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# Conventional and non-conventional disinfection methods to prevent microbial contamination in minimally processed fruits and vegetables

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## ABSTRACT

Pandemic COVID-19 warned the importance of preparing the immune system to prevent diseases. Therefore, consuming fresh fruits and vegetables is essential for a healthy and balanced diet due to their diverse compositions of vitamins, minerals, fiber, and bioactive compounds. However, these fresh products grew close to manure and irrigation water and are harvested with equipment or by hand, representing a high risk of microbial, physical, and chemical contamination. The handling of fruits and vegetables exposed them to various wet surfaces of equipment and utensils, an ideal environment for biofilm formation and a potential risk for microbial contamination and foodborne illnesses. In this sense, this review presents an overview of the main problems associated with microbial contamination and the several chemicals, physical, and biological disinfection methods concerning their ability to avoid food contamination. This work has discussed using chemical products such as chlorine compounds, peroxyacetic acid, and quaternary ammonium compounds. Moreover, newer techniques including ozone, electrolyzed water, ultraviolet light, ultrasound, high hydrostatic pressure, cold plasma technology, and microbial surfactants have also been illustrated here. Finally, future trends in disinfection with a sustainable approach such as combined methods were also described. Therefore, the fruit and vegetable industries can be informed about their main microbial risks to establish optimal and efficient procedures to ensure food safety.

## 1. Introduction

The pandemic caused by COVID-19 (SARS COV 2 disease, 2019) has influenced worldwide our food security, safety, and nutrition. In a nutritional context, researchers found fruits and vegetables (FV) to be an essential food source of micronutrients, minerals, and phytochemicals that can be used as a proactive dietary supplement and consumed fresh or minimally processed (Moreb et al., 2021). The minimally processed fruits and vegetables (MPFV) is defined as any fruit or vegetable that has been physically modified from its original form through several processes such as peeling, slicing, chopping, shredding, coring, trimming, mashing, and washing to obtain an edible product that is subsequently packaged and stored under refrigeration (Alzamora et al., 2015; Fardet,

2018). The significance of MPFV is determined by their quality attributes, i.e., freshness, retention of vital nutrients, convenience, and sensory attributes, along with enhancement of shelf-life (Troyo & Acedo, 2019). In addition, the consumption of MPFV has played an essential role in maintaining a healthy and balanced diet (Bhilwadikar et al., 2019). Despite the advantages of MPFV, there is a food safety concern due to the high risk of contamination caused by the presence of microorganisms and their toxic compounds (Callejón et al., 2015). Consequently, contamination can happen in any part of the process from harvest to distribution, so the supply chain behind raw FV requires specific parameters to be strictly followed. FV can be contaminated using infected irrigation water, potent pesticides, cross-contamination during processing, and improper handling (Yeni et al., 2016). In

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addition, the microbiota during the minimally processing of FV might vary on the function of their chemical composition, pH, water activity, and extrinsic preservation parameters such as refrigeration temperature. According to (Al-Tayyar et al., 2020), when it comes to MPFV, the growth of microorganisms on product surfaces is related to the family *Enterobacteriaceae* and *Pseudomonadaceae*, lactic acid species, and species of yeast belonging to *Torulaspota*, *Pichia*, *Candida*, among others. On the other hand, one of the food security challenges of the MPFV industry is the biofilm formation on surfaces such as plastic, glass, stainless steel, and food products. Biofilms are complex structure formed by a variety of microorganisms that produces a three-dimensional (3D) network constituted by extracellular polymeric substances (EPS) (e.g., protein, nucleic acids, lipids, and polysaccharides) (Pu et al., 2020; Yuan et al., 2019). Pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Vibrio cholerae*, and *Yersinia enterocolitica* could form biofilm and cause illness (Mai-Prochnow, 2020; Rossi et al., 2020). Likewise, biofilms are sources of spoiling microorganisms, reducing product shelf life (Rossi et al., 2020).

Therefore, the MPFV's main objective is food safety assurance and quality control through the inactivation of pathogenic and spoilage microorganisms. It is critical to avoid biofilm formation that could threaten consumers' health. Different strategies have been used to avoid microbial contamination in MPFV. Historically, the first cleaning approach was washing with water to remove dirt, foreign materials, and tissue fluids from cut surfaces and adding soap to reduce microorganisms from the surface (Castro-Ibáñez et al., 2017). Indeed, the literature indicated that washing performed with or without disinfectants reduces the microbial load on the product surface (Gil et al., 2009). Total bacterial counts after the storage are also similar when the product is washed with tap water or when it is sanitized (Allende et al., 2008). However, Castro-Ibáñez et al. (2017) noticed that water use in raw FV had been identified as a potential source for cross-contamination with fecal indicator organisms (e.g., *E. coli* O157:H7) and human enteric pathogens. Thus, some situations require disinfectant agents or physical techniques to reduce FV microbial counts. The disinfectant compounds and disinfection methods must assure that MPFV achieves fresh-like quality, safety, and low residue. Hence, MPFV industries must implement different strategies by introducing or combining sustainable techniques, especially standard procedures for disinfection. Therefore, this review aims to discuss the primary microbial contamination associated with the MPFV, including biofilm problems, and offer a comprehensive review of the most conventional disinfection methods (e.g., chemical compounds) to the newly developed disinfection technologies to preserve MPFV. In addition, future trends with a sustainable approach for MPFV disinfection were discussed (Fig. 1).

## 2. Microbial contamination

The benefits of consuming fresh FV and MPFV have been recognized and widely published. MPFV provides the natural FV characteristics, high moisture, and high nutrient content (e.g., vitamins, minerals, fibers, and antioxidants). De Corato (2020) hypothesized two challenges in MPFV, keeping fresh products and improving the shelf-life. Microorganisms are strictly related to both challenges. For this reason, unit operations have been implemented to reduce the initial microorganism numbers with cleaning, washing, trimming, peeling, and disinfection (Mritunjay & Kumar, 2015). Although, raw FV may be contaminated at any point during the production and transformation chain. The first preventive measure is to identify the potential contamination sources, classified into two broader groups—preharvest and postharvest (Balali et al., 2020). Preharvest sources of contamination are considered pathogens through the uptake from soils or groundwater, use of raw manure and compost, exposure to contaminated water (e.g., feces and insects), and human interaction (Alegbeleye et al., 2018; Gil et al., 2015). Hence, there are significant risk factors for microbial contamination across the whole production chain (Mostafidi et al., 2020). FV composition also favors the presence and growth of several microorganisms. On the other hand, some postharvest practices in MPFV (e.g., peeling, slicing, chopping, shredding) can damage the tissue, releasing cellular fluids, which provide water and nutrients that promote the microorganism's growth (Francis et al., 2012). A broad spectrum of postharvest contamination sources has been reported feces, harvesting equipment, human handling, insects, wild and domestic animals, methods of transportation, processing equipment, the condition of the processing environment, and rinse water (Balali et al., 2020; Gil et al., 2015; Xylia et al., 2019). Microorganisms can distribute throughout the production chain due to the lack of thermal operations. The long distance between the farms and the processing to commercialization might contribute to the microbial count and, afterward, the risk of foodborne illness. These microorganisms can be pathogenic and non-pathogenic, associated with bacteria, yeasts, fungi, and viruses.

The most common pathogens found in FV are Gram-positive and Gram-negative bacteria, which may be psychotropic or mesophilic. This initial microflora consists of *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum*, *Enterobacter*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., and *Yersinia enterocolitica* (Vivek et al., 2019). Recently, Krishnasamy et al. (2020) reported *Salmonella* outbreaks in multiple states of the USA in 14 and 24 types of FV. On the other hand, the bacteria most associated with MPFV spoilage by aerobically stored refrigeration include *Pseudomonas marginalis*, *Pseudomonas fluorescens*, and *Pseudomonas viridiflava* (Benner, 2014). *L. monocytogenes* is also a challenging problem in FV and MPFV due to its frequent presence in the environment and the ability to grow at refrigeration temperatures (Chan & Wiedmann, 2009; Ziegler et al.,

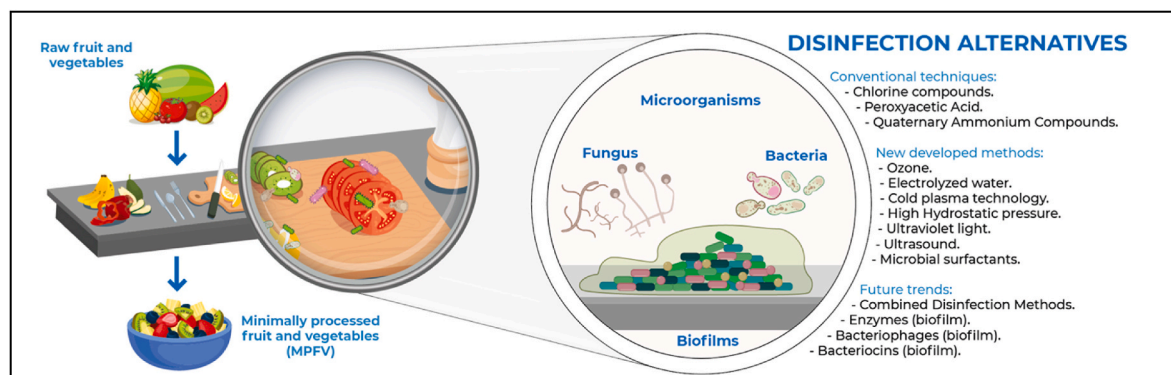


Fig. 1. Relationships between the potential microbial contamination include biofilm problems in minimally processed fruits and vegetables and possible methods to avoid potential contamination.

2019). The most recent multi-state outbreaks of listeriosis were reported in February 2022 and December 2021 in the USA. Both were linked to packaged salads, resulting in 53 infected people and three fatal cases in total (CDC, 2022). Similarly, Silva et al. (2017) found that *L. monocytogenes* was the primary pathogen in packed or unpacked vegetables sold at retail establishments in Europe. In the same line, in fresh-cut FV, the pathogens of major concern were *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. (Francis et al., 2012). For example, in packaged fresh leafy green vegetables, *Yersinia enterocolitica*, *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* were also identified (Noussainen et al., 2016; Korir et al. (2016).

