

Neutral electrolyzed water (NEW), chlorine dioxide, organic acid based product, and ultraviolet-C for inactivation of microbes in fresh-cut vegetable washing waters

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Abstract

The effect of decontamination methods on fresh-cut vegetable washing waters was evaluated. NEW, ClO₂, organic acid-based product FPW, and UV-C were tested with and without an interfering carrot juice of 1% (IS), on *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, *Escherichia coli*, and yeast *Candida lambica*. The use of ClO₂ (50 ppm active chlorine) resulted in >4 log reduction of *Y. enterocolitica*, *Y. pseudotuberculosis*, *E. coli* and >3 log reduction of *C. lambica*. The antibacterial effect of NEW was less effective in the presence of IS when compared with ClO₂. The inactivation of *C. lambica* by FPW reached a maximum of 2.8 log cfu/mL (concentration 0.125%), but the antimicrobial effect was delayed by the IS. The effect of FPW on *E. coli* was significantly reduced by 1% IS. The inactivation of *E. coli* and *C. lambica* with UV-C IS decreased the inactivation and lengthened its time. Filtration improved the effect of UV-C inactivation.

Practical applications

When chemical decontamination methods were used in fresh-cut vegetable processing, the presence of organic matter in process water increased the reaction times and the need for higher concentrations of the chemical decontamination and the time of physical decontamination. *Yersinia* required longer inactivation times than *E. coli*. When UV-C is used for decontamination of process waters, waters should be filtered to enhance the disinfection efficacy.

KEYWORDS

ClO₂, NEW, organic acid-based product, UV-C, vegetable washing water

1 | INTRODUCTION

Fresh-cut vegetables have caused several epidemics (Monaghan, Thomas, Goodburn, & Hutchison, 2009; Olaimat & Holley, 2012). Contamination with pathogens can occur anywhere in the food chain (Berger et al., 2010; FDA, 2001). In many industrialized countries, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* have caused infections in humans. For example raw carrots have been traced back as sources of a number of extensive *Y. pseudotuberculosis* outbreaks in the 2010 decade, and a large number of sporadic infections have been caused primarily by *Y. enterocolitica* (Hallanvuori, 2009). Also *Y. enterocolitica* O:9 caused an outbreak of illness that was linked to ready-to-eat salad mix (MacDonald et al., 2012). Previous published results concerning *Yersinia* are few. Preventing cross-contamination of fresh-cut produce is essential in maintaining the microbiological safety and quality

of these commodities (EC-SCF, 2002; EFSA, 2013; López-Gálvez, Gil, Truchado, Selma, & Allende, 2010; Sapers, 2003). It is very important to promote hygiene throughout the entire production chain, including processing (Lehto, Kuisma, Määttä, Kymäläinen, & Mäki, 2011).

The processing plants of ready-to-eat, fresh-cut vegetables consume a significant amount drinking water in the course of the washing and rinsing of vegetables and their processing (Lehto, Sipilä, Alakukku, & Kymäläinen, 2014). Raw material is generally washed with cold water of a temperature between 4°C and 12°C, because low temperatures retard plant respiration, transpiration, warming and microbial activity (Nicola, Tibaldi, & Fontana, 2009). Reusing or recirculating of washing water increases the importance of the issue of decontamination. Decontamination of produce washing waters are one way to ensure product safety (Banach, Sampers, Van Haute, & van der Fels-Klerx, 2015; Gil, Selma, & López-Gálvez Allende, 2009), because inadequacies

in water control can cause food safety problems in fresh produce (Holvoet, Jacxens, Sampers, & Uyttendaele, 2012). In the fresh-cut industry various chemical, physical and biological disinfection methods and technological means of their applications for disinfection of the processing water as well as the product themselves have been studied (Gil et al., 2009; Gómez-López, 2012; Goodborne and Wallace, 2013; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). Disinfection processes may alter the physiochemical quality of water by the presence of disinfection residuals, the build-up of toxic by-products or modification of organic content (Chaidez et al., 2012; EPA, 2011). Chlorine-based chemicals are probably the most widely used sanitizers in the fresh-cut industry. In some European countries the use of chlorine in ready-to-use products is prohibited (Rico et al., 2007). Sanitizing methods such as ultraviolet radiation (UV), chlorine dioxide and electrolysed oxidising water have been shown to be more effective against microorganisms present in water than against those attached to vegetable produce surfaces (Sapers, 2003). For example, UV and ozonation or their combination has been shown to be effective in the disinfection of processing water and wastewater (Selma, Allende, López-Gálvez, Conesa, & Gil, 2008; Wert, Rosario-Ortiz, Drury, & Snyder, 2007). Sanitizing agents should be used to prevent the cross-contamination (Banach et al., 2015; Gil et al., 2009).

