Crop Protection 146 (2021) 105679

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Chlorine dioxide, peroxyacetic acid, and calcium oxychloride for post-harvest decontamination of citrus fruit against *Xanthomonas citri* subsp. *citri*, causal agent of citrus canker

Franklin Behlau [a,*], Alexandre Paloschi [b], Tamiris G.S. Marin [a], Talita A. Santos [a], Henrique Ferreira [c], Lenice M. do Nascimento [d]

[a] Fundo de Defesa da Citricultura (Fundecitrus), Araraquara, SP, 14807-040, Brazil
[b] Coordenadoria de Defesa Agropecuária do Estado de São Paulo (CDA), Catanduva, SP, 15803-060, Brazil
[c] Departamento de Biologia Geral e Aplicada, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, 13506-900, Brazil
[d] Associação Brasileira de Citros de Mesa (ABCM), Leme, SP, 13610-070, Brazil

ARTICLE INFO

Keywords:
Sanitization
Sodium hypochlorite
Packing house
Lime
Orange
Lemon

ABSTRACT

After a new regulation on the control of citrus canker (*Xanthomonas citri* subsp. *citri*) came into force in Brazil in 2017, the post-harvest sanitization of citrus fruit became mandatory to prevent dissemination of the causal pathogen and enable the commercial trade of citrus to other states and countries. Although several sanitizers are available worldwide for decontamination of fresh produce, only sodium hypochlorite is legally required by the country for decontamination of citrus fruit traded with other states and countries against *X. citri* in the packing house. Thus, the objectives of this study were to assess the efficiency of chlorine dioxide, peracetic acid, and calcium oxychloride as alternative sanitizers and exposure times of 1 and 2 min for the post-harvest decontamination of citrus fruit against *X. citri*. Sodium hypochlorite, the legally required standard, was used as control. The sanitizers were evaluated regarding the capacity to eliminate live *X. citri* in solution immediately or 1 h after exposure to the products and the efficiency in disinfesting artificially and naturally contaminated lime fruit. An additional assessment was carried out to determine the infective potential of *X. citri* suspensions at different concentrations in order to evaluate the risk of disease dissemination by the remaining bacterial population on the fruit following treatment. All the assessed sanitizers were able to completely eliminate *X. citri* in suspension immediately after treatment. In artificially and naturally contaminated fruit, the sanitizers promoted a significant reduction of 2.4–2.8 and 1.1 to 1.5 $\log_{10}$ cfu/mL, respectively, in the population of live bacteria when compared to the untreated control. In these experiments, the amount of *X. citri* that remained on the fruit ranged from 0.5 to 1.0 and 0.1 to 0.3 $\log_{10}$ cfu/mL, respectively, regardless of the exposure time. The labeled concentrations and treatment periods assessed effectively decontaminated fruit with *X. citri* and demonstrated little or no risk of pathogen spread from treated fruit.

1. Introduction

Brazil is the largest citrus juice producer and exporter in the world (Neves et al., 2020), but accounts for a minor share in the global fresh citrus fruit market, where it ranks well below countries with less expressive citrus growing area, such as Argentina, South Africa and Spain (FAO, 2021; USDA, 2021a). Despite the minor participation in the global market, citrus ranks third in fresh fruit exports from Brazil (Abrafrutas, 2021) with a potential to increase in the near future. The main factors limiting citrus exports from the country relate to phytosanitary restrictions mostly resulting from the occurrence of black spot caused by *Phyllosticta citricarpa*, and citrus canker caused by *Xanthomonas citri* subsp. *citri* (Behlau and Belasque, 2014; Gottwald et al. 2009, 2020; Neves et al., 2010).

Required by the Brazilian Mistry of Agriculture and international trade partners, a new legislation, known as Normative Instruction 21 (IN21), was issued to regulate the control of citrus canker in Brazil after the disease become endemic in some citrus-producing states (Behlau, 2021; Brasil, 2018). For the first time in the country, IN21 allows trees with citrus canker to remain in orchards, but oblige the post-harvest

* Corresponding author.
E-mail address: franklin.behlau@fundecitrus.com.br (F. Behlau).

https://doi.org/10.1016/j.cropro.2021.105679
Received 19 February 2021; Received in revised form 23 April 2021; Accepted 24 April 2021
Available online 29 April 2021
0261-2194/© 2021 Elsevier Ltd. All rights reserved.

decontamination of fruit in order to make the commercialization of fresh citrus fruit viable between federation states and other countries (Behlau, 2021). Prior to the IN21, decontamination of fruit in packing houses was carried out by few growers that aimed specifically to reduce or inhibit the microbiota present on fruit surface in order to prolong produce shelf life (Narciso et al., 2005). The importance of decontamination of foods consumed fresh does not only relate to preventing the spread of plant pathogens but also to consumers' health and post-harvest stability and durability of the produce. In addition to pathogens that affect crops, fresh food surfaces may carry microorganisms that are pathogenic to people or may accelerate decay (Gómez-López et al., 2009). Therefore, despite incurring investments on infrastructure by growers, the IN21 that regulates citrus canker in Brazil has been contributing to an important development in the quality of products and processes in the whole production chain of fresh citrus fruit in Brazil.

