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

2.1. Biological-based methods

2.1.1. Bacteriocins

One possibility to prevent the growth of both spoilage and pathogenic microorganisms is the exploitation of their competition with other microorganisms, typically with beneficial ones (Ramos et al., 2013). Lactic acid bacteria (LAB) are an example of such beneficial microorganisms, having GRAS (Generally Recognized as Safe) status. Furthermore, LAB produce antimicrobial compounds, such as organic acids and bacteriocins, which can be used as antimicrobials (Rodgers, 2001, 2008). A well-known example of such compound is the bacteriocin nisin. This natural preservative is produced by *Lactococcus lactis* and is effective mainly against Gram positive bacteria (Arevalos-Sánchez, Regalado, Martin, Domínguez-Domínguez, & García-Almendárez, 2012; Davies & Delves-Broughton, 1999; Hansen, 1994; Magalhães, Mena, Ferreira, Silva, Almeida, Gibbs, & Teixeira, 2014). The Gram negative bacteria are not affected by this peptide due to their outer membrane. Nevertheless, this drawback can be overcome by exposing the cells to chelating agents or osmotic shock that destabilize the outer membrane before the use of nisin (Angiolillo, Conte, & Del Nobile, 2014; Delves-Broughton, 2005). Nisin acts on the cell membrane forming pores that result in cell death (Arevalos-Sánchez et al., 2012; Bari, Ukuku, Kawasaki, Inatsu, Isshiki, & Kawamoto, 2005). The major advantage on the use of nisin as a disinfectant is the fact that it is harmless and is already used as a food preservative (O'Keeffe & Hill, 1999). With regard to fresh produce, Bari et al. (2005) used nisin at 50 ppm for 1 min on mung bean and broccoli and achieved the reduction of *L. monocytogenes* by 2.20 and 4.35 log CFU/g, respectively. The disinfecting efficacy observed with 1 min contact time was higher for broccoli. Allende, Martínez, Selma, Gil, Suárez, and Rodríguez (2007) incorporated nisin and coagulin individually and combined in tryptic soy agar (TSA) plates and obtained reductions of *L. monocytogenes* of 1.0-1.5 log colony forming units (CFU) after 48 hours of storage at 4 ^{0}C. Bacteriocins have also been used to disinfect stainless steel (SS) and glass surfaces. Arevalos-Sánchez et al. (2012) used nisin at 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 have GRAS status and are FDA and EC (European Commission) approved. Besides their use as preservatives, they are also used as antioxidants, flavoring agents, acidulants and pH regulators (Carpenter & Broadbent, 2009; Theron & Lues, 2011). Citric, acetic and lactic acids are the most common acids applied in the food industry (Ölmez & Kretzschmar, 2009; Rico et al., 2007). Their mode of action is based on the acidification of the cytoplasm, disruption of proton motive force, osmotic stress and inhibition of macromolecule synthesis (Brul & Coote, 1999; Carpenter & Broadbent, 2009; Hirshfield et al., 2003). Furthermore, they have a quick mode of action against an extensive range of bacteria grown under varying temperatures (Hirshfield et al., 2003; Sagong et al., 2011). Organic acids have advantages towards sodium hypochlorite when used as disinfectants for the fresh-cut industry, as they interaction with organic molecules do not produce toxic or carcinogenic compounds (Lianou et al., 2012). Their possible disadvantage could be the change in the flavor of the product that could influence its sensorial analysis. Furthermore, when organic acids are used to disinfect fresh-produce, the wastewater may present high values of both chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (Ölmez & Kretzschmar, 2009). Other pointed disadvantages are their high cost and the corrosiveness of the processing equipment that they may provoke (Sagong et al., 2011).

Citric acid is a preservative and flavoring agent usually applied in the food and pharmaceutical industries (Ölmez & Kretzschmar, 2009). Contrary to the action of the other acids, this acts as a chelating agent of metallic ions present in the medium, preventing microbial proliferation (Gurtler & Mai, 2014). Samara and Koutsoumanis (2009) used citric acid (5×10^3 to 1×10^4 ppm) at 20 ^{0}C for 1 to 5 min to control *L. monocytogenes* from lettuce and obtained 1 log CFU/cm^2 reduction. Acetic acid is soluble in lipids and therefore is able to diffuse through the cytoplasmic membrane, affecting the intracellular pH of microorganisms, causing cell death (Lianou et al., 2012). Akbas and Olmez (2007) tested concentrations of 5×10^3 to 1×10^4 ppm of acetic and lactic acids (used in separate) (20 ^{0}C for 2 to 5 min) in order to decontaminate lettuce leafs. They were able to reduce the populations of *E. coli* and *L. monocytogenes* with acetic acid by 2.2 and 1.3 log CFU/g, respectively; lactic acid caused reductions of 2.8 and 2.1 log CFU/g, respectively (Akbas & Olmez, 2007). The available research clearly shows that in order to have significantly antimicrobial effects these organic acids 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 reduced heating of the food (Ramos et al., 2013). This method has not yet been adopted in the fresh produce industry mainly because: i) further research is needed to determine the necessary doses for different products (Goodburn & Wallace, 2013); ii) the consumer still have a strong negative perception of irradiated foods (Goodburn & Wallace, 2013; Ramos et al., 2013); iii) the quality of the fresh produce can be affected, especially at high doses (Ramos et al., 2013).