UV-C (254 nm) is widely used in food applications due to its antimicrobial capacity and cost efficiency. UV-C is easy to use, cheap, and lacks residues (Ben-Yehosua & Mercier, 2005; Ignat, Manzocco, Bartolomeoli, Maifreni, & Nicolii, 2015) as toxic or nontoxic by-products (Keyser, Müller, Cilliers, Nel, & Gouws, 2008). However, its disinfection efficiency is limited by dissolved organics and inorganics, the turbidity and color of the water, which prevent the penetration of light and inactivation of microorganisms (Gómez-López, 2012). Unlike chlorine, the efficacy of UV for disinfection is largely independent of water temperature and pH (EPA, 2011).

Neutral electrolysed water (NEW) can be produced by different methods and its pH can vary between 5 and 8. Compared with electrolysed oxidizing water EOW (pH <3), NEW has less of a corrosive effect, and it does not lose as much chlorine (Al-Haq & Gómez-López, 2012; Ayebach & Hung, 2005).

Chlorine dioxide (ClO₂) is a strong oxidant which has several advantages over chlorine: 2.5 times the oxidation capacity, lower reac-

tivity with organic matter and high effectiveness at low concentrations (Beuchat, Adler, & Lang, 2004). ClO₂ has been shown to have efficacy on a wide variety of microorganisms and viruses (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009).

Commercial organic acid-based products have been tested on processed lettuce. Lactic acid (2.5 g/L) alone or in combination with UV-C effectively reduced microbial counts (Nogales-Delgado, Fernández-León, Dekgado-Adámez, Hernández-Méndez, & Bohoyo-Gil, 2012). In the study by Hellström, Kervinen, Lyly, Ahvenainen-Rantala, and Korkeala (2006) a commercial citric acid-based produce wash at 0.25% was as effective as 100 ppm chlorinated water against *L. monocytogenes*. Akbaz and Ölmez (2007) showed that dipping of iceberg lettuce in 0.5% lactic or citric acid solution for 2 min was as effective as 100 ppm chlorine for reducing numbers of *E. coli* and *L. monocytogenes*.

Although results from the research into the effect of different treatment methods on microbial of fresh-cut vegetables is available, the data concerning treatment of processing water is very scarce, in particular concerning the effect of treatments on *Y. enterocolitica* and *Y. pseudotuberculosis*. The aim of this study was to evaluate decontamination methods utilizing NEW, ClO₂, organic acids and UV-C, in washing waters of fresh-cut vegetables, specifically on *Y. enterocolitica* and *Y. pseudotuberculosis*, *E. coli* and *Candida lambica*. The microbes tested were selected as representatives of potential pathogens (*Yersinia* and *E. coli*) or as spoilage organisms (yeast *C. lambica*) that could contaminate vegetables.

2 | MATERIALS AND METHODS

The efficiency of decontamination methods on processing water was studied by means of two methods, suspension tests (EN1276, 1998) and the test of industrial washing water. The tested decontamination treatments are shown in Table 1. First, suspension tests on pure cultures of microbes were conducted with and without 1% of sterile carrot juice as an interfering substance (IS) at a low temperature between 5 and 10°C. Suspension tests are useful for indicating general disinfectant efficacy (Holah, 2014). Second, washing water from the carrot processing company was tested with UV-C with and without filtration in a laboratory experiment. Available chlorine in NEW and ClO₂ stock

TABLE 1 Decontamination method of water used in suspension tests

Treatment (abbreviation)	Product, manufacturer/supplier	Concentrations
Neutral electrolyzed water (NEW)	NEW, XerChem Oy and Envirolite Finland Oy	30, 50, 100 ppm free chlorine ^b
Commercial wash ^a (FPW)	Fresh Produce wash™ (FPW) 100-1 dilution, Forsfood Oy	0.125, 0.25, 0.5% dilutions
UV-C (254 nm)	Wedeco 4 UV ₂₅₄ Spektrotherm® lamp and Cintropur TIO-UV ECO lamp, 600 W, Ozair Oy	–30 mJ/cm ²
UV-C (254 nm + filter 150 µm)	TIO-UV ECO, Ozair Oy	-
Chlorine dioxide (ClO ₂)	ClearKlens® Bi-Spore 250 ppm ClO ₂ , Sealed Air	10, 50, 100 ppm free chlorine ^b

^aCommercial citric acid-based produce wash™ (FPW).