Another point that needs to be addressed is fungal species contamination after harvesting FV, which can alter the plant tissues and help the growth of pathogenic bacteria. The presence of fungus postharvest can cause corrosion on the product surface, favoring the growth, and production of toxic compounds, diminishing the shelf life, and contaminating other fresh products. Fungal species are the most dangerous pathogens in FV, and MPFV can colonize the products since plant growth and remain latent until storage, transportation, distribution, and consumption (Adeyeye, 2016). For instance, the genera of *Aspergillus*, *Penicillium*, and *Alternaria* are frequently contaminating FV and MPFV (Sanzani et al., 2016). *Aspergillus* genera is a common pathogen of FV in the postharvest, causing the disease called black mold, especially by *Aspergillus niger* (Jahani et al., 2020). The fruit infection can initiate from flowering to fruiting. The infection is related to enzymatic cell-wall degradation (Abdel-Aziz et al., 2019; Lorrai & Ferrari, 2021). *A. niger* can also produce a variety of resistance compounds (Yehia, 2013). In addition, *Penicillium* genera also contaminate FV and MPFV. For example, *Penicillium digitatum* is a significant source of postharvest fruit decay and mainly produces green mold in citrus. *P. digitatum* initiates its infection using mainly spores as infective units. After settling over the fruit surface, the infection cycle starts and will produce green mold after 2-days (Z. Wang et al., 2018; Yang et al., 2019). Other fungi genera that can cause postharvest disease are the species of *Alternaria*, *Botrytis*, *Lasiodiplodia*, *Colletotrichum*, *Geotrichum*, *Fusarium*, *Dothiorella*, and *Phomopsis* (Firas et al., 2017). *Alternaria* genera contaminate refrigerated fruits, especially citrus (Nabi et al., 2017; Yan et al., 2015). *Botrytis* genera are the most destructive postharvest disease, causing grey mold in FV (e.g., apple, cherry, peach, pear, strawberry, tomato, and grape). *Botrytis cinerea* causes significant worldwide economic FV losses, the second pathogenic fungus of importance (Dean et al., 2012; Hua et al., 2018). Another problem related to fungal is the production of mycotoxins (e.g., ochratoxin, citrinin, ergot, patulin, and fusaria) with low molecular weight, causing disease and death in humans and animals (A. Gallo et al., 2015). Finally, viruses are also responsible for FV contamination. Norovirus (NoV) was the primary pathogen associated with FV outbreaks in the USA (59%) and European Union (53%) between 2004 and 2012. In the USA, the outbreaks were related to salads, and in the EU were mainly for berries. Other viruses (e.g., hepatitis A, adenovirus, and rotavirus) are correlated with foodborne but in a lesser percentage (Callejón et al., 2015).

### 2.1. Biofilm in fresh fruits and vegetables

Biofilms are a complex structure that gives new survival advantages to microorganisms, allowing them to grow into diverse microbial communities. It is essential to mention that not every microorganism can produce biofilms (Flemming & Wingender, 2010). FV production requires environmental conditions (e.g., humidity, temperature, and solar radiation), which are suitable for developing many microorganisms and forming biofilms. Biofilm formation starts with the microbial adhesion to the plant, being accessible for the bacteria that have flagella, allowing chemotaxis and swimming motility for colonization through surface adhesion (McLandsborough et al., 2006). Researchers have demonstrated that *Bacillus*, *Salmonella*, *Listeria*, *Staphylococcus*, and *Escherichia* presented in FV are capable of biofilm formation, which is crucial for the

virulence of the pathogenic strains (Amrutha et al., 2017). Recently, Sun, Ye, et al. (2021) demonstrated that *E. coli* O157:H7 formed a denser biofilm on cucumber tissues, especially in the vascular tissues. Similarly, *E. coli* O157:H7 and *S. enterica* are the most common illness outbreaks in alfalfa sprouts and lettuce leaves, using their fimbriae to attach to the cell plant (Yaron & Römling, 2014). In this context, the diversity of microorganisms in vegetable plants is related to the content of  $\beta$ -carotene on the surface of the plant leaf, where the bacterial community is high. For instance, Pometto Antony & Demicri Ali (2015) described that spinach has a large amount of  $\beta$ -carotene in its leaves and reported the relationship with an extensive bacterial community in this environment. Moreover, pathogens produce extracellular polysaccharides (e.g., cellulose), a principal component of the biofilm formation in tomatoes and alfalfa (Limoli et al., 2015). On the contrary, *Salmonella enteritidis* uses lipopolysaccharides to colonize alfalfa, whereas *E. coli* O157:H7 uses colonic acid to colonize the same vegetable (Pometto Antony & Demicri Ali, 2015).

Research about outbreaks produced by FV showed that biofilms formed by human pathogens are highly resistant to sanitizing products. Bacteria adherence to fresh products is also favored by the irregularities of the surface product (e.g., roughness, crevices, pits), reducing washing and sanitization effectiveness to remove or eliminate attached cells (Giaouris & Simões, 2018). The biofilm formation has also shown antimicrobial resistance compared to the planktonic bacteria, enhancing the potential of MPFV cross-contamination.

### 2.2. Biofilm on processing surfaces of FV

During the MPFV industrial processing, all food contact surfaces (e.g., cutting boards, knives, tables) should be frequently cleaned to ensure food safety and avoid cross-contamination. Without appropriate disinfection, increase the risk of cross-contamination with the inherent growth of microorganisms and the formation of highly resistant biofilms (Mritunjay & Kumar, 2015). It is important to note that once the microorganisms are attached to the surface and start growing the biofilm, they become more challenging to eliminate (Limoli et al., 2015). Thus, contamination is caused by stage one of biofilm formation, which is the detachment of cells from biofilm, followed by the dispersion to nearby areas to form a new biofilm (Rossi et al., 2020). Biofilms in food industries involve a community of several bacterial species, where cells are more withstand to disinfectants than microorganisms involved in single-species-biofilms (Y. Yuan et al., 2019). Biofilms have been associated with food spoilage, foodborne illness, machinery damage, and other matters—material corrosion, reduction of heat transfer, mechanical obstruction, and changes in the permeability of filter membranes. In addition, the energy cost could increase due to biofilm presence (Rossi et al., 2020; L.; Yuan et al., 2020). Thus, one of the main challenges for MPFV microbial safety is microbial biofilms in food, packaging, and machinery surface, requiring manufacturing protocols o control biofilms.

Many surfaces with less porosity are specifically used in the food industry to ensure an effective cleaning (e.g., stainless steel, cast iron, polypropylene). However, microorganisms can still grow biofilms despite proper cleaning and sanitization procedures and survive for prolonged periods (De Araujo et al., 2016; Shi & Zhu, 2009; Amrutha et al., 2017). The surface of equipment is constantly exposed to microbial contamination as bacteria form a matrix, mainly with polysaccharides, proteins, lipids, and extracellular DNA. The cells adhere to and initiate the interaction between different microbial species. Those interactions create new conditions as biofilm aggregates, unknown as individual cells, unpredicted before the aggregation (Fleming & Rumbaugh, 2017). The biofilm is formed on a solid surface with appropriate characteristics to favor the attachment process (Annous et al., 2009). As mentioned, roughness is a surface property that favors the microbial colonization expansion due to the larger surface area, which acts as an anchor for the already attached bacteria (Dong et al., 2015). The

hydrophobic surfaces used in the food industry (e.g., plastics, Teflon, polystyrene, and polyethylene) favored the microbial community adherence because of the hydrophobic interactions between the surface and bacteria (Adlhart et al., 2018). The adhesion mechanism starts due to forces–van der Waals and electrostatic interactions at the surface. van der Waals forces promote their attraction until a point, but the electrostatic forces determine the repulsion or closer distance (Coronel-León et al., 2016; Papa et al., 2013; Shakerifard et al., 2009). The negative charge groups in species (e.g., carboxyl and amino groups) might interact with the surface positively charged (Zezi do Valle Gomes & Nitschke, 2012). This mechanism is associated with the adequate adherence of *Pseudomonas*, *E. coli*, and *Staphylococcus aureus* on surfaces with a positive charge than with a negative charge (Zhu et al., 2015).

Finally, the food industry uses different methods to remove biofilms in food contact surfaces–disinfection, cleaning, enzymes, bacteriophages, quorum sensing inhibitors, and physical treatments. However, using a single technique is insufficient for biofilm removal. The efficiency of the techniques for the biofilm removal is affected by some factors–disinfectant applied, concentration, period time, microorganisms involved, pH, temperature, relative humidity, food that remains on the surface, and type of surface (L. Yuan et al., 2020).

### 3. Conventional disinfection methods: chemical compounds

As mentioned, MPFVs have significant nutritional benefits for consumers, but they can be a higher risk product due to pathogenic contamination, making the consumers' safety uncertain. For this reason, this section provides food processors and consumers with a description of conventional disinfection techniques implemented to improve FV's shelf life. Disinfection is the inactivation or destruction of microorganisms that could cause foodborne illness or non-desired changes in food products. The most conventional and widely used method to disinfect FV is the application of chemical disinfectants, as shown in Table 1. These disinfectants are used for FV washing to reduce the risk of microbial contamination. However, it is necessary to understand that diverse factors, such as concentration, contact time, temperature, organic load, pH, type, and a load of microorganisms, affect the antimicrobial efficacy of disinfectants. Chemical compounds used in the food industry are oxidative and non-oxidizing disinfectants. The most frequent oxidative disinfectants are chlorine compounds and peroxyacetic acid. Otherwise, the most representative non-oxidizing disinfectants are Quaternary Ammonium Compounds (QACs or Quats). These three disinfectant compounds are described below.