2.3.2. Membrane filtration

Membrane separation can be used to treat the process water, to avoid cross-contamination of the produce (Allende, Selma, Lopez-Galvez, Villaescusa, & Gil, 2008; Gil et al., 2009). This procedure involves the flow of water through a semipermeable membrane and the consequent retention of the undesired contaminants on the membrane. Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO), are membrane unit operations that can be applied in the food industry to treat the process water (Cassano & Basile, 2013). MF is a dead-end filtration, where the feed flows vertical to the membrane and all the solids become retained on the membrane. In MF the membrane pore size is between 0.05 and 10 µm (Berk, 2009; Salehi, 2014), the molecular weight cut off (MWCO) is 200 kDa (Singh, 2015) and the pressure used is below 0.2 MPa (Salehi, 2014). This membrane process is usually applied for the retention of microorganisms (Salehi, 2014). UF membranes have a pore size of 0.001-0.05 µm, a MWCO between 1 and 300 kDa (Singh, 2015) and a pressure of 0.2-0.5 MPa. UF is usually used to separate proteins and other high molecular weight organic compounds (Salehi, 2014; Singh, 2015). Regarding NF, the membrane pore size is lower than 2 nm (Salehi, 2014), the MWCO is between 100 and 1000 Da (Singh, 2015) and the pressure is of 0.5-1.5 MPa (Salehi, 2014). Normally NF is used for water softening and the removal of salts (Singh, 2015). In RO the membrane has a pore size of 0.6 nm (Singh, 2015), with a MWCO of 100 Da (Salehi, 2014) and the pressure used is between 1.5 and 10 MPa (Berk, 2009; Salehi, 2014; Singh, 2015). The main disadvantage of these methods is the high investment costs associated with the implementation of a membrane separation unit (Casani, Rouhany, & Knøchel, 2005). Moreover, the costs (acquisition, energy and maintenance) of membrane 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, &

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

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

This physical method has been extensively studied in the food industry for produce decontamination and water disinfection. Seymour et al. (2002) reported 1.5 log CFU/g reduction of *S.* Typhimurium on cut iceberg lettuce at 32-40 kHz for 10 min. These authors tested the effects of different frequencies (25, 32 and 70 kHz) and found no statistical significant differences (Seymour et al., 2002). Birmpa, Sfika, and Vantarakis (2013) achieved 2.30, 5.72 and 1.88 log CFU/g reduction of *E. coli*, *S.* Enteritidis, *L. innocua*, respectively, on lettuce at 37 kHz for 30 min. Kim, Feng, Kushad, and Fan (2006) obtained a lower log CFU/g reduction (1.08) of *E. coli* O157:H7 on broccoli seeds using the same treatment time and a higher frequency (40 kHz, at 23 ^{0}C for 30 min). The disinfection of the washing water is also important and Elizaquível, Sánchez, Selma, and Aznar (2012) found a 4.4 log CFU/mL reduction of *E. coli* O157:H7 in the washing water using US at 20 kHz for 53 min, which is obviously a high exposure time and therefore not appropriate for the fresh-cut industry.