^bThe available chlorine was analyzed by titration with 0.1 M sodium thiosulphate (Tamime, 2008).

solutions was determined by titration with 0.1 M sodium thiosulphate (Tamime, 2008). The pH of all solutions was measured.

The UV-C treatment was applied using a Wedeco A4 Spectrotherm® lamp (suspension test) or by a TIO-UV ECO 2000 lamp (processing water). In the suspension test, UV-C treatment alone was used, whereas in the processing water test it was used both alone and in combination with a filter. The commercial product Fresh Produce Wash™ (FPW) at a 100–1 dilution contains sucrose esters (E473), sodium citrate (E331) and glycerol (E422). The manufacturer recommends a concentration of 0.25%. NEW was supplied in the form of a concentrate by XerChem Oy and Envirolyte Finland Oy. ClO₂ was prepared from ClearKlens® Bi-Spore (Sealed Air), which is a 250 ppm ClO₂ generating system.

2.1 | Suspension tests

Pure cultures of three Gram-negative bacteria and one yeast (*Candida lambica* VTTC-00360) were inoculated into growth media from stock cultures stored at –70°C (Table 2). *Yersinia*, *E. coli* and *C. lambica* were grown at 30, 37, and 25°C, respectively for 18 hr before the test. Suspensions of the test organisms were prepared, containing 10⁷–10⁸ cfu/mL. The numbers of colony-forming units (cfu) were determined by cultivation of serial decimal dilutions on plate count agar (PCA). The suspensions were diluted to contain approximately 10⁵cfu/mL in test solution at the beginning of the test.

The test solutions were prepared using sterile, moderately hard water (EN1276, 1998). As an interfering substance (IS), carrot juice, dry matter content 8.6%, pressed from carrots and sterilized at 121°C for 15 min, was used. The test reagents were kept at a low temperature in an ice water bath, the temperature of which ranged from 5 to 10°C. The tests on NEW, FPW and ClO₂ were conducted in 50 ml plastic centrifuge tubes in which the total volume of reagents was 30 ml. UV-C treatment was conducted in a 5 L container in which water was circulated with a pump. 1 ml samples for the measurement of total colony counts were added to 9 ml of Ringer's 1/4-strength solution which contained 0.5% of 0.1 M sodium thiosulphate solution as a neutralizer. In the test of UV-C-treatment, no neutralizer was used in dilution solution. After 5 min, 1 mL was applied to petri dishes for the determination of colony counts. Suspension tests on FPW and UV were done on *E. coli* and *C. lambica*, whereas NEW and ClO₂ were also tested on *Yersinia*.

TABLE 2 Microbes used in the suspension test and their growth media

Microbe	Medium
<i>Y. enterocolitica</i> EELA 56	BHI (Brain Heart Infusion Broth, Difco 237500)
<i>Y. pseudotuberculosis</i> EELA 472	BHI
<i>E. coli</i> DSM 787 (= ATCC 11229)	PCA (Plate Count Agar, Difco 247940)
<i>C. lambica</i> VTTC-00360	PCA

2.2 | UV-C treatment of industrial processing water

The industrial processing water sample (30 L) was taken the day before the testing from a container in which whole carrots had been washed before they went to packaging machines. The water sample was stored overnight below 6°C. For the UV treatment, a 12 L sample was poured into 25 L container. A filter was placed in a container in which larger particles were first removed by centrifugal force and after that water was filtrated. The UV-lamp was integrated into the filter and placed in a sealed container. The water was continuously filtered through a 150 µm filter before UV-treatment and for comparison the water was circulated without a filter. The time for UV-C exposure was calculated from the time that the lamp was switched on, which took place after the water was circulated in the system for 5 min. Microbiological samples were taken before the water was circulated and periodically from the water container during the 30 min treatment.

3 | RESULTS

3.1 | Suspension tests

The logarithmic reduction was calculated by the following equation:

$$\text{Log reduction} = \log_{10}(N/N_0),$$

where N is the colony count after treatment and N₀ is the initial colony count.