**Table 1**  
Conventional disinfection techniques with different chemicals compounds, produce, and microbial reduction.

Conventional treatments	Produce	Microbial log reduction	Reference	
Chlorine compounds	NaOCl, 100 mg/L, 5 min	Spinach	<i>E. coli</i> O157:H7 ~1.7 log CFU/g <i>L. monocytogenes</i> ~1.7 log CFU/g	Rahman et al. (2010)
	NaOCl, 100 mg/L, 1 min	Fresh-cut Lettuce	<i>E. coli</i> O157:H7 0.7 log CFU/g	(López-Gálvez et al., 2010)
	NaOCl, 100 mg/L, 10 min, 22 °C	Cabbage	<i>E. coli</i> O157:H7 0.94 log CFU/g <i>L. monocytogenes</i> 0.73 log CFU/g <i>S. Typhimurium</i> 0.94 log CFU/g	Choi et al. (2008)
	NaOCl, 25 mg/L, 1 min, 20 °C	Fresh-cut red chard	<i>E. coli</i> O157:H7 0.85 log CFU/g <i>S. Typhimurium</i> 1.50 log CFU/g	Tomás-Callejas et al. (2012)
Chlorine compounds	NaOCl, 25, 50, 100 mg/L, 1 min, 25 °C	Tomato	<i>S. enterica</i> 4.3, 5.0, and 5.5 log CFU/g	Chang and Schneider (2012)
	ClO <sub>2</sub> , 10 mg/L, 3 min, 25 °C	Tomato	<i>S. enterica</i> 4.87 log CFU/g	(Sun et al., 2019; Trinetta et al., 2012)
	ClO <sub>2</sub> , 5 mg/L, 10 min, 22 °C	Strawberry	<i>L. monocytogenes</i> 4.7 log CFU/g	(Mahmoud et al., 2007; X; Sun et al., 2019)
	ClO <sub>2</sub> , 120 mg/L, 10 min, 21 °C	Apple	<i>E. coli</i> O157:H7 8 log CFU/g	(Du et al., 2003; X; Sun et al., 2019)
Peroxyacetic Acid	ClO <sub>2</sub> , 0.75, 1, 1.25, 1.5 mL, 1 h, 25 °C	Green coffee beans	<i>A. flavus</i> 1.1–2.2 log CFU/bean	Lee et al. (2020)
	ClO <sub>2</sub> , 5 mg/L, 2 min	Fresh-cut iceberg lettuce	Total psychrotrophic plate count >3 log CFU/g <i>E. coli</i> >5 log CFU/g	(van Haute et al., 2017)
	ClO <sub>2</sub> , 5 mg/L, 3 min	Radish seeds	<i>Cronobacter</i> spp 2.4, 3.6 log CFU/g	(E. G. Kim et al., 2013)
	ClO <sub>2</sub> , 50, 100 mg/L, 5 min	Mungbean sprouts	<i>S. Typhimurium</i> 4.6–3 log CFU/g <i>L. monocytogenes</i> 5.6–1.5 log CFU/g	(Yu Neo et al., 2013)
Peroxyacetic Acid	ClO <sub>2</sub> , 100 ppm, 5 min	Fresh-cut apple	<i>E. coli</i> O157:H7 and <i>L. innocua</i> >4 log CFU/g	Abadias et al. (2011)
	20 mg/L, 5 min, 20 °C	Iceberg lettuce	<i>E. coli</i> O157:H7 0.97–1.74 log CFU/g	Davidson et al. (2017)
	50 ppm, 90 s, 25 °C	Lettuce	<i>S. Typhimurium</i> 0.99 log CFU/g	Ge et al. (2013)
	40 ppm, 5 min	Jalapeno	Aerobic bacteria 2 log CFU/g Coliforms 1.4 log CFU/g	Ruiz-cruz et al. (2010)
Quaternary Ammonium Compounds	80 mL/L, 2 min	Lettuce	Mesophilic aerobics 3 log CFU/g	Bachelli et al. (2013)
	100 mg/L, 15 min	Watercress	Aerobic mesophiles 5.1 log CFU/g	(De São José & Vanetti, 2015)
	40 mg/L, 10 min	Tomato	<i>E. coli</i> O157:H7 2.06 log CFU/g <i>Yersinia enterocolitica</i> 4.21 log CFU/g	Velázquez et al. (2009)
	Benzalkonium chloride, 0.1 mg/mL	Cantaloupe	<i>Salmonella</i> 5.16 log CFU/mL	Saucedo-Alderete et al. (2018)
Quaternary Ammonium Compounds	Cetylpyridinium chloride, 1.0% vol/vol, 15 min	Spinach leaves	<i>L. monocytogenes</i> 4.54 log CFU/g, <i>E. coli</i> O157:H7 3.33 log CFU/g, <i>S. Typhimurium</i> 3.28 log CFU/g	Kang et al. (2019)
	Cetylpyridinium chloride, 80 mg/L, 3 min	Apple	<i>L. monocytogenes</i> biofilm 2.41 log CFU/g (200 ppm) and 3.06 log CFU/g (400 ppm)	Korany et al. (2018)
	Quaternary ammonium compound solution, 200 and 400 ppm, 1 min, 22 °C	Simulated wash water (for FV)	<i>Salmonella</i> 99.999% (MBC value)	Pablos et al. (2018)
	Benzalkonium chloride, 100 mg/L, 90 s	Simulated wash water (for FV)	<i>Salmonella</i> 99.999% (MBC value)	Pablos et al. (2018)
Quaternary Ammonium Compounds	Didecylidimethylammonium chloride, 30 mg/L, 90 s	Simulated wash water (for FV)	<i>E. fecalis</i> 99.999% (MBC value)	Pablos et al. (2018)
	Benzalkonium chloride, 50 mg/L, 90 s	Simulated wash water (for FV)	<i>E. fecalis</i> 99.999% (MBC value)	Pablos et al. (2018)

### 3.1. Chlorine compounds

Sodium hypochlorite (NaOCl), chlorine dioxide (ClO<sub>2</sub>), and chlorine (Cl<sub>2</sub>) are the most employed aqueous disinfectant (Zamuner et al., 2020). In water, these compounds produce hypochlorous acid (HOCl) and other Reactive Chlorine Species (RCS) that simultaneously damage multiple cellular components of bacteria. However, the specific mechanism depends on the microorganism's species (Gray et al., 2013). For instance, free chlorine damages viral capsids enabling this compound to access and damage viral genomes (Sigstam et al., 2013; Wigginton et al., 2012). In vegetative bacteria, chlorine compounds can lethally damage the bacteria cells' inner membrane, which is the primary mechanism for antimicrobial activity (Gray et al., 2013). ClO<sub>2</sub> increases the permeability of outer and cytoplasmic cell membranes without necessarily causing cell lysis, leading to the release of cell components and, consequently, cell death (Ofori et al., 2018).

According to the MPFV product, sanitation process, and initial microbial load, the most common concentrations of chlorine compounds range from 50 to 200 ppm (Ryther, 2014). Recently a research group reported that chlorine-based disinfectants and gaseous chlorine dioxide exhibited an average microbial reduction of 1.12 and 4.07 log CFU/g, respectively, after cleaning different FV (Yoon & Lee, 2018). In addition, disinfection application methods can enhance disinfectants' results. For instance, after using an overhead spray and brush roller system, the sodium hypochlorite effectiveness at 100 ppm increased to 5.5 log reductions of *Salmonella enterica* on tomato surfaces (Chang & Schneider, 2012).

Nevertheless, the chlorine compounds' application in food has raised some concerns that lead to limiting their use. Bhilwadikar et al. (2019) reported that the interaction between NaOCl and the organic components of food products could form carcinogenic compounds. Chlorine is a powerful oxidant, able to oxidize chemical compounds and react with organic matter, forming carcinogenic by-products (e.g., trihalomethanes) and chloramines in the presence of ammonia (Artés et al., 2009). Consequently, there are currently efforts to replace chlorine with more sustainable and safe options. In this context, chlorine dioxide (ClO<sub>2</sub>) seems a better alternative than other chlorine compounds. The ClO<sub>2</sub> carcinogenicity for humans has not been sufficiently demonstrated due to a limited database of relevant investigations on humans or animals. Gaseous ClO<sub>2</sub> is recommended in FV processing for being less corrosive than aqueous chlorine formulations. The gaseous ClO<sub>2</sub> operation can take seconds to minutes, has pH tolerance from acid to alkaline, and can introduce into product surfaces and even biofilms (Chen et al., 2020). Different application studies of ClO<sub>2</sub> solutions up to 200 ppm in some MPFV effectively reduced counts of natural or inoculated microorganisms in 1–5 log CFU/g (Praeger et al., 2018). For instance, X. Sun et al. (2017) evaluated the action of gaseous ClO<sub>2</sub> in tomatoes, strawberries, and apples, getting a microbial log reduction of *S. enterica*, *L. monocytogenes*, and *E. coli* O157:H7 from 4.5 log–8 log CFU/g. In addition, to control vegetative bacteria, molds, and yeast, ClO<sub>2</sub> also showed effective results in controlling spore-forming bacteria (Trinetta et al., 2012). However, practical application's limitations should be considered. ClO<sub>2</sub> has an explosiveness risk at higher concentrations, at partial pressures of >0.1 bar, and represents a toxic risk to humans at concentrations ≥1000 ppm. Thus, it cannot be transported and must be generated on-site, significantly impacting operations costs (Praeger et al., 2018).

Studies demonstrate that the sensory quality of FV is not significantly affected when used sodium hypochlorite or aqueous chlorine dioxide in the washing water treatment (Delaquis et al., 2004; Gómez-López et al., 2013). Nevertheless, gaseous chlorine dioxide could induce bleaching and browning in FV depending on the time and concentration used (Gómez-López et al., 2009). Consequently, adequate disinfectant selection, time, and concentration are needed to prevent damage to FV sensory properties.

### 3.2. Peroxyacetic acid

Peroxyacetic acid (PAA) is an equilibrium mixture of hydrogen peroxide and acetic acid. It was patented to treat fruit and vegetable surfaces and reduce spoilage from bacteria and fungi (Zoellner et al., 2018). PAA-based disinfectants are primarily used in combination with hydrogen peroxide. It affects the microorganism cell membranes by disrupting their chemical bonds and oxidizing membrane proteins (Singh et al., 2018); it also releases Reactive Oxygen Species (ROS), which cause DNA and lipid damage (Small et al., 2007).

PAA-based disinfectants show some advantages over chlorine compounds, such as efficacy within a wide pH range, higher stability in the presence of organic matter, and fewer disinfection by-products (W. N. Lee & Huang, 2019). PAA effectively reduces pathogen loads of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. from different MPFV (P. Singh et al., 2018; Brilhante São José & Dantas Vanetti, 2012).

P. Singh et al. (2018) reported 1.8–6.7 log reductions of *E. coli* O157:H7 after using concentrations between 45–100 ppm in lettuce, lemon, tomatoes, and blueberry. Meanwhile, *S. Typhimurium* reached 3.6–6.8 log reductions in lettuce, lemon, cantaloupe, and blueberry at the same concentrations. *L. monocytogenes* was tested in lettuce and cantaloupe, reaching up to 2.4 and 4.5 log CFU/g reduction at 100 ppm (Singh et al., 2018). Additional advantages include PAA-based disinfectant's ability to unmodified the organoleptic characteristics of the final product since PAA's odor can be removed in the final production steps (Mills et al., 2018). The remaining subproducts of the disinfectant's degradation, such as water and acetic acid, are harmless against MPFV organoleptic characteristics (Alvaro et al., 2009; Ölmez & Kretzschmar, 2009). PAA is also environmentally friendly and without a report on cytotoxic effects.

### 3.3. Quaternary ammonium compounds

Quaternary ammonium compounds (QACs) are human-made cationic surfactants commonly used in industrial products. QACs are effective against many microorganisms, especially Gram-positive bacteria, but with lesser results for spore-forming species. Their mechanism interferes with the lipid bilayer of bacteria and the outer membrane of Gram-negative bacteria, leading to loss of cytoplasmic elements and, consequently, bacteria lysis (Gilbert & Moore, 2005). In addition, QACs can change the membrane permeability of bacteria and stimulate ROS production, promoting DNA bacteria damage (Han et al., 2019). Bacteria membrane's ability to absorb nutrients is also affected by QACs because they bind with acidic phospholipids in the microbial cell wall (Kwaśniewska et al., 2020).

QACs-based disinfectants are used from 100 to 400 ppm for applications, mainly on food contact surfaces. After drying the surfaces, a residue of QACs remains and provides germicidal activity until degradation occurs (C. Zhang et al., 2015). The effectiveness of QACs in eliminating food pathogens has been extensively studied. It is reported 3–5 log reductions of *Salmonella*, *E. coli*, *L. monocytogenes*, and *Yersinia enterocolitica* in different MPFV (Kang et al., 2019; Saucedo-Alderete et al., 2018; Velázquez et al., 2009). For instance, Kang et al. (2019) reported 3.33, 3.28, and 4.54 log reductions against *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *L. monocytogenes*, respectively, on spinach leaves. Regarding the organoleptic properties of MPFV, some studies showed no effect when QACs-based disinfectants were used on spinach leaves and cantaloupe rind plugs (Kang et al., 2019; Saucedo-Alderete et al., 2018), but Velázquez et al. (2009) reported small spots of yellowish appearance on lettuce leaves and tomatoes.

Some studies have addressed the potential environmental risk that QACs' residues can represent to the ecosystem due to their toxicity for aquatic and terrestrial organisms. For instance, Zhang et al. (2015), after reviewing QACs concentrations in different sources of surface water, sewage, and sediments of wastewater from water treatment facilities, concluded that QACs were accumulated in the environment at levels that are toxic and lead to antibiotic-resistant bacteria for humans. This

severe problem raises several concerns among consumers, industries, and government agencies.

