



## Review

## 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 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.

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**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.

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## 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 (Lampe, 1999; Randhawa et al., 2015; Williams, 1995). 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 et al., 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 provoking 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 *Salmonella* 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 et al., 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) found that “longer wash times (from 5 to 30 min) did not result in increased removal of 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 et al., 2011; Van Haute, Samper, 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 et al., 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 et al., 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 Commission, 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 et al., 2015). Different methods to reduce and/or replace the use of chlorine have already been 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 et al., 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. Biological-based methods

### 2.1. 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.,

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