The test solutions were prepared so that the 5 log reduction could have been detected. *C. lambica* in suspensions the cell concentration was lower, around 3 log cfu/mL. The activity of NEW was measured as the amount of active chlorine in the stock solution. Three dilutions, at differing strengths 30, 50 and 100 ppm active chlorine, were prepared. The limit of quantitation (LOQ) in figures shows the lowest detectable log cfu/mL of the test organism reliably measured in test solution. The highest concentration 100 ppm efficiently killed the bacteria. The results showed (Figure 1a) that 50 ppm NEW inactivated *Y. pseudotuberculosis* in two minutes and in 5 min in the presence of IS. At the lower concentration of 30 ppm, inactivation took 5 and 15 min, correspondingly. A 5 log cfu/mL reduction of *Y. enterocolitica* was achieved by 30 ppm NEW in 2.5 min and in 5 min in the presence of IS (Figure 1b). *E. coli* (Figure 1c) was more sensitive than *Yersinia*, because it was inactivated in 1 min by 30 ppm NEW and in 3 min in the presence of IS. A 3 log cfu/mL reduction of *C. lambica* took 0.5 min; IS did not delay the effect (Figure 1d).

ClO₂ decreased *Y. enterocolitica*, *Y. pseudotuberculosis* and *E. coli* counts in water efficiently (>4 log cfu/mL reduction) but at 10 ppm of ClO₂ concentration IS impaired the effect (Table 3). Reaction time was also longer (0.5 min, 1.0 min and 0.5 min, respectively) (Table 3) in the presence of IS. In this test the reduction efficiency on *C. lambica* was weaker (less than 2 log), than on the other microbes examined. In the presence of IS at 10 ppm, the effect of ClO₂ was mild or showed no effect on *C. lambica*.

All FPW solutions reduced the numbers of *E. coli* by 5 log cfu/mL within 3 min. IS diminished the effect so that the maximum reduction was attained in less than 3 min: 4.2 log cfu/mL reduction for 0.5%