#### 4. New developed disinfection methods

New strategies to conserve MPFV include advanced techniques and components used in disinfection procedures. For instance, newly developed disinfection methods have received attention in several studies of common contaminations observed in MPFV due to their minimal impact on health and the environment. These methods propose a more sustainable approach to this problem and are divided into heat and non-heat treatment methods. The present review describes the non-heat treatment methods such as ozone, electrolyzed water, cold plasma technology, high hydrostatic pressure, ultraviolet, ultrasound, and microbial surfactants. In addition, complementary information about the effect of these disinfection techniques is shown in Table 2.

##### 4.1. Ozone

Ozone is the triatomic molecule of oxygen, with an average half-life of approximately 20 min (Kim et al., 1999). It has excellent oxidizing properties because it can react efficiently with other components by adding an atom of oxygen to its molecular structure. Therefore, it has been widely used as an antimicrobial agent in the food industry since its approval as Generally Recognized as Safe (GRAS) in the United States (Botondi et al., 2015; Khadre et al., 2001). Ozone processing carried out on FV can be done in a gaseous or aqueous state and must be generated on-site. Ozone has a 1.5 higher oxidizing potential than chlorine, and it decomposes rapidly to oxygen, leaving no residues in food (Tzortzakis & Chrysargyris, 2017). Ozone oxidizes the bacterial cell components, such as proteins, unsaturated lipids, and respiratory enzymes. Shezi and collaborators extensively reviewed the changes in the biochemistry of fresh produce after ozone treatment. The authors report that microbial inactivation is mainly due to changes in double bonds of unsaturated membrane lipids (Shezi et al., 2020). This review also summarized that the efficacy of ozone treatment depends on the type of product, maturity of FV, time, and method of exposure. Gaseous oxygen is more effective than ozone in liquid systems because gaseous ozone is pure, highly reactive, and has a longer half-life. In the case of aqueous ozone, water quality can affect its effectiveness, and impurities such as organic and inorganic substances can also affect its antimicrobial action (Shezi et al., 2020). Ahmad and collaborators recently reported a 6 log CFU/mL bacterial reduction in kiwi after ozone treatment of 19.8 mg/mL with 60 min exposure (Ahmad et al., 2019). Another study has shown a reduction of *E. coli* O157:H7 at 3.5 log CFU/g in blueberries by applying 1.5 mg/L ozone for 5 min (Pangloli & Hung, 2013).

##### 4.2. Electrolyzed water

Electrolyzed water (EW) is typically generated by passing a salt solution (~1% NaCl) through an osmosis electrochemical cell which contains positively and negatively inert charged platinum electrodes (Zhao et al., 2021). Anode and cathode are separated by a membrane during the process, producing acidic and alkaline EW. Acidic EW has high oxidation-reduction potential (>1000 mV) and low pH (2.3–2.7). Whereas alkaline EW has low oxidation-reduction potential (800–900 mV) and high pH (10.0–11.5) (Rahman et al., 2010). As a result, the species chloride ion and water molecules are converted into active chlorine (AC), which contains oxidant compounds (e.g., Cl<sub>2</sub>, HOCl, and ClO) (Guentzel et al., 2008). EW's mechanism action is related to these oxidant compounds' ability to damage the outer and inner membrane, causing inhibiting enzymes, breaking down DNA, and disrupting cell metabolic processes (S. Wu et al., 2018). EW has recently become a popular sanitizer in the food chain. It was developed for water decontamination and regeneration. Therefore, it is considered an environmentally friendly decontamination agent. EW product is simple,

requiring water and salts (Zhao et al., 2021). Acidic and highly acidic electrolyzed waters have been reported as an effective method to reduce the microbial activity on the surface of FV (Mostafidi et al., 2020). However, Deng et al. (2020) recommended that factors such as available chlorine content (ACC), pH, exposure time, FV types, and temperature could influence the EW efficiency against microorganisms. In addition, some disadvantages need to be considered, like strong acidic electrolyzed water (AEW) and free chlorine content, which may be corrosive to metals. Both may induce synthetic resin degradation (Feliziani et al., 2016).

Studies reported using EW to reduce the population of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica serovar* Typhimurium on tomatoes, resulting in the above 3.0 log reductions of the initial load (Cao et al., 2009). EW's treatment is also a promising option for removing pesticides from fresh vegetables due to its strong oxidizing nature. It was also reported to cause an effective removal of various pesticides, such as diazinon and cyprodinil, in snap beans, grapes, and spinach (Graça et al., 2011). On the other hand, Hao et al. (2015) described the AEW effect, applied for 5 min, on the microbial load of fresh-cut cilantro. This study showed a microbial reduction of 2.5 log CFU/g for aerobic bacteria and 1.86 log CFU/g for molds and yeasts (Hao et al., 2015). Likewise, Seo et al. (2019) reported an average aerobic bacteria reduction of 2.1 log CFU/g of yeasts and molds after exposing collards to AEW for 10 min.

##### 4.3. Cold plasma technology

Cold plasma is a partially ionized gas formed by a high voltage, high frequency, or both. It has been studied as a method to decontaminate food at room temperature and atmospheric pressure (Bourke et al., 2018; Hertwig et al., 2018). The gas surrounding food is used as the starting material to produce reactive plasma species (RPS), such as peroxides, nitrites, nitrates, ozone, and other species described elsewhere (Connolly et al., 2012; Misra et al., 2018). These RPS are formed by serial reactions of colliding electrons, atoms, and molecules of the initial gas within seconds and minutes. The gas composition influences the RPS generation by cold plasma technology. Therefore, the air is the main gas used to produce reactive oxygen species (ROS) and reactive nitrogen species (RNS). These species are known for their effect on DNA damage, lipid oxidation, or loss of secondary structure of proteins (Fig. 2). Cold plasma can also produce electrostatic disruption, electroporation, cell apoptosis, and death due to the high energy level during direct treatments (Liao et al., 2017).

The effectiveness of cold plasma as a novel processing technology to eliminate food pathogens and contaminants has been extensively studied. A 5 log reduction of a microbial population has been reported with *Salmonella enterica serovar* Typhimurium, *E. coli*, *Listeria innocua*, and *S. aureus* on media exposed to cold plasma treatment (Kawamura et al., 2012; Wan et al., 2019; Xu et al., 2017; Ziuzina et al., 2013). Cold plasma is a suitable processing alternative for decontaminating food products sensitive to thermal processes, such as MPFV. Fresh cut cantaloupe treated with cold plasma for 1.5 min showed no bacterial, yeast, and mold count within 2-days of storage at 4 °C and no significant changes in flavor and color (Zhou et al., 2022). Extended shelf life of tomatoes was achieved with cold plasma treatment, when tomatoes were inoculated with *E. coli* and showed a 6 log CFU/mL reduction with a 60 kV treatment for 15 min and stored for 2-days at room temperature (Prasad et al., 2017). The type of surface is an important factor for cold plasma treatments. For example, the smooth surface of tomatoes allows a microbial reduction up to a nondetectable count with *E. coli* and *Salmonella*, in contrast to the complex surface of strawberries, which requires a longer treatment time to achieve the same reduction level (Ziuzina et al., 2014). Recently, Shah and collaborators reported complete inactivation of *E. coli* O157:H7 after applying 25 kV/2500 Hz for 300 s (Shah et al., 2019). In addition to the microbial decontamination, the effect on quality attributes will depend on gas composition and RSP type formed. For instance, leafy greens such as spinach are susceptible to

**Table 2**  
Non-Conventional disinfection techniques with different produce and microbial reduction.

Non-conventional treatments	Produce	Microbial log reduction	Reference
Ozone	1.5 mg/L of O <sub>3</sub> , 5 min	Blueberries	<i>E. coli</i> O157:H7 3.5 log CFU/g
	19.8 mg/mL of O <sub>3</sub> , 60 min	Kiwi	Gram-negative bacteria $1.5 \times 10^8$ – $1.2 \times 10^2$ CFU/mL
Electrolyzed water	ozonated water, 0.3 ppm, 80 g sample/30 L ozonated water	Strawberries	Total mesophiles 1.21 log CFU/g
	60 µmol/mol, 120 min	Extruded food	<i>A. flavus</i> 98.3%
	13.0 mg/L, 120 min	Brazil nuts	<i>A. flavus</i> 1.25–1.28 log CFU/g
	60 mg/L, 180 min	Wheat grain	Aflatoxins 95% AFB1, 29.6% AFB2
	13.5 ppm, 20 min	Wheat grain	Aflatoxins 53.9% AFG2
	Gaseous ozone $32 \pm 1$ µL/L, 24 h	Wine grapes	<i>Brettanomyces bruxellensis</i> 2.1 log
	150 ppb during the day, and 300 ppb during the night	Chestnuts	<i>Gnomoniopsis castanea</i> 75%
	LcAE (low concentration acidic electrolyzed water), 3 min	Mushrooms	Total bacterium counts 4.31–2.94 log CFU/g
	Slightly acidic electrolyzed water, 5 min	Cilantro	Yeast and molds 3.4–2.94 log CFU/g
	AcEW (acidic electrolyzed water), 10 min	Collards	Aerobic bacteria 2.53 log CFU/g
	NEW (neutral electrolyzed water), 12 mg/L ACC, 3, 5, 10 min	Pineapple	Yeasts and molds 1.86 log CFU/g
	NEW (neutral electrolyzed water), 12 mg/L ACC (available chlorine content), 3, 5, 10 min	Tomato	Aerobic bacteria 2.2 log CFU/g
	NEW, 12 mg/L ACC, 3, 5, 10 min	Tomato	Yeasts and molds 2.09 log CFU/g
	NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>Alternaria alternata</i> 100%
	NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>Botrytis cinerea</i> 100%
NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>Cladosporium australiense</i> 100%	
NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>Colletotrichum gloeosporioides</i> 100%	
NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>C. siamense</i> 100%	
NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>Fusarium solani</i> 100%	
NEW, 12 mg/L ACC, 3, 5, 10 min	Banana	<i>F. oxysporum</i> 100%	
NEW (neutral electrolyzed water), 12 mg/L ACC, 3, 5, 10 min	Banana	<i>Lasiodiplodia theobromae</i> 100%	
NEW, 53 mg/L ACC, 3, 5, 10 min	Tomato	<i>Aspergillus niger</i> 100%	
NEW, 53 mg/L ACC, 3, 5, 10 min	Peach	<i>A. tamarii</i> 100%	
NEW, 53 mg/L ACC, 3, 5, 10 min	Strawberry	<i>Rhizopus stolonifer</i> ~25%	
AIEW (alkaline electrolysed water) with sodium metabisulfite (SM), potassium sorbate (PS), potassium carbonate (PC) and sodium chloride (SC) at 30 min	Orange	<i>Penicillium digitatum</i> ; 98% (SM), 78%(PS), 80%(PC), and 89%(SC)	
AcEW (acidic electrolysed water) with sodium metabisulfite (SM), potassium sorbate (PS), potassium carbonate (PC) and sodium chloride (SC) at 30 min	Orange	<i>Penicillium italicum</i> ; 95% (SM), 96%(PS), 100%(PC), and 81%(SC)	
Cold plasma technology	APCP (atmospheric pressure cold plasma), 60 Hz, 12.83 kV, 10 min	Lettuce	<i>Penicillium digitatum</i> ; 99% (SM), 98%(PS), 85%(PC), and 87%(SC)
	APCP, 60 Hz, 12.83 kV, 10 min	Tomato	<i>Penicillium italicum</i> ; 88% (SM), 100%(PS), 100%(PC), and 100%(SC)
	26 kV, 2500Hz, 300 s	Baby kale leaves	<i>E. coli</i> 1.5 log CFU/g
	Cold plasma, 47 kHz, 549 W, 120 s	Blueberries	<i>E. coli</i> 1.7 log CFU/g
	Pressure: 0.2 mbar, atmospheric air, 0–30 min; 40, 60 W; 13.56 MHz; RH: $45.3 \pm 0.3\%$	Groundnut	<i>E. coli</i> O157:H7 complete inactivation
	Cold atmospheric plasma jet, argon; 5, 10, 15, 20, 25 min; 10 L/min; power: 20, 40 W; 50–600 MHz	Brown rice cereal bars and malt extract aga	Aerobic bacteria 1.6 log CFU/g
	AP-CCP (Atmospheric pressure capacitive coupled plasma) argon; 2, 6, 10 min; 50, 75, 100, 150 W; 13.65 MHz	Pistachio	<i>A. parasiticus</i> 97.9%
	APFBP (Atmospheric pressure fluidized bed plasma), dry air and nitrogen, 1–5 min, 3000 L/h, 460–655 W, 5–10 kV; 18–25 kHz	Hazelnut	<i>A. flavus</i> preventing growth by at least 20-days
	APFBP, dry air and nitrogen, 1–5 min, 3000 L/h, 460–655 W, 5–10 kV, 18–25 kHz	Hazelnut	<i>A. flavus</i> 66.6%
	APFBP, dry air and nitrogen, 1–5 min, 3000 L/h, 460–655 W, 5–10 kV, 18–25 kHz	Maize	<i>A. parasiticus</i> 4.5 log CFU/g, 5 min
High Hydrostatic pressure	HPP, 600 MPa, 8 min, 45 °C	Peach	<i>A. flavus</i> 4.19 log CFU/g for 5 min
	HPP, 600 MPa, 8 min, 23–27 °C	Cherry	<i>A. parasiticus</i> >5 log CFU/g for 5 min
	HPP, 500 MPa, 20 min ( <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> ), 5 min ( <i>Salmonella typhimurium</i> )	Carrot	Mesophilic aerobic bacteria 2.8 log cycles CFU/mL; Yeasts and molds 3.1 log cycles CFU/mL
	HPP, 500 MPa, 20 min ( <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> ), 5 min ( <i>Salmonella typhimurium</i> )	Spinach	Total aerobic mesophilic counts 4.65 log cycles; Yeasts and molds 6.51 log CFU/g–undetectable level
	HPP, 500 MPa, 10 min	Mulberry juice	<i>Listeria monocytogenes</i> 4.1 log CFU/g
			<i>Staphylococcus aureus</i> >5 log CFU/g
			<i>Salmonella Typhimurium</i> >5 log CFU/g

