresh-cut food industry, applied on the food-contact surfaces, on the produce and on the
2 water

Target	Method	Results	Reference
	Nisin (6.75×10^{-3} ppm, 5 min, 20 ^{0}C)	Reduction of 2.58 log CFU/cm^2 of *L. monocytogenes* on SS surfaces	(Arevalos-Sánchez et al., 2012)
	Nisin (6.75×10^{-3} ppm, 20 min, 20 ^{0}C)	Reduction of 1.92 log CFU/cm^2 of *L. monocytogenes* on glass surfaces	(Arevalos-Sánchez et al., 2012)
	Lytic phage (phage ϕ S1)	Reduction of 80% of *P. fluorescens* biofilm on SS surfaces	(Sillankorva et al., 2004)
	Carvacrol (0.05 to 0.1%, 1 hour)	Reduction of 7 log CFU of *Salmonella* sp. on PS and SS surfaces	(Soni et al., 2013)
	Carvacrol (2 mM)	Reduction of 2-3 log CFU of bacteria (listeriae and salmonellae) on SS surfaces	(Knowles & Roller, 2001)
	ClO_2 (200 µg/mL)	Reduction of 4.42 log CFU of *B. cereus* on SS surfaces	(Kreske et al., 2006)
	ClO_2 (5%, 10 min)	Reduction of 4.14 log CFU/chip of *L. monocytogenes* biofilm	(Robbins et al., 2005)
	EOW (56 ppm of free chlorine, 5 min)	Reduction of 9 log CFU/cm^2 of *L. monocytogenes* on SS surfaces	(Kim et al., 2001)
	NEOW (63 ppm of free chlorine, 1 min)	Reduction of 6 log CFU/cm^2 of *E. coli*, *P. aeruginosa*, *Staphylococcus aureus* and *L. monocytogenes* on SS and glass surfaces	(Deza et al., 2005)
	NEOW (70 ppm of free chlorine, 3 min)	*L. monocytogenes* biofilms (on SS surfaces) completely inhibited	(Arevalos-Sánchez et al., 2013)
	Aqueous ozone (5.9 ppm, 1 min)	*B. subtilis* and *P. fluorescence* were completely eliminated from SS surfaces	(Khadre & Yousef, 2001)
	Sodium bicarbonate (5×10^4 ppm, 1 min)	Reduction of 99.22% of feline calicivirus on food contact surfaces	(Malik & Goyal, 2006)
	US (40 kHz)	30% of *E. coli* biofilm removal on SS	(Oulahal-Lagsir et al., 2003)
	α-amylase + Realco B (30 min)	Reduction of 2.98 log CFU/cm^2 of *B. mycoides* on SS	(Lequette et al., 2010)

		surfaces	
	Sodium bicarbonate (2×10^4 ppm) + H_2O_2 (2×10^4 ppm), for 10 min	Reduction of 99.68% of feline calcivirus on food contact surfaces	(Malik & Goyal, 2006)
	US (40 kHz) + trypsin (7600 U/mL)	76% of *E. coli* biofilm removal on SS	(Oulahal-Lagsir et al., 2003)
	Nisin (50 ppm, 1 min)	Reduction of 2.20 and 4.35 log CFU of *Listeria monocytogenes* on mung bean and broccoli, respectively	(Bari et al., 2005)
	Lytic bacteriophages (UAB_Phi 20, UAB_Phi78, and UAB_Phi87) (60 min at room temperature)	Reduction of 3.9 and 2.2 log CFU/g for *S.* Typhimurium and *S.* Enteritidis, respectively, on lettuce	(Spricigo et al., 2013)
	Lytic *L. monocytogenes*-specific phages	Reduction of 2.0-4.6 log CFU of *L. monocytogenes* per melon sample	(Leverentz et al., 2003)
	Carvacrol (150 ppm)	Reduction of the total viable counts in 4.6 log CFU/g on kiwi	(Roller & Seedhar, 2002)
	Cinnamic acid (150 ppm)	Reduction of the total viable counts in 4.6 log CFU/g on kiwi	(Roller & Seedhar, 2002)
	Oregano oil (25, 40 and 75 ppm, at 5, 10, 15 and 20 min)	Reduction of 1.92 log CFU/g of *S.* Typhimurium on lettuce	(Gündüz et al., 2010)
	ClO_2 (10 ppm, 5 min)	Reduction of 1.2 log CFU/g of *E. coli* O157:H7 on lettuce	(Singh et al., 2002a)
	ClO_2 (100 ppm)	Reduction of 3.5-4.0 log CFU/g of total bacterial and coliform counts on lettuce	(Chung et al., 2011)
	ClO_2 (100 ppm)	Reduction of 1.25 log CFU/g of *E. coli* O157:H7 on lettuce	(Keskinen et al., 2009)
	AcEOW (pH 2.6, at 50 ppm (free chlorine), 2 min)	Reduction of 1 log CFU/g of *E. coli* O157:H7 on lettuce	(Keskinen et al., 2009)
	AcEOW (pH 2.06, at 37.5 ppm (free chlorine), 1 min)	Reduction of 4.45 log CFU/g of *E. coli* O157:H7 on green onions	(Park et al., 2008)
	NEOW (89 ppm (free chlorine), 5 min treatment)	Reduction of 6 log CFU/mL of *E. coli* O157:H7, *S.* Enteritidis and *L. monocytogenes* on tomatoes	(Deza et al., 2003)
	NEOW (20 ppm (free chlorine), 10 min)	Reduction of 6 log CFU/mL of *E. coli*, *S.* Typhimurium, *Staphylococcus aureus*, *L. monocytogenes* and *Enterococcus faecalis*, on lettuce	(Guentzel et al., 2008)
	NEW (50 ppm free chlorine)	Reduction of 1-2 log CFU/mL of *E. coli* O157:H7, *Salmonella*, *L. innocua* and *Erwinia carotovora* on lettuce	(Abadias et al., 2008)