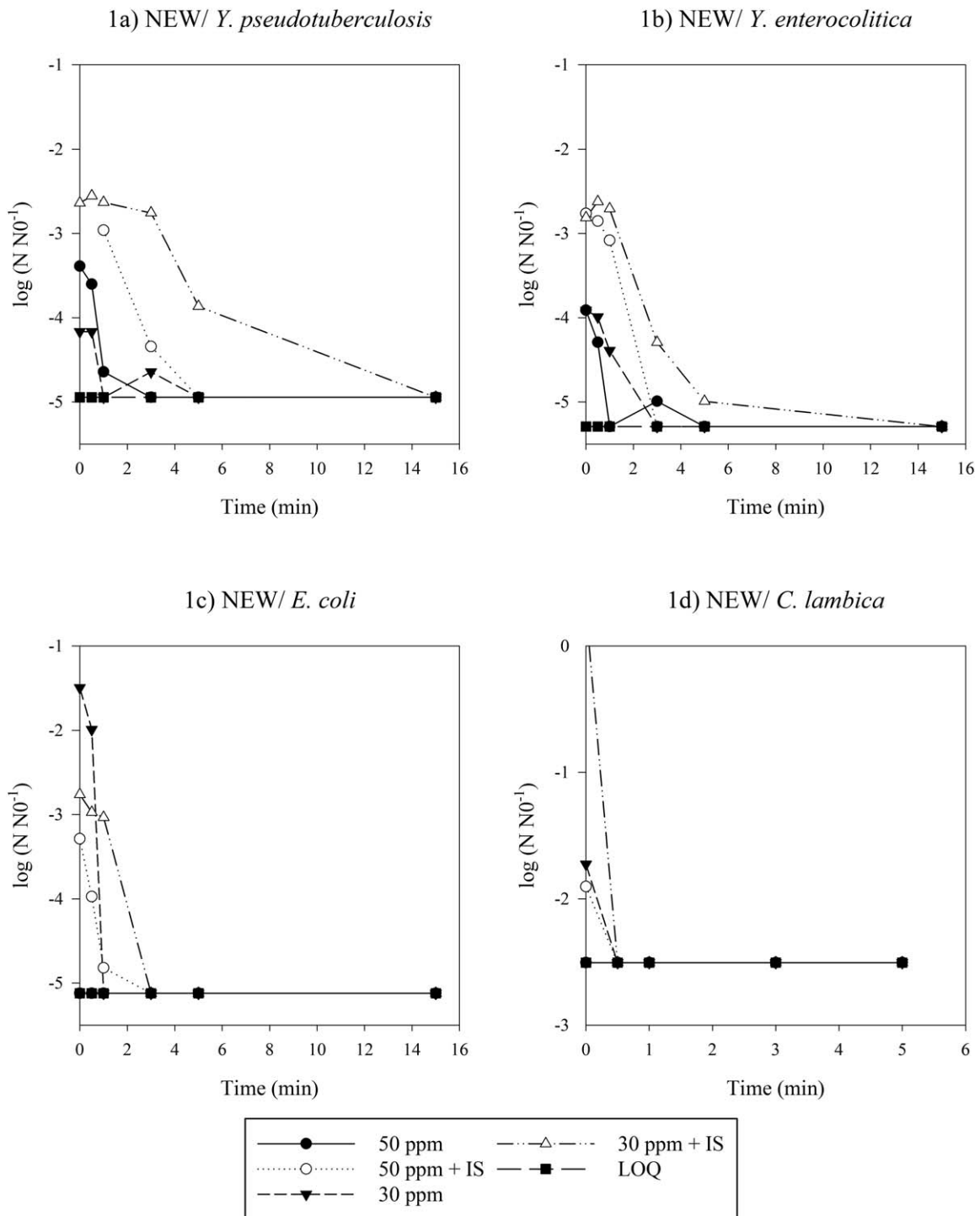


FIGURE 1 Log reduction of (a) *Y. pseudotuberculosis*, (b) *Y. enterocolitica*, and (c) *E. coli*, (d) *C. lambica* colony counts in water suspension during the exposure to NEW with or without the presence of interfering substance (IS, carrot juice). LOQ = limit of quantitation

FPW solution and 2.5 log cfu/mL reduction for 0.25 and 0.125% FPW solutions (Figure 2a). *C. lambica* was inhibited to the limit of detection (2.8 log cfu/mL), at all concentrations with or without IS, except for the 0.125% solution with IS, with which the maximum log reduction was achieved in 15 min (Figure 2b).

The effect of UV-C on logarithmic reductions of *E. coli* was more than 2 log cfu/mL in 5 min and 5 log cfu/mL in 15 min. The influence of the interfering substance was minor. The reduction of *C. lambica* counts

in water suspension treated with UV-C was 2.0 in 5 min and reached the limit of detection, 2.5 in 15 min. When the interfering substance was used, the respective reductions were 1.5 and 1.8 log cfu/mL (Figure 2c).

3.2 | UV-C treatment of industrial processing water

Before the beginning of the test, the total colony count of the processing water was 7.1×10^5 cfu/mL and pH 6.58. After 10 min, the colony

TABLE 3 Effect of chlorine dioxide (ClO₂) on the amounts of four microbes examined in water with or without the interfering substance (IS)

Strain	Concentration of ClO ₂ , ppm	IS 1%	Initial log (cfu ml ⁻¹)	Time, min			
				0	0.5	1	3
<i>Y. enterocolitica</i>	100	No	5.70	***	***	***	***
		Yes	5.70	***	***	***	***
	50	No	5.70	***	***	***	***
		Yes	5.70	**	***	***	***
	10	No	5.70	NE	***	***	***
		Yes	5.70	*	*	*	*
<i>Y. pseudotuberculosis</i>	100	No	5.38	***	***	***	***
		Yes	5.38	***	***	***	***
	50	No	5.38	***	***	***	***
		Yes	5.38	**	**	***	***
	10	No	5.38	*	**	***	***
		Yes	5.38	NE	NE	NE	NE
<i>E. coli</i>	100	No	5.60	***	***	***	***
		Yes	5.60	***	***	***	***
	50	No	5.60	***	***	***	***
		Yes	5.60	***	***	***	***
	10	No	5.60	*	***	***	***
		Yes	5.60	*	*	*	*
<i>C. lambica</i>	100	No	3.65	**	**	**	**
		Yes	3.65	**	**	**	**
	50	No	3.65	**	**	**	**
		Yes	3.65	**	**	**	**
	10	No	3.65	**	**	**	**
		Yes	3.65	NE	NE	NE	NE

*** > 4 log reduction, ** > 3 log reduction, * > 2 log reduction, NE = mild or no effect, less than 2 log reduction.

count decreased by 2.5 log cfu/mL using UV-C treatment and by 3.5 log cfu/mL when the UV-C was combined with filtration. After 30 min, the reductions were 2.5 and 5.0, respectively (Figure 3).

4 | DISCUSSION

The main purpose of this study was to evaluate the effect of chemical and physical decontamination methods on fresh-cut vegetable washing water. The evaluation was conducted by a modified standard method for testing disinfectants (EN 1276, 1998). The main challenges in this field of operation are low temperatures below 10°C, and organic material which both decrease the effect of the treatments.