(continued on next page)



Table 2 (continued)

Non-conventional treatments	Produce	Microbial log reduction	Reference	
Ultraviolet	UV-C, 0.14 kJ/m <sup>2</sup> , 10 s	Apple	<i>E. coli</i> O157:H7 2.1 ± 0.4 log CFU/g <i>L. monocytogenes</i> 1.6 log CFU/g	Adhikari et al. (2015)
	UV-C, 7.56 kJ/m <sup>2</sup> , 4 min	Pear	<i>E. coli</i> O157:H7 3.7 log CFU/g	Syamaladevi et al. (2013)
	UV-C, 253.7 nm, 2 h	Roasted coffee beans	<i>A. flavus</i> : 1.58 log CFU/g	Byun et al. (2020)
	UV-C, 253.7 nm, 2 h 6.4 mW/cm <sup>2</sup> , 40 min 55–60 mW/cm <sup>2</sup> , 30 min	Roasted coffee beans Peanut oil Peanut oil	<i>A. parasiticus</i> 0.72 log CFU/g Aflatoxins: AFB1 89% Aflatoxins: AFB1 96%	Diao et al. (2015) Mao et al. (2016)
Ultrasound	US, 40 kHz	Melon	<i>E. coli</i> 1.6 log CFU/g <i>S. enterica</i> enteritidis 1.9 log CFU/g	São José et al. (2014)
	US, 40 kHz	Green peppers	<i>E. coli</i> 2.3 log CFU/g <i>S. enterica</i> enteritidis 1.8 log CFU/g	
	US, 20 kHz, 400 W, 20 °C, 15 min	Cucumber	Total number of colonies 1.02 log CFU/g Molds and yeasts 0.84 log CFU/g	Fan, Zhang, Bhandari, and Jiang (2019)
Microbial surfactants	40 kHz, 80 W L 1, 20 min	Shiitake mushrooms (Lentinula edodes)	Loss of hardness of post-harvest shiitake mushrooms: 48.5% and 37.4%	Ni et al. (2018)
	Sophorolipids (SOs), 2h, 1%; Thiamine dilauryl sulfate (TDS), 1 min, 1%	Baby spinach leaves	<i>E. coli</i> O157:H7: from 7.1 log CFU/ml to undetectable level	Zhang et al. (2016)
	Lipopeptides (LP) from <i>B. amyloliquefaciens</i> , pH 6.8, 30 °C containing 4 g/L NH <sub>4</sub> NO <sub>3</sub> and sparged with 21% O <sub>2</sub>	Grape	<i>Botrytis cinerea</i> : 100%	Pretorius et al. (2015)
	<i>Bacillus subtilis</i> : 0.05–20 µg/disk, 10 days, 25 °C	Wheat Blast	<i>Magnaporthe oryzae Triticum</i> : 60.9 ± 2.5%	Chakraborty et al. (2020)
	LP <i>Bacillus altitudinis</i> , 2 mg/mL	Apple	<i>Alternaria alternata</i> : 83.2%	(M. Sun, Ye, et al., 2021)
	LP <i>Bacillus velezensis</i> , 24 h, 30 °C	Pome fruits	<i>Alternaria alternata</i> : 59%	Cozzolino et al. (2020)
	LP <i>Bacillus velezensis</i> , 24 h, 30 °C	Pome fruits	<i>Botrytis cinerea</i> : 72.67% <i>Penicillium expansum</i> : 67%	

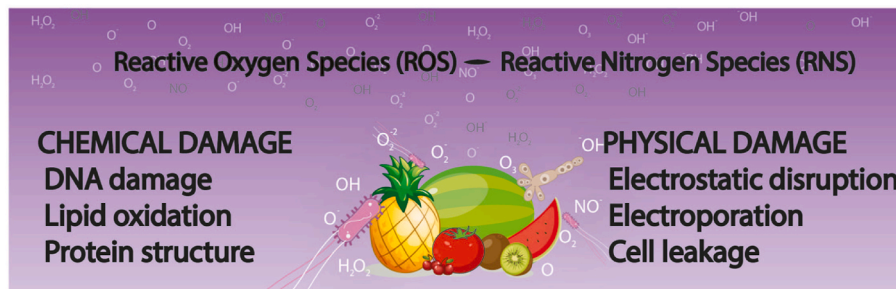


Fig. 2. Principal chemical and physical damages induced by Cold Plasma technology on the microorganism.

chlorophyll degradation when ROS are present, in contrast with RNS, which is less harmful to these components (Sudarsan & Keener, 2022).

Cold plasma is an effective surface treatment that can achieve disruption of the 3D structure of a biofilm. For instance, a study showed that PRS could diffuse within 10–15 mm of a polymeric matrix and decontaminate *Salmonella* up to 5 log CFU/cm<sup>2</sup> with a dielectric barrier discharge system that operates at 90 kV with air (Xu et al., 2020). Furthermore, Govaert and collaborators reported a 2.7 log CFU/cm<sup>2</sup> reduction of a *Listeria* biofilm (Govaert et al., 2019). In the same study, cold plasma was combined with a 0.2% H<sub>2</sub>O<sub>2</sub> solution, and this approach reduced by more than 5 log CFU/cm<sup>2</sup> the population of *Listeria* with a dielectric DBD system that operates at 6.5 kV/7W. Additionally, Patange et al. (2019) investigated the efficiency of cold plasma on both mono- and mixed-cultures biofilms of fresh processing, showing a significant reduction of viable cells by the application of 80 kV/60 s, even a level of nondetectable bacteria was achieved with a 120 s treatment. The application of cold plasma enables the disintegration of the biofilm conformation, including the EPS network, by breaking the bonds of biofilm components. The latter triggers the dispersal of the residing microorganisms, leading to its direct exposure to cold plasma. Consequently, the death of microorganisms is achieved due to plasma's excessive electrostatic stress in the cell membrane (Liu et al., 2021).

#### 4.4. High hydrostatic pressure

High hydrostatic pressure (HPP) is a non-thermal technology for microbial inactivation in foods without negatively affecting their quality

attributes. HPP applies a pressure treatment in the range of 100–800 MPa, with or without the combination of heat, from a few seconds to several minutes (Balasubramaniam et al., 2015). HPP causes damage to cell membranes and denaturation of cell components, inactivating the initial load of foodborne microorganisms. Some factors can affect HPP treatment's efficacy, such as the magnitude of the force generated, water activity, and treatment time (Bhilwadikar et al., 2019). In the case of FV, several studies have been reported on microbial inactivation by HPP. For example, alfalfa seeds inoculated with *Salmonella* spp. were treated with 600 MPa for 25 min, showing a 4.5 log CFU/g microbial reduction (Barba et al., 2017). In addition, Grande Burgos et al. (2017) studied the HPP influence on the reduction of mesophilic aerobic bacteria and yeasts and molds in peaches. After the HPP treatment of 600 MPa for 8 min, mesophilic aerobic bacteria, yeasts, and molds were reduced on average by 2.9 log CFU/mL. Furthermore, HPP has shown effectiveness against total aerobic mesophilic yeasts and molds up to the undetectable level in cherry after applying 600 MPa (Toledo delÁrbol et al., 2016). In addition, Jung et al. (2013) reported an average reduction of 2.2 log CFU/g against *Listeria monocytogenes* and *S. aureus* in spinach treated by HPP for 20 min. The authors also treated carrots with HPP for 20 min and found an average reduction of 4.6 log CFU/g against *Listeria monocytogenes* and *S. aureus*. They showed that Gram-positive bacteria were more resistant to HPP than Gram-negative bacteria. Consequently, the use of HPP in FVMP is appropriate to inactivate pathogenic and spoilage microorganisms, increasing their shelf life.