	H_2O_2 (3×10^4 ppm, 5 min)	Reduction of 1.6 log CFU/g reduction of *E. coli* O157:H7 on baby spinach leaves	(Huang et al., 2012)
	H_2O_2 (5×10^4 ppm, 2 min)	Reduction of 2.0-3.5 log CFU/cm^2 of *L. monocytogenes* from melon surfaces	(Ukuku & Fett, 2002)
	H_2O_2 (2×10^4 ppm)	Reduction of 1.5 log CFU/g of *E. coli* O157:H7 on baby spinach leaves	(Huang & Chen, 2011)
	Citric acid (5×10^3 to 1×10^4 ppm, at 20 ^{0}C for 1 to 5 min)	Reduction of 1 log CFU/cm^2 of *Listeria monocytogenes* from lettuce	(Samara & Koutsoumanis, 2009)
	Acetic acid (20 ^{0}C for 2-5 min)	Reduction 2.2 and 1.3 log CFU/g of *E. coli* and *L. monocytogenes* respectively, from lettuce	(Akbas & Olmez, 2007)
	Lactic acid (20 ^{0}C for 2-5 min)	Reduction of 2.8 and 2.1 log CFU/g of *E. coli* and *L. monocytogenes*, respectively, from lettuce	(Akbas & Olmez, 2007)
	PAA (120 ppm)	Reduction of the microbial load in 1.2 log CFU/g on fresh-cut iceberg lettuce	(Vandekinderen et al., 2009)
	PAA (40 ppm, 5 min)	Reduction of 0.99 log CFU/g of *S.* Typhimurium on lettuce	(Ge et al., 2013)
	Propionic acid (1×10^4 ppm, 10 min)	Reduction of 0.93-1.52 log CFU/g of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on organic fresh lettuce	(Park et al., 2011)
	Acetic acid (1×10^4 ppm, 10 min)	Reduction of 1.13-1.74 log CFU/g of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on organic fresh lettuce	(Park et al., 2011)
	Lactic acid (1×10^4 ppm, 10 min)	Reduction of 1.87-2.54 log CFU/g of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on organic fresh lettuce	(Park et al., 2011)
	Malic acid (1×10^4 ppm, 10 min)	Reduction of 2.32-2.98 log CFU/g of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on organic fresh lettuce	(Park et al., 2011)
	Citric acid (1×10^4 ppm, 10 min)	Reduction of 1.85-2.86 log CFU/g of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* on organic fresh lettuce	(Park et al., 2011)
	Benzalkonium chloride (2 ppm)	Growth of *B. cereus*, *Staphylococcus aureus* and *E. coli* (isolated from fresh vegetables) completely inhibited	(Park et al., 2013)
	Cetylpyridinium chloride (5×10^3 ppm)	Reduction of 3.70, 3.15 and 1.56 log CFU/g for *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7, respectively, from broccoli, cauliflower, and radishes	(Wang et al., 2001)
	Aqueous ozone (5 ppm, 5 min)	Reduction of 1.8 log CFU of *Shigella sonnei* from shredded lettuce	(Selma et al., 2007)