According to our study the interfering carrot juice (IS) extended the reaction times in the suspension tests. The 50 ppm concentration of NEW inactivated *Y. pseudotuberculosis* in water after two minutes and in the presence of IS in five minutes. Reduction of 3.6 log cfu/mL

of *E. coli* took 1 min (NEW 30 ppm) and in the presence of IS, 3 min. A 3 log cfu/mL reduction of *C. lambica* took 0.5 min (30, 50, and 100 ppm); IS did not delay the effect. Publications concerning the bactericidal effect of NEW on *Y. pseudotuberculosis* and *Y. enterocolitica* in suspension tests in water were not found in the existing literature. In the study by Abadias, Usall, Oliveira, Alegre, and Viñas (2008) a NEW solution effectively reduced the numbers of *E. coli*, *Listeria innocua*, *Salmonella* and *Erwinia carotovora* at 48 or 89 ppm of free chlorine (>5 log cfu/mL) in water after 1 or 3 min of contact time, but at a lower concentration (28 ppm), smaller reductions were detected during the 5 min of contact time. Interestingly, the reduction was greater in cold water at 5 ± 2°C than at room temperature at 20 ± 2°C.

The commercial citric acid-based product FPW reduced the numbers of *E. coli* at the concentration of 0.25% or higher (>5 log cfu/mL), recommended by the producer, during the first minute of contact time when no IS was present. However, in the presence of IS, the effect on *E. coli* was reduced noticeably, from 1 to 3 log cfu/mL, and only the