2.3.6. Ultraviolet light

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

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

3. Combination of disinfection methods

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

Ionizing irradiation is a method to decontaminate fresh produce that is mandatorily combined with a chemical method. Foley, Dufour, Rodriguez, Caporaso, and Prakash (2002) combined this method with chlorine and proved that applying 0.55 kGy of gamma-rays together with 200 ppm of chlorine on shredded iceberg lettuce were able to reduce the population of *E. coli* O157:H7 by 5.4 log CFU/g. However, they used a high concentration of chlorine which is not the purpose of combining the two methods. Huang and Chen (2011) combined a physical process (heating at 50 ^{0}C) with H_2O_2 (2×10^4 ppm) to decontaminate baby spinach. When the physical-chemical combination 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*, *respectively. However, when this was combined with citric acid higher reductions were observed: 2.31 and 1.84 log CFU/g for E. coli O157:H7 and L. monocytogenes, respectively (Yuk et al., 2006). Combining sodium bicarbonate with H_2O_2 proved to be more efficient than using sodium bicarbonate alone. Malik and Goyal (2006) reduced 99.68% the population of feline calcivirus by combining 2×10^4 ppm of sodium bicarbonate with 2×10^4 ppm of H_2O_2 for 10 min. Van Haute, López-Gálvez, Gómez-López, Eriksson, Devlieghere, Allende, and Sampers (2015) combined peracetic acid (20 ppm) and lactic acid (4000 ppm) to treat the wash water of fresh-cut leafy vegetables. The authors proved that the use of this treatment is better than the use of chlorine, however, higher disinfectant concentrations are needed because the inactivation of E. coli O157 is slower, when compared to the chlorine kinetics. Enzymes have also been used in combination with US in order to remove E. coli biofilms from SS surfaces (Oulahal-Lagsir, Martial-Gros, Bonneau, & Blum, 2003). When US (40 kHz) were used alone, 30% of biofilm mass removal was achieved. However, when US were used in combination with enzymes (trypsin) biofilm removal reached 76% (Oulahal-Lagsir et al., 2003). The combination of UV and ozone was also applied by Selma, Allende, López-Gálvez, Conesa, and Gil (2008) achieving 6.6 log CFU/mL microbial reduction in escarole wash water. Ge et al. (2013) studied the combination of UV-C with chlorine and UV-C with PAA. When they combined UV-C (irradiation fluency of 900 mJ/cm^2, 10 min) with chlorine (200 ppm, 10 min) 2.40 log CFU/g reduction of S. Typhimurium on lettuce was achieved; when combining UV-C (irradiation fluency of 900 mJ/cm^2, 10 min) with PAA (80 ppm, 10 min) 2.52 log CFU/g was obtained. The treatments applied alone caused 2.29, 0.99 and 0.95 log CFU/g reduction for UV-C (irradiation fluency of 900 mJ/cm^2, 10 min), chlorine and PAA, respectively (Ge et al., 2013). From all the previous examples, it becomes rather clear that the combination of methods is a promising effort to replace and/or reduce the use of chlorine.*

4. Conclusions and future perspectives

The environmental and public health concerns on the use of chlorine are constantly increasing, with some European countries having already prohibited its use (Bilek & 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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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).

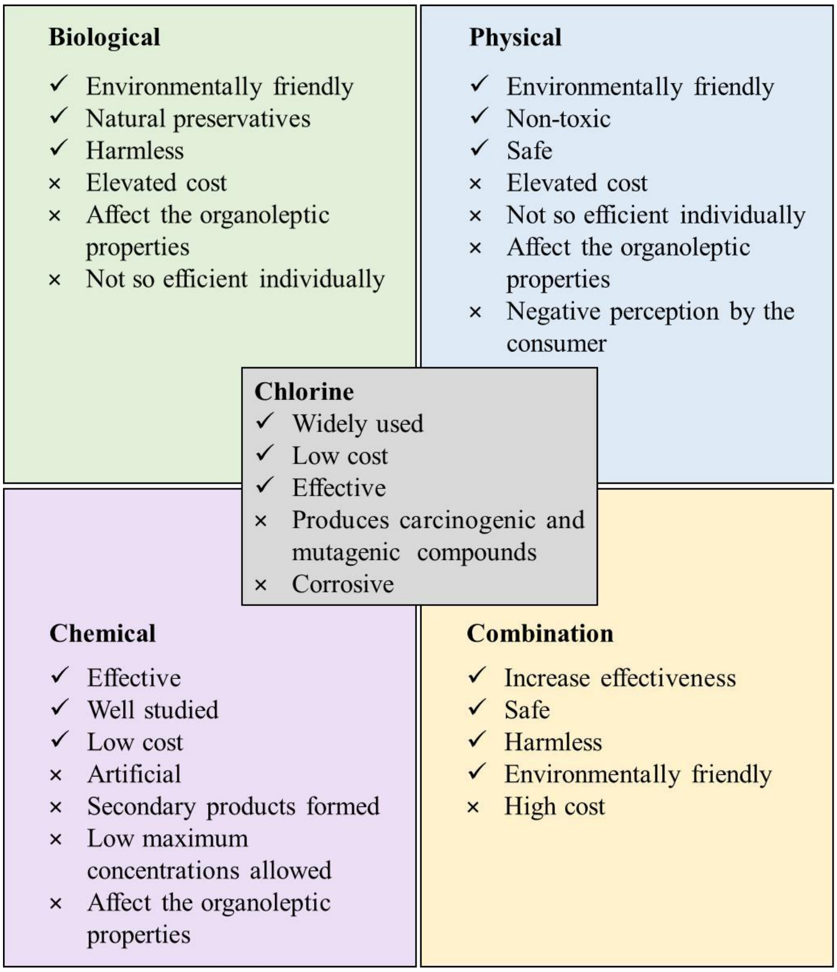
Biological
✓ Environmentally friendly
✓ Natural preservatives
✓ Harmless
× Elevated cost
× Affect the organoleptic properties
× Not so efficient individually
Physical
✓ Environmentally friendly
✓ Non-toxic
✓ Safe
× Elevated cost
× Not so efficient individually
× Affect the organoleptic properties
× Negative 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

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