	Gaseous ozone (5-10 ppm)	Reduction 1.8 log CFU/g of *E. coli* O157:H7 on spinach leaves	(Vurma et al., 2009)
	Sodium bicarbonate (2×10^4 to 4×10^4 ppm, 150 seconds at room temperature)	Reduction of 50% of blue mold by *Penicillium italicum* in citrus	(Palou et al., 2001)
	UV lamp (254 nm)	Reductions of 1.75, 1.27, 1.39 and 1.21 log CFU/g of *E. coli*, *L. innocua*, *S.* Enteritidis and *Staphylococcus aureus*, respectively, on lettuce	(Birmpa et al., 2013)
	UV lamp (253.7 nm, 60 min)	Reduction of 1.7 log CFU/g of *E. coli* on lettuce	(Bermúdez-Aguirre & Barbosa-Cánovas, 2013)
	UV-C lamp (254 nm, irradiation fluency of 450 mJ/cm^2, 5 min)	Reduction of 2.28 log CFU/g of *S.* Typhimurium on lettuce by applying a.	(Ge et al., 2013)
	US (32-40 kHz, 10 min)	Reduction of 1.5 log CFU/g of *S.* Typhimurium on cut iceberg lettuce	(Seymour et al., 2002)
	US (37 kHz, 30 min)	Reduction of 2.30, 5.72 and 1.88 log CFU/g of *E. coli*, *S.* Enteritidis, *L. innocua*, respectively, on lettuce.	(Birmpa et al., 2013)
	US (40 kHz, at 23 ^{0}C, for 30 min)	Reduction of 1.08 log CFU/g of *E. coli* O157:H7 on broccoli seeds	(Kim et al., 2006)
	Irradiation (0.55 kGy) + chlorine (200 ppm)	Reduction of 5.4 log CFU/g of *E. coli* O157:H7 on shredded iceberg lettuce	(Foley et al., 2002)
	Heating (50 ^{0}C) + H_2O_2 (2×10^4 ppm)	Reduction of 2.2 log CFU/g of *E. coli* O157:H7 on baby spinach	(Huang & Chen, 2011)
	Heating (50 ^{0}C, 1 min) + chlorine (100 ppm)	Reduction of 2.0 log CFU/g of total microbial populations on fresh-cut iceberg lettuce	(Delaquis et al., 2004)
	US (32-40 kHz) + chlorine (25 ppm) (10 min treatment)	Reduction of 2.7 log CFU/g of *S.* Typhimurium from cut lettuce	(Seymour et al., 2002)
	US (40 kHz) + lactic acid (2%), for 5 min at 20 ^{0}C	Reduction of 2.75, 2.71 and 2.50 log CFU/g of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, respectively, on organic lettuces	(Sagong et al., 2011)
	ClO_2 (20-40 ppm) + US (170 kHz)	Reduction of 2.6 and 1.8 log CFU/g of *Salmonella* spp. and *E. coli* O157:H7 on lettuce	(Huang et al., 2006)
	ClO_2 (40 ppm) + ozone (5 ppm) (5 min treatment)	Reduction of 2.17 log CFU/g of total bacterial count on	(Ibrahim et al., 2008)

		turnip greens	
	Copper (40 ppm) + lactic acid (2×10^3 ppm)	Reduction of 3.93 log CFU/cm^2 of *E. coli* O157:H7 on lettuce surface	(Gyawali et al., 2011)
	AlEOW (100 ppm) + citric acid (1×10^4 ppm) at 50 ^{0}C	Reduction of 3.99 log CFU/g and 4.19 log CFU/g of *L. monocytogenes* and *E. coli* O157:H7, respectively, on cabbage	(Rahman et al., 2010)
	Citric acid (1×10^4 ppm) + ozonated water (3 ppm), for 1 min	Reduction of 2.31 and 1.84 log CFU/g of *E. coli* O157:H7 and *L. monocytogenes* on lettuce	(Yuk et al., 2006)
	UV-C (254 nm, irradiation fluency of 900 mJ/cm^2, 10 min) + chlorine (200 ppm, 10 min)	Reduction of 2.40 log CFU/g of *S.* Typhimurium on lettuce	(Ge et al., 2013)
	UV-C (254 nm, irradiation fluency of 900 mJ/cm^2, 10 min) + PAA (80 ppm, 10 min)	Reduction of 2.52 log CFU/g of *S.* Typhimurium on lettuce	(Ge et al., 2013)
	Ozone (2 ppm, 10 min)	Reduction of 1.56 log CFU/mL of *B. subtilis*	(Rosenblum et al., 2012)
	US (20 kHz, 53 min)	Reduction of 4.4 log CFU/mL of *E. coli* O157:H7 on fresh-cut vegetables wash water	(Elizaquível et al., 2012)
	UV and ozone (60 min)	Microbial reduction of 6.6 log CFU/mL in escarole wash water	(Selma et al., 2008)
	QACs (n-alkyl dimethyl benzyl ammonium chloride sulfosuccinate dioetil and urea) (100 and 200 ppm, 30 and 120 seconds)	Reduction of 99.99% of *E. coli* and *Staphylococcus aureus* (for 100 and 200 ppm, for 30 and 120 seconds, for low and high turbidity), except for *E. coli* with high turbidity in the disinfection process of 100 ppm at 30 and 120 seconds (20.78% and 87.94%, respectively).	(Chaidez et al., 2007)

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