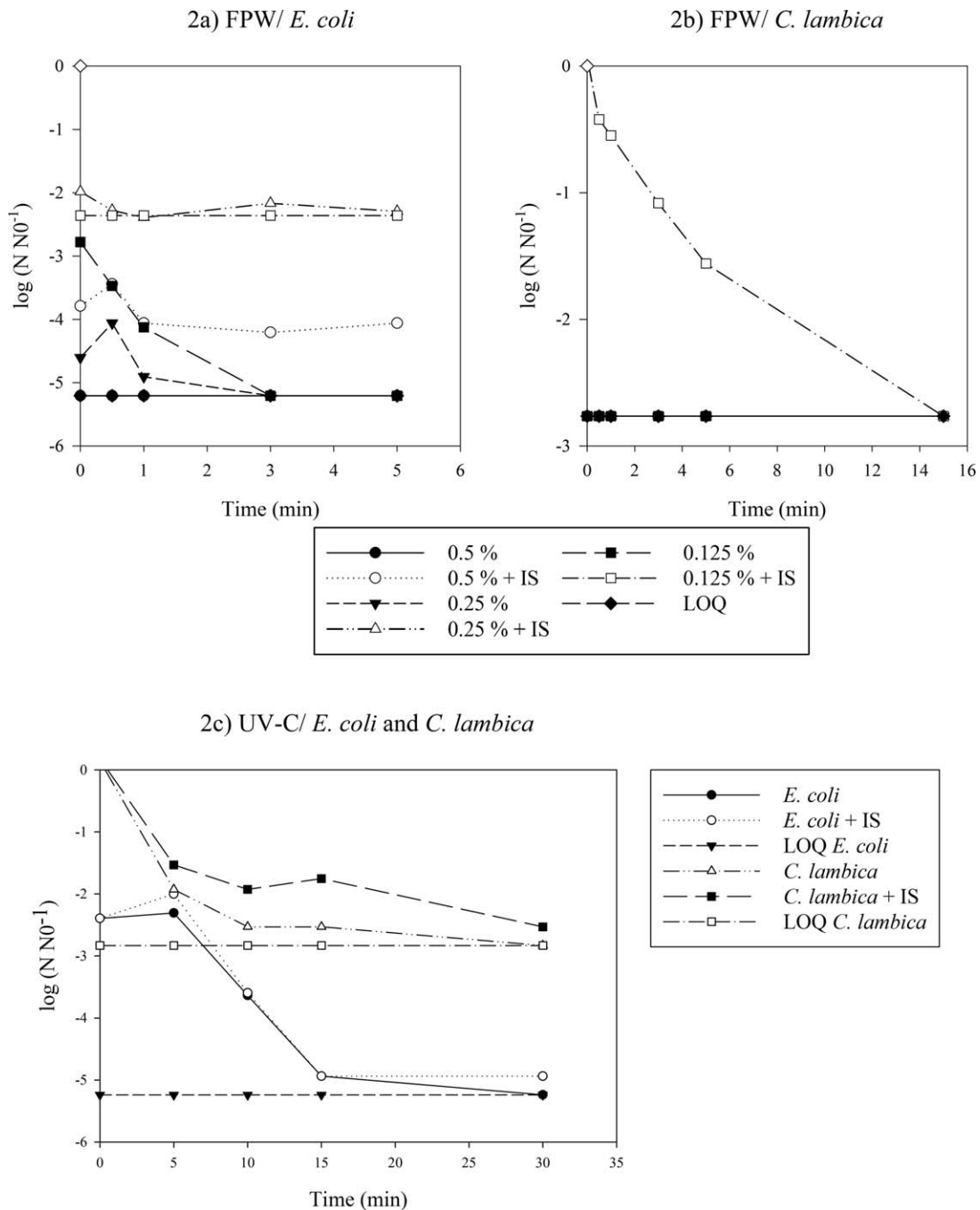


FIGURE 2 Log reduction of (a) *E. coli*, and (b) *C. lambica* colony counts in water suspension during the exposure to FPW or (c) UV365-light with or without the presence of interfering substance (IS, carrot juice). LOQ = limit of quantitation

highest concentration (0.5% + IS) showed over a 4 log cfu/mL reduction. As for *C. lambica*, the maximum reduction of 2.8 log cfu/mL was detected both without IS and in the presence of IS at the recommended concentration. At a concentration of 0.125%, IS delayed the reduction by 15 min. There are only few studies of similar products containing organic acids such as FPV. More generally, organic acids such as lactic, citric, malic and acetic acids, among others, are stable in high organic loading and are classified by the FDA as Generally Regarded As Safe (GRAS) (FDA, 2015a). The effect of citric and lactic acids on inactivation of *L. monocytogenes* and *E. coli* has been observed

to depend on time and temperature of exposition and on acid concentration (Virto, Sanz, Álvarez, Cordon, & Raso, 2006). In that study lactic acid was more effective than citric acid and *E. coli* was more sensitive to both acids than *L. monocytogenes*. In the study by van Haute, Uyttendaele, and Sampers (2013) weak organic acids in general were inefficient water disinfectants.

The reductions of *E. coli* and *C. lambica* after the UV-C treatment were 2 and 1.5 log cfu/mL (respectively) in 5 min and 5 and 1.8 log cfu/mL in 15 min when IS was used. The IS weakened the effect by 0–0.7 log. In our experiment the UV-C dose 0.3 kJ/m² used (reduction of

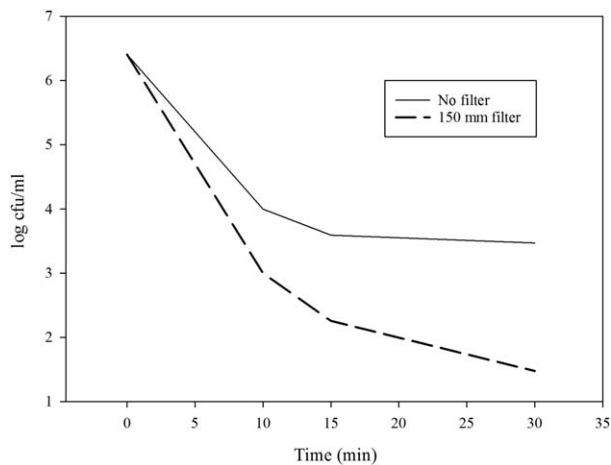


FIGURE 3 The effect of UV-treatment and UV + filtration on the total numbers of four microorganisms examined

5 log cfu/mL for *E. coli* in 15 min) showed similar results as that by Ignat et al. (2015), who obtained a 5 log cfu reduction for the most photoresistant bacteria (*E. coli*) at UV-C dose corresponding to 0.4 kJ/m² in the laboratory tests.

In our study ClO₂ produced a good (> 4 log) reduction of *Y. enterocolitica*, *Y. pseudotuberculosis* and *E. coli* after 0 or 0.5 min contact time at 100 and 50 ppm, both with or without IS. At 10 ppm, the effect was slower and was reduced by IS. The inhibition of *C. lambica* was not as clear due to the lower initial cell concentration. However, at 10 ppm the effect was not improved by a longer contact time. In the study by Petri, Rodriguez, and Garcia (2015) ClO₂ at concentration of 2 ppm was insufficient for maintaining the microbial quality (*E. coli*) of wash water in the washing process for both fresh-cut lettuce and shredded carrots.