#### 4.5. Ultraviolet light

Ultraviolet light (UV) provides a high intensity of light. It has been used in the food industry for several purposes like surface sterilization, fluid disinfestation, air treatment, waste treatment, and insect trapping (Darré et al., 2022). This technology works with a wavelength range of 190–280 nm and results in germicidal and antimicrobial activities used to decontaminate water, fruits, and root vegetables (Cutler & Zimmerman, 2011). Consequently, the antimicrobial effect of UV light is through the generation of pyrimidine dimers, which distorts DNA helix and interferes with cell replication of exposed microorganisms (Lado & Yousef, 2002). The UV technology produces lethality against various microorganisms, including bacteria, fungi, and viruses (C. Liu, Huang, & Chen, 2015). Several research reports suggest that UV light may stimulate the production of phenolic compounds such as ascorbic acid, glucosinolates, or carotenoids in fresh food. These effects keep the attention of researchers (Darré et al., 2022). In this sense, the wavelength approved by the FDA is 254 nm to use in food products and juices (J.A. Otter, 2014). However, the UV process has disadvantages such as shallow penetration ability, sample heating, and shadowing effect that limits its application in the decontamination of the fresh product (Liu, Huang, & Chen, 2015). On the other hand, Guo et al. (2017) developed a water-assisted UV decontamination system for fresh produce, where this system enabled fresh produce to move and rotate randomly during UV treatment to achieve total exposure of all surfaces of fresh produce to UV.

Furthermore, different reports of UV light reducing <4.0 log microbial load of *Salmonella* spp. on lettuce using an intensity of 7 J/cm<sup>2</sup> for 30 min (Birmpa et al., 2013). Additionally, Adhikari et al. (2015) reported that a UV treatment on apples reduced 1.6 log CFU/g of *L. monocytogenes*. Under those circumstances, the reduction of *E. coli* was carried out by UV-C (UV light of shorter wavelength, 100–279 nm) for 10 s at 0.14 kJ/m<sup>2</sup> and UV-C for 5 min at 3.75 kJ/m<sup>2</sup> for *L. monocytogenes*. Similar results were obtained with pear exposed to UV-C at 7.56 kJ/m<sup>2</sup> for 4 min. Consequently, *E. coli* O157:H7 showed a reduction of 3.7 log CFU/g (Syamaladevi et al., 2013). Regarding water-assisted UV has shown a higher efficacy than conventional process UV. For instance, the water-assisted UV treatment achieved 4.97 and 2.79 log reduction of *Salmonella* spot-inoculated on tomatoes and lettuce, while only <1 log reduction was achieved by conventional UV treatments (Guo et al., 2017).

Few studies have reported adverse effects in MPFV using UV light. For instance, the experiments with pineapple resulted in the lowest expression of vitamin C (Pan & Zu, 2012). The biggest challenge of this technology should focus on compatibility within a continuous food processing process and the time of exposure of these foods to UV light without causing alteration to the final product.

#### 4.6. Ultrasound

Ultrasound (US) is considered a safe, non-toxic, and environmentally friendly decontamination technology, which works with pressure waves with a frequency ranging from 20 to 100 kHz in a liquid media (Bhilwadikar et al., 2019; Gallo et al., 2018). The high-pressure waves induce acoustic cavitation liberating a high amount of energy that destroys the microbial cell walls and damages the DNA via free radical production (Hulsmans et al., 2010; Nicolau-Lapeña et al., 2019; Bhargava et al., 2021). Some studies regarding the efficiency of this technology reported a microbial load reduction of <1.0 log CFU/g by US treatment at 45 kHz for 10 min (Bilek & Turantaş, 2013). Silva et al. (2018) reported a low microbial reduction on fresh lettuce by a US treatment alone at 40 kHz and 500 W for 5 min at room temperature. The authors showed a reduction of 0.6 and 0.3 log CFU/g against mesophilic aerobic bacteria and molds, respectively. Moreover (Fan, Zhang, Bhandari, & Jiang, 2019), reported that US treatment decreased slightly (<1.0 log CFU/g) the total number of colonies, mold and yeast, and total coliform counts

of fresh-cut lettuce stored at 4 °C for 12-days. They treated the lettuce by the US at 20 kHz and volumetric power of 23 W/L for 10 min. Furthermore, Fan, Zhang, and Jiang (2019) described the effect of US treatment for 15 min with a 20 Hz frequency at 20 °C, addressing microbial reduction in cucumber, which resulted in an average reduction of 0.9 log CFU/g in molds and yeasts. The limited efficiency of US treatment alone in FV could be attributed to the ability of pathogens to penetrate fruits and vegetables and become inaccessible to sound waves (Silva et al., 2018). Finally, literature has reported that the intensity of US treatment could influence the firmness and color changes of fresh fruits and vegetables, which could be due to the acoustic cavitation and the inactivation of phenol oxidase and polyphenol oxidase enzymes, respectively (Bhargava et al., 2021).

#### 4.7. Microbial surfactants

Microbial surfactants (MS) are surface-active molecules that comprise a hydrophobic and hydrophilic moiety produced by microorganisms, applying biotechnological fermentation processes (Vecino et al., 2021). These compounds have surface activities, helping reduce interfacial tension and producing detergency and emulsifying properties. MS are new friendly-ecological surfactants and biodegradable molecules with low toxicity, which could be used as cleansers, sanitizers, emulsifiers, pesticides, and detergents (Gayathiri et al., 2022). MS can also be obtained from the different microbial genera, including *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Candida*. MS show antimicrobial, anti-biofilm, and antiviral activity, which can help prevent food spoilage (André et al., 2007). Comparatively, Table 2 shows the future trends in treatments for the MS antimicrobial action in MPFV. Lipopeptides (LPs) are among the most effective and efficient MS types. These compounds can be used in therapeutic, cosmetic, and agri-food industries because the interest in LPs is growing. LPs' structure contains a fatty acid connected to a peptide chain. Additionally, their production is related to the mode of action against other microorganisms ((Dey et al., 2015; Wu et al., 2019). According to Coronel et al. (2016), the LPs' fungal action is very likely to rely on osmotic perturbation, including ion-pore formation, and compounds like surfactins induce the disruption of the membrane or its solubilization. Several studies showed the activity of LPs compounds as antifungal properties from several bacterial genus (Gutiérrez-Chávez et al., 2021). The LPs' inhibitory activity from *Bacillus amyloliquefaciens* DSM23117 has been reported against *Botrytis cinerea*, a major phytopathogen in the table grape industry. These LPs also have similar efficacy studies against phytopathogens agents like *Penicillium digitatum*, causing damage to the fungus mycelium (Pretorius et al., 2015). Likewise, the antifungal metabolites produced by *B. subtilis* V26 significantly inhibited the growth of *B. cinerea*, avoiding the grey mold disease (Kilani-Feki et al., 2016). In another study, the lipopeptide fraction composed of iturin and fengycin produced through *B. subtilis* inhibited the mycelial growth and conidia germination of fungus like *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium stilboides*, and *Penicillium expansum*, being causal agents of the postharvest blue mold in apple fruit (Rodríguez-Chávez et al., 2019). More recently, lipopeptide extract produced by the *B. velezensis* FZ06 showed a positive effect on Gram-positive bacteria at a concentration from 512 to 2048 µg/mL. In contrast, over Gram-negative bacteria, the effect was about 1024 µg/mL (F. Z. Li et al., 2020).

Another potential MS are rhamnolipids (RL), composed of one or two rhamnose molecules linked to one or two fatty acids alkyl chains produced mainly by *Pseudomonas* and *Burkholderia* (Boubakri, 2020). Some studies reported RL's effectiveness in controlling the growth of the plant pathogen *Alternaria alternata* on cherry tomatoes through the inhibition of mycelium growth and spore germination (Yan et al., 2015). In addition, the RL activity between *B. cereus* and *L. monocytogenes* was clearly demonstrated at a concentration of minimum inhibitory from 19.5 to 156.2 µg/mL and minimum bactericidal concentration from 39.1 to 312.5 µg/mL, meaning effective reduction (De Freitas Ferreira et al.,

2019). Similarly, another MS is sophorolipids, produced from *Starmerella bombicola*, which has antifungal activity against *A. flavus*, *A. melleus*, *A. ochraceus*, *A. parasiticus*, *A. niger*, *F. oxysporum*, *B. cinerea*, and *Rhizopus* spp., in concentrations between 225729  $\mu\text{g/mL}$  (Hipólito et al., 2020). Developing an edible coating using aloe vera and RL significantly effectively controlled *P. digitatum* NSP01. The process combined the aloe vera's limitation effect on the transpiration process and the RLs' antimicrobial activity (Adetunji et al., 2019).

Alternatively, microbial surfactants can be used in the food chain for biofilm disruption. Biofilm production has great importance and ecological function, including resistance to the environment, cell division, and proliferation, among others (Balcázar et al., 2015; Raaijmakers, 1983). These molecules synthesized by microorganisms also have anti-adherence properties against certain microorganisms. The action of these molecules (Fig. 3) in the biofilm prevention could result from the repulsion forces between the negative charges of the microbial surface and the negative charge of the surface coated with MS molecules. Whereas the disruption effect is due to MS penetration and absorption at the interface between the solid surface and the attached biofilm-forming bacteria, reducing the interfacial tension and facilitating biofilm removal (Coronel-León et al., 2016; Nitschke & Silva, 2018). Singh and Sharma (2020) found that applying 6 mg/mL lipopeptide produced by *Bacillus tequilensis* strain SDS21 resulted in the microorganism removal of >99% on stainless steel. In addition, it is believed that within the mechanisms of action of biosurfactants, they may cross the 3D structure of biofilms, reducing the surface tension between the biofilm and the solid substrate (Nitschke & Silva, 2018). De Araujo et al. (2016) evaluated the potential of two biological surfactants against film formation by *L. monocytogenes* and *P. fluorescens* on different surfaces—stainless steel and polystyrene. The surfactin treatment inhibited the adhesion of *P. fluorescens* into stainless steel by 73%, and *Listeria monocytogenes* biofilm formation on both materials was significantly reduced by the treatment with MS.

## 5. Combined disinfection methods

Novel decontamination methods may be used alone or in combination with other chemical treatments such as organic acids, chlorine, surfactants, hydrogen peroxide, or multiple products (Table 3). The combination of different methods, called hurdle technology, enhances fruits and vegetables' microbiological safety, nutritional, and sensory qualities (Khan et al., 2017). Alenyorege et al. (2019) found that sweeping frequency ultrasound from 28 to 68 kHz with NaOCl solutions from 20 to 100 mg/L for 10 min reduced on average 0.7 log CFU/g of *L. innocua* population in fresh-cut chinese cabbage. Both treatments showed synergistic reductions in 85% of *L. innocua*, reaching up to 3.35 log CFU/g, without adverse quality changes to leaf color, texture, pigments, and microstructure. Likewise, São José et al. (2014) observed that 40 kHz US, lactic acid, and acetic acid reduced the presence of *E. coli* on the surface of green pepper on average by 1.9 log CFU/cm<sup>2</sup>, as well as

the presence of *S. enterica Enteritidis* on the surface of melon on average by 1.7 log CFU/cm<sup>2</sup>, respectively. The combination of US with lactic acid or acetic acid in green pepper decreased the population on average by 2.8 log CFU/cm<sup>2</sup> of *E. coli*. In melon, the same combined treatments were reduced to 3.1 (US and lactic acid) and 2.4 (US and acetic acid) log CFU/cm<sup>2</sup> of *S. enterica Enteritidis*. These results indicated that it is feasible to enhance the effect of organic acid sanitizers using ultrasound and emerging technology.

Guo et al. (2017) experimented with the combination of water-assisted UV treatment ( $\sim 29 \text{ mW/cm}^2$ , 2 min) and chlorine, which maintained a *Salmonella enterica* free environment in the wash water for lettuce, spinach, and baby-cut carrots. The combination of both methods generally showed more effectiveness than the treatments alone. However, water-assisted UV inactivation of *Salmonella* was unable on tomatoes. Huang and Chen (2018) also applied water-assisted UV treatment (23 and 28 mW/cm<sup>2</sup>, 2 min) combined with chlorine or peroxyacetic acid, reducing *Salmonella enterica* in wash water below the detection limit (2 CFU/mL) in lettuce, tomato, blueberry, and carrot. Hence, the type of product might influence the inactivation effect of *S. enterica* by water-assisted UV treatments, and the combined technology could prevent cross-contamination with *S. enterica* significantly, eliminating the risk (Guo et al., 2017; R.; Huang & Chen, 2018).

A combination of technology is also reported by Ramos-Villarreal et al. (2015). The study investigated pulsed light (180–1100 nm, 12 J/cm<sup>2</sup>) and malic acid, reducing the *L. innocua* and *E. coli* population in avocado, watermelon, and mushrooms, up to more than 5 log reductions compared to separated treatments. In addition, the study found that applying sequential treatments is feasible to effectively control bacteria growth on the product's surface for at least 2-weeks.

The improvement of microbial reduction on products due to the combined treatments is related to the synergistic effect of microbial cell damages. For example, Park et al. (2018) observed that *S. Typhimurium* cells treated with UV and ClO<sub>2</sub> gas demonstrated an uneven distribution and aggregation of internal cellular substances and slight separations of the cell membrane from the cytoplasm. However, the cells treated with both showed a more pronounced damage effect, such as a severe rupturing of cell membranes and leakages of intracellular contents.

Therefore, a significant synergistic benefit could be achieved from combined decontamination treatments to reduce and eliminate food-borne pathogens from FV. Similarly, combined treatments could minimize the concentration of chemical products and obtain safe FV and sensory acceptable with a long shelf life. However, more studies on combining decontamination techniques for FV are needed—cold plasma, pulsed electric field, and HPP to prove the higher synergistic effect among these technologies.

## 6. Trends in disruption and prevention of biofilm

Despite advantages in the modern disinfection process described earlier, the MPFV industry is continuously challenged by the threat of

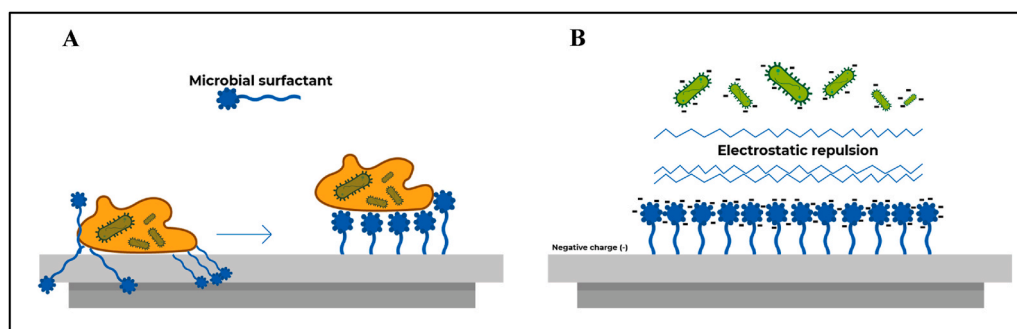


Fig. 3. Schematic representation of the mechanism action of microbial surfactant to disruption (A) and prevention (B) of biofilm formation on the surface in contact with fruits and vegetables.

**Table 3**  
Combined disinfection methods for fruit and vegetables.

Non-conventional treatments	Chemical treatments		Produce	Microbial log reduction	Reference	
UV	70.68 $\mu\text{W}/\text{cm}^2$ ; 253.7 nm; 20 min	Chloride	$\text{ClO}_2$ gas; 10 ppmv	Spinach (leaves)	<i>E. coli</i> O157:H7 5.17log CFU/g <i>S. Typhimurium</i> 5.47log CFU/g <i>L. monocytogenes</i> 4.32log CFU/g	Park et al. (2018)
				Tomato	<i>E. coli</i> O157:H7 4.8log CFU/g <i>S. Typhimurium</i> 4.28log CFU/g <i>L. monocytogenes</i> 2.7log CFU/g	
	29 mW/cm <sup>2</sup> ; 2 min	Chloride	10 ppm free chlorine	Tomato (grape)	<i>Salmonella enterica</i> 5.93 log CFU/g	Guo et al. (2017)
	7.9 mW/cm <sup>2</sup> ; 254 nm; 10 min	Chloride	10 ppm free chlorine; 4 C	Blueberry	<i>Salmonella enterica</i> 2.56log CFU/g <i>E. coli</i> O157:H7 ~2 log CFU/g <i>Salmonella enterica</i> ~1.75log CFU/g	Liu, Li, and Chen (2015)
	10 kJ/m <sup>2</sup> ; UV-C; 18.24 min	Chloride	$\text{ClO}_2$ gas; 15 ppmv; 20 min	Plums	<i>E. coli</i> O157:H7 3.27log CFU/g <i>L. monocytogenes</i> 4log CFU/g	(Kim & Song, 2017)
	23–28 mW/cm <sup>2</sup> ; 2 min	Chloride	10 ppm free chlorine	Spinach (Baby, leaves)	<i>Salmonella enterica</i> 1.35log CFU/g	(R. Huang & Chen, 2018)
				Lettuce (iceberg)	<i>Salmonella enterica</i> 2.52log CFU/g	
				Tomato (grape)	<i>Salmonella enterica</i> > 3.76log CFU/g	
				Blueberry	<i>Salmonella enterica</i> 2.52log CFU/g	
				Carrot (baby-cut)	<i>Salmonella enterica</i> 3.4log CFU/g	
	29 mW/cm <sup>2</sup> ; 2 min	Peroxide	Hydrogen peroxide; 1%	Tomato (grape)	<i>Salmonella enterica</i> 5.74log CFU/g	Guo et al. (2017)
	23–28 mW/cm <sup>2</sup> ; 2 min	Peroxide	Hydrogen peroxide; 1%	Tomato (grape)	<i>Salmonella enterica</i> 3.12log CFU/g	(R. Huang & Chen, 2018)
				Blueberry	<i>Salmonella enterica</i> 1.61log CFU/g	
				Carrot (baby-cut)	<i>Salmonella enterica</i> 1.37log CFU/g	
	56.7 mJ/cm <sup>2</sup> ; 254 nm	Peroxide	Hydrogen peroxide; 2%; 50 C	Mushroom (fresh-cut)	<i>L. monocytogenes</i> ~0.5log CFU/g	Murray et al. (2015)
	0.3 kJ/m <sup>2</sup> ; UV-C	Peroxyacetic	Peroxyacetic acid; 80 mg/L	Broccoli (fresh-cut)	<i>Listeria innocua</i> 1.7 log CFU/g	Collazo et al. (2019)
	23–28 mW/cm <sup>2</sup> ; 2 min	Peroxyacetic	Peroxyacetic acid; 80 ppm	Spinach (Baby, leaves)	<i>Salmonella enterica</i> 1.33log CFU/g	(R. Huang & Chen, 2018)
				Lettuce (iceberg)	<i>Salmonella enterica</i> 2.99log CFU/g	
				Tomato (grape)	<i>Salmonella enterica</i> 3.48log CFU/g	
				Blueberry	<i>Salmonella enterica</i> 2.6log CFU/g	
				Carrot (baby-cut)	<i>Salmonella enterica</i> 3.65log CFU/g	
	7.9 mW/cm <sup>2</sup> ; 254 nm; 10 min	Organic acid	Levulinic acid; 0.5%; 4 C	Blueberry	<i>E. coli</i> O157:H7 ~11log CFU/g <i>Salmonella enterica</i> ~0.5log CFU/g	Liu, Li, and Chen (2015)
	7.9 mW/cm <sup>2</sup> ; 254 nm; 10 min	Surfactant	Sodium dodecyl sulfate; 100 ppm; 4 C		<i>E. coli</i> O157:H7 ~1.75log CFU/g <i>Salmonella enterica</i> ~1.25log CFU/g	
	10 kJ/m <sup>2</sup> ; UV-C; 18.24 min	Multiple	$\text{ClO}_2$ gas; 15 ppmv; 20 min; fumaric acid; 0.5%	Plums	<i>E. coli</i> O157:H7 4.37log CFU/g <i>L. monocytogenes</i> 5.36log CFU/g	(Kim & Song, 2017)
	5 kJ/m <sup>2</sup> ; UV-C; 30 s	Multiple	Lemongrass EO; citrus extract; lactic acid; 0.01:0.1:1	Cauliflower	<i>E. coli</i> O157:H7 0.82log CFU/g <i>L. monocytogenes</i> 1.45log CFU/g	Tawema et al. (2016)
	5 kJ/m <sup>2</sup> ; UV-C; 30 s	Multiple				

(continued on next page)

Table 3 (continued)