According to the Finnish Food Safety Authority Evira UV-C is suitable for disinfection of processing water of vegetables (Kekki, 2013). We measured the effect of UV-C (273 nm) treatment and UV + filtration (150 μm) on the total numbers of microorganisms in carrot washing water (Figure 3). Filtering improved the disinfecting effect of UV-C. The total amount of solids in the water affected the effectiveness of the filtering. Selma et al. (2008) examined the treatment of washing water of vegetables with UV and ozone. The mesophilic reduction in onion washing water was 0.67 ± 0.1 log cfu/mL, and in escarole washing water 3.57 ± 0.3 log cfu/mL after 20 min. After 60 min treatments of escarole washing water, reductions of mesophilic bacteria, coliforms, and moulds were achieved using UV treatment 3.9, 2.8 and 1 log cfu/mL. In the present study a reduction of 2 and 2.5 log cfu/mL was achieved in 5 min, and 2 and 4 log cfu/mL in 15 min (UV-C and UV-C + filter, respectively).

In a standard suspension test, the disinfectant effect is currently calculated in terms of logarithmic reduction. The most widely accepted requirement is a microbicidal effect that equals or is greater than 5, which means that at least 99.999% of the germs have been killed (EN 1276, 1998; Reybrouck, 2007). However, the decontamination treatments typically decrease microbial populations by no more than 1–2 logs in microbial populations under laboratory conditions. Reductions

can be substantially smaller with commercial produce washing systems (Sapers, 2001). The lack of a standardized methodology and validation procedure makes it difficult to evaluate and select the most adequate disinfection method for fresh-cut produce (Gil et al., 2009).

Chlorine has generally been used in the fresh-cut industry in many countries, while in other countries its use is prohibited. According to Gómez-López, Lannoo, Gil, and Allende (2014) minimal chlorine doses (<7 mg/L) produce trihalomethane concentrations above the current standards for potable water. In many countries when chlorine is used, the trend is to eliminate it from the disinfection process because of harmful by-products (Ölmez & Krezschmar, 2009). NEW and ClO₂ contain chlorine as well, even if in quite low concentrations. The commercial product FPV contains organic acids and is classified as Generally Regarded As Safe (GRAS) by the FDA. Likewise, UV-C does not produce harmful chemical residues (FDA, 2008).

In the EU, there are no regulations concerning ClO₂ application in fresh-cut produce washing, but individual member states will be given the ability to establish enforcement levels at the national level until risk management can take place based on European Food Safety Authority (EFSA) scientific opinion and monitoring data (Banach et al., 2015; EFSA, 2014). In the United States, a ClO₂ concentration of 3 mg/L in water is the maximum allowable for contact with whole produce. Treatment of produce with ClO₂ must be followed by a potable water rinse or blanching, cooking, or canning (FDA, 2015b). In addition, worker safety requirements with regard to the amount of ClO₂ in workplace environments are subject to national regulations (Gómez-López, Rajkovic, Ragert, Smigic, & Devlieghere, 2009).

5 | CONCLUSION

The efficacy of NEW (neutral electrolysed water), chlorine dioxide, FPW (commercial product Fresh Produce Wash), and UV-C on the hygiene of vegetable washing water including several microbes, with and without an interfering substance (IS, carrot juice) was examined in this study. In most cases, the IS impaired the effect of the treatments, the reaction times were longer, and concentrations needed to be stronger to cause inactivation. The inactivation of *Y. pseudotuberculosis* in water was very slow with NEW, with a concentration of 50 ppm of free chlorine inactivation took two minutes, and in the presence of IS inactivation took five minutes to occur. Chlorine dioxide (concentration of 50 ppm of free chlorine) inactivated (>4 log cfu/mL) *Y. pseudotuberculosis* in one minute with the presence of IS. ClO₂ at 50 ppm was also effective in the reduction of *Y. enterocolitica*, *E. coli* and *C. lambica* in water, but when the ClO₂ concentration was 10 ppm, the interfering substance impaired the reducing effect. The numbers of *E. coli* reduced 2.5 log cfu/mL with the 0.25 FPW solutions with IS, and FPW 0.25% was good at inactivation of *C. lambica*. Inactivation of *E. coli* with UV-C took 15 min to occur and 2 log reduction of *C. lambica* took 10 min to occur. The reduction of the turbidity of the water by filtration was found to provide less shielding of the micro-organisms from the effects of disinfection chemicals and UV.

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