Non-conventional treatments	Chemical treatments		Produce	Microbial log reduction	Reference		
Pulsed light	200–1100 nm; 60 s	Chloride	Oregano EO; citrus extract; lactic acid; 0.01:0.1:1	<i>E. coli</i> O157:H7 >1.82log CFU/g <i>L. monocytogenes</i> 1.37log CFU/g	(R. Huang & Chen, 2018) (Y. Huang & Chen, 2015)		
			10 ppm free chlorine	Lettuce (iceberg, shreds)		<i>Salmonella enterica</i> 1.48log CFU/g	
	200–1100 nm; 60 s	Peroxide	Hydrogen peroxide; 1%	Strawberry		<i>E. coli</i> O157:H7 3.3log CFU/g <i>Salmonella enterica</i> 2.8log CFU/g <i>Murine norovirus</i> 2.2log PFU/g	
						Raspberry	<i>E. coli</i> O157:H7 5.3log CFU/g <i>Salmonella enterica</i> 4.9log CFU/g <i>Murine norovirus</i> 2.5log PFU/g
	180–1100 nm; 12 J/cm <sup>2</sup>	Organic acid	Malic acid; 2%	Avocado (fresh-cut)		<i>L. innocua</i> 2.46 CFU/g <i>E. coli</i> 3.14log CFU/g	Ramos-Villarreal et al. (2015)
				Watermelon (fresh-cut)		<i>L. innocua</i> 2.68 CFU/g <i>E. coli</i> 3.48log CFU/g	
180–1100 nm; 12 J/cm <sup>2</sup>	Organic acid	Malic acid; 2%	Mushroom (fresh-cut)	<i>L. innocua</i> 2.64 CFU/g <i>E. coli</i> 3.43log CFU/g	Salinas-Roca et al. (2016)		
			Mango (fresh-cut)	<i>L. innocua</i> 4.5 CFU/g			
200–1100 nm; 60 s	Surfactant	Sodiumdodecyl sulfate; 100 ppm	Strawberry	<i>E. coli</i> O157:H7 2.3log CFU/g	Huang and Chen (2015)		
			Raspberry	<i>E. coli</i> O157:H7 5.1log CFU/g			
Ultrasound	180–1000 nm; 31.5 J/cm <sup>2</sup> ; 30 s 47.8 J/cm; 22–40 s	Multiple	Shortchain organic acid; ethylenediaminete-traacetic acid; nisin	Tomato (cherry)	<i>Salmonella enterica</i> > 5log CFU/g	Leng et al. (2020)	
			Strawberries	Postharvest molds:16–42%	Duarte-Molina et al. (2016)		
	40 kHz S; 120 W/L; 10 min	Chloride	Sodium hypochlorite; 100 mg/L	Cabbage (Chinese, fresh-cut)	<i>Listeria innocua</i> 3.35log CFU/g	Alenyorege et al. (2019)	
				40 kHz; 24 W/L; 1 min	Chloride		10 ppm free chlorine
	40 kHz	Organic acid	Lactic acid; 1%	Green peppers		<i>S. enterica Enteritidis</i> 2.8log/cm <sup>2</sup> <i>E. coli</i> 2.9log/cm <sup>2</sup> <i>S. enterica Enteritidis</i> 3.1log/cm <sup>2</sup> <i>E. coli</i> 2.5log/cm <sup>2</sup>	
	40 kHz			Organic acid	Acetic acid; 1%	Green peppers	<i>S. enterica Enteritidis</i> 2.4log/cm <sup>2</sup> <i>E. coli</i> 2.6log/cm <sup>2</sup> <i>S. enterica Enteritidis</i> 2.4log/cm <sup>2</sup> <i>E. coli</i> 2.1log/cm <sup>2</sup>
40 kHz; 30 W/L; 5 min	Surfactant	Tween 20; 0.1%	Lettuce (iceberg)			<i>B. cereus</i> spores 2.45log CFU/g	Sagong et al. (2013)
40 kHz			Surfactant	Tween 20; 0.1%	Carrots	<i>B. cereus</i> spores 2.28log CFU/g	
HHP	500 MPa; 1 min	Chloride			200 ppm; 15 min; in water	Tomato (cherry)	<i>Salmonella</i> Typhimurium 5.47log CFU/g
PEF	2 kV/cm; 100 pulses/s; 4 min	Multiple	Peroxyacetic acid (5.2%); hydrogen peroxide (11.2%); 0.25%	Blueberry	<i>E. coli</i> K12 ~2.9log CFU/g <i>L. innocua</i> ~2.9log CFU/g	Jin et al. (2017)	
Gamma	0.5 kGy; 16.74 kGy/h; cobalt-60	Multiple	Lemongrass EO; citrus extract; lactic acid; 0.01:0.1:1	Cauliflower	<i>E. coli</i> O157:H7 > 1.82log CFU/g <i>L. monocytogenes</i> >1.93log CFU/g	Tawema et al. (2016)	
				0.5 kGy; 16.74 kGy/h; cobalt-60	Multiple	Oregano EO; citrus extract; lactic acid; 0.01:0.1:1	Cauliflower

microbial contamination. In this context, the ability of microorganisms to build biofilm as a shield against disinfection techniques could develop antimicrobial-resistant foodborne pathogens (L. Yuan et al., 2020). The food industries remove biofilms with diverse methods, including biological techniques (e.g., bacteriophages, anti-biofilms enzymes, lactic acid bacteria bacteriocins) (Y. Yuan et al., 2019), biosurfactants (e.g.,

lipopeptides) (Nitschke & Silva, 2018), physical technique (e.g., plasma) (Liu et al., 2021). Therefore, this section analyzes different biological and sustainable methods with efficiency against biofilm disruption.

Enzymes are an interesting eco-friendly strategy for controlling biofilms in food industries. The use of anti-biofilm enzymes enables the degradation of extracellular polymeric substances (EPS) structure

components, leading to the access of cleaners and disinfectants within the biofilm (Giaouris & Simões, 2018). Different anti-biofilm enzymes are involved in the biofilm degradation—proteases, DNA, polysaccharides, Quorum sensing molecules using proteases, oxidative enzymes, polysaccharide-degrading enzymes, and anti-QS enzymes (Meireles et al., 2016). Better results were obtained by combining different enzymes for biofilm removal (Y. Yuan et al., 2019). (Wang et al., 2016) have reported a reduction of  $6.22 \pm 0.16 \log \text{CFU/cm}^2$  of residing microorganisms from a mature *Salmonella* biofilm on stainless steel after applying cellulase, followed by cetyltrimethylammonium bromide (CTAB).

In other matters, bacteriophages are viruses that have antimicrobial activity and require bacteria host cells to reproduce, causing bacteria infection (Cacciatore et al., 2021). One disadvantage of using bacteriophages is that these phages are rather specific regarding the target cell, so the bacteria detection involved before the treatment is essential. Bacteriophages can also access bacteria within the biofilm by penetrating the matrix structure and infecting the cells' target. Salmofresh, a phage product, oppresses some *Salmonella* strains and has shown a diminution of 2.1 log to 4.3 log CFU/mL after 5 min (Cacciatore et al., 2021; Y.; Yuan et al., 2019). On the other hand, some microorganisms produce bacteriocins, especially lactic acid bacteria. These bacteriocins are proteins or peptides with antibacterial properties and act against biofilm formation, generally in which Gram-negative bacteria are involved. Bacteriocins bind to a particular cell wall constituent in the target cell, affecting its regular cycle (Niaz et al., 2019). These molecules are also responsible for the pores formed in the bacterial cell wall, and consequently, cell death occurs. Moreover, bacteriocins' activity against biofilm formation is based on the enzymatic activity disturbance of RNA polymerase, aspartyl-tRNA synthetase, and DNA gyrase in some microorganisms, being involved in the metabolisms of DNA, RNA, and proteins (Toushik et al., 2020). Kim et al. (2019) studied the effect of bacteriocin obtained from *Lactobacillus brevis* DF01 on *E. coli* and *S. Typhimurium* biofilm formation. They reduced 50% *E. coli* KCTC 1039 and *S. Typhimurium* from  $2.0 \times 10^5 \text{CFU}$  in stainless steel coupon.

## 7. Conclusions and future perspectives

The food industry is responsible to society by covering the food demand with nutritional value, high quality, and safe products. As mentioned in this review, MPFVs have the healthy, functional, and sensory characteristics that the consumers are looking for (Fardet, 2018). However, the agricultural crop conditions (e.g., manures and irrigation water) and processing operations (e.g., cross-contamination) of MPFV could favor the contamination with antimicrobial-resistant microorganisms, one of the biggest concerns in the last years (Donaghy et al., 2019). Thus, the food processors face the invisible and latent risk, the presence of microorganisms in their natural form or complex aggregates (e.g., biofilms) (L. Yuan et al., 2020). In response to these needs, extensive work between researchers and industry independently and in a collaborative format has been done. The results of conventional and new strategies developed so far have different efficacy ranges against various microorganisms (Shezi et al., 2020; Zamuner et al., 2020) and is discussed in this comprehensive review. Nevertheless, these techniques have aspects that must be improved for large-scale industrial applications.

For instance, in conventional chemical products, one improvement aspect is the exposure time and concentrations used (Nerín et al., 2016) because microbial resistance has been associated with those factors (Donaghy et al., 2019). The extreme exposure of MPFV to techniques can also affect the sensory characteristics, especially color, flavor, and aroma (Khan et al., 2017; Velázquez et al., 2009). The effects can also be observed in the food operator, where inappropriate contact with these substances can generate a series of allergic reactions. Likewise, surfaces in contact with MPFV can be affected by corrosion problems. In the case of excessive compound concentrations, it can affect the characteristics of

the food and increase the use of water, having an industrial impact (Bhagwat, 2019). Therefore, future research should focus on optimizing the time-concentration parameters of chemical compounds and their effectiveness in controlling microorganisms, including studies in biofilms, and considering that the nutritional and functional characteristics are minimally affected.

In the specific case of the new technologies described, their positive disinfection effects are undeniable; however, the exposure time and concentration are crucial. In addition, it is necessary to identify if the disinfection strategy selected requires additional conservation operations to maintain food safety and ensure the extension of the shelf life of MPFV. It is critical to understand that if the technology used does not reach the desired disinfection levels, the microorganisms can survive and affect the quality of MPFV. Similarly, if the operating conditions are inadequate, FV's nutritional and functional characteristics will be affected. Another aspect that must be considered is the texture and shape of the products to be processed, especially since there are technologies whose limitations are related to the poor penetration capacity of the treatment. At last, the operational parameters should be optimized to maintain the quality of the product. Meanwhile, investment and cost should be analyzed for large-scale applications.

Finally, disinfection in MPFV is a necessary process that needs to be properly correctly done to ensure food safety, extend shelf-life, provide high-quality products for consumers, and reduce food losses and waste. This process has been done for many years with current techniques and is widely practiced in the industry. Nevertheless, there has been a new wave of technological advances and research looking forward to improving and giving more options using different sources. New approaches to disinfection techniques must find more suitable production methods that compete economically with the standard procedures. That is one of the significant disadvantages of using more environmentally friendly products (e.g., microbial surfactants, cold plasma technology). Besides, combining disinfection techniques is an innovative way to decrease the use of high quantities of chemical compounds and avoid adverse effects on MPFV quality. For instance, combined biological products such as essential oils (EOs) and edible coatings could be considered preventive actions to reduce losses by microbial contamination of minimally processed fruits and vegetables. The EOs contain monoterpenes, sesquiterpenes, alcohols, esters, ethers, aldehydes, amines, amides, phenols, and ketones that cause membrane destruction, permeabilization, and cellular death of microorganisms (Dwivedy et al., 2016; Z. H. Li et al. (2019). Thus, the edible coatings can incorporate antimicrobial agents such as EOs, natural seed extracts, probiotics, bacteriocins, and organic acids, among others (Otoni et al., 2017; Riva et al., 2020). As a result, EOs can improve specific properties of edible coatings, for example, enhance moisture and gas barriers, helping to preserve the color, texture, and moisture of MPFV (Grande-Tovar et al., 2018; Sánchez-González et al., 2011). Therefore, combined methods are a promising strategy to improve disinfection efficacy with minimal impact on the quality of MPFV.

## CRedit authorship contribution statement

**Iana Cruz Mendoza:** Conceptualization, Investigation, Writing – original draft, preparation. **Esther Ortiz Luna:** Writing – original draft, preparation, Investigation. **María Dreher Pozo:** Writing – original draft, preparation, Investigation. **Mirian Villavicencio Vásquez:** Writing – original draft, preparation, Investigation. **Diana Coello Montoya:** Writing – review & editing, Visualization, Investigation. **Galo Chuchoa Moran:** Writing – review & editing, Visualization, Investigation. **Luis Galarza Romero:** Writing – review & editing, Visualization, Investigation. **Ximena Yépez:** Writing – review & editing, Visualization, Investigation. **Rómulo Salazar:** Writing – review & editing, Visualization, Investigation. **María Romero-Peña:** Writing – review & editing, Supervision, Investigation. **Jonathan Coronel León:** Conceptualization, Project administration, Supervision, Investigation.



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