The sanitizing products most commonly used worldwide to reduce surface microorganisms on harvested fruit and vegetables have chlorine as a component (Gómez-López et al., 2009; Mathew et al., 2018; Simons and Sanguansri, 1997). In conjunction with other workers' hygiene and disease prevention practices, chlorination is usually very effective, comparatively inexpensive, promptly available and may be implemented in operations of any size or scale. Since chlorine-based products have a contact rather than a systemic action against microorganisms, their application in packing houses should always be associated with sorting and discarding of fruit with injuries or cracks caused by mechanical action, insects or diseases, including citrus canker itself (Behlau, 2021; Brasil, 2018). In fruit wounds, lesions and parts protected by fruit wax, the biocide action of chlorine on microorganisms is low or inexistent, although it can be maximized by both the application of surfactants and prewashing to remove impurities before the decontamination process (Gottwald et al., 2009; Moretti, 2007).

Regardless of there being numerous bactericides of potential use in cleaning fresh food, the IN21 authorizes only sodium hypochlorite (NaClO) to decontaminate citrus fruit against *X. citri* in Brazil (Brasil, 2018). Sodium hypochlorite is one of the most used fresh fruit sanitizers in the world. Hypochlorite causes high microbial mortality due to the capacity to damage the outer membrane, which leads to loss of permeability and cell lysis (Beuchat, 1992; Venkobachar et al., 1977). This bactericide, however, poses important disadvantages such as the need for monitoring and maintaining the solution pH between 6.0 and 7.5, loss of efficacy in the presence of suspended solids and organic matter (Chen and Hung, 2017), and high potential to cause metal corrosion, thus reducing the lifetime of machineries and equipments used in the packing house (Ritenour et al., 2014). Moreover, sodium hypochlorite may be the precursor of toxic chlorinated compounds such as organic chloramines, trihalomethanes and dioxins, which are claimed to be harmful to human health due to their carcinogenic potential (López-Gálvez et al., 2010; Mcneal et al., 1995). Therefore, it is important to assess alternative sanitizers to decontaminate citrus fruit in packing houses. Recently, Zamuner et al. (2020) showed the potential of a cinnamaldehyde-based formulation as an alternative to sodium hypochlorite in the decontamination of fruit against *X. citri* at post-harvest. However, the use of that product has not yet been regulated in Brazil.

Other sanitizing formulations either based on chlorine or composed of different active ingredients are used in the decontamination against microorganisms in fresh fruit and vegetable production chains in different countries. Such products have been extensively characterized and approved in regard to toxicity, environmental impact and residues on treated surfaces, thus gathering credentials that qualifies them to be used against *X. citri* in packing houses, as substitutes or alternatives for sodium hypochlorite. Calcium oxychloride ($Ca(OCl)_2$), chlorine dioxide (ClO_2) and peracetic acid ($C_2H_4O_3$) stand out among those products (Aday and Caner, 2011; Alvaro et al., 2009 López-Gálvez et al., 2010; Mathew et al., 2018; Mishra et al., 2018; Pao et al., 2007; Srebernich, 2007; Zoellner et al., 2008).

Properties and applications of calcium oxychloride (also known as calcium hypochlorite) are similar to those of sodium hypochlorite. Upon being added to water, both form hypochlorous acid (HOCl), which has a high biocide capacity within a narrow pH range but loses efficacy in the presence of organic matter. Calcium oxychloride differs from sodium hypochlorite for being formulated in powder or tablets, which makes storage easier but requires stirring before use to fully dissolve in water (Mishra et al., 2018). Moreover, the calcium present in the formulation may contribute to a higher resistance of the treated surface to the post-harvest attack of microorganisms, thus prolonging shelf life (Martin-Diana et al., 2007; Poovaiah, 1986). Finally, the low cost is an important appeal for the use of calcium oxychloride in fresh food production chains (Sun et al., 2019).

Chlorine dioxide is a powerful disinfectant that gained more attention as food processing companies started to search for more efficient and less harmful products. This is the chlorinated bactericide with fastest expanding use to decontaminate fresh produce (Chen, 2017). The use of chlorine dioxide has been encouraged due to its 2.5 higher oxidation capacity, enabling the use of a lower product concentration (Sun et al., 2014), and the fact that it generates lower amounts of the supposedly carcinogenic by-products, as compared to sodium hypochlorite (Gómez-López et al., 2009; Hua and Reckhow, 2007; Kaur et al., 2015; Richardson et al., 2000). Moreover, chlorine dioxide in a gas formulation has the advantage of being more stable in the presence of organic matter, tolerating a wider solution pH range (5.0–10.0) and being less corrosive than the hypochlorite (Medir and Giralt, 1982; Sun et al., 2019). On the other hand, chlorine dioxide in aqueous formulations can be as corrosive as sodium hypochlorite (Kang et al., 2012; Sun et al., 2019).

Peracetic acid, also known as acetic acid peroxide or peroxyacetic acid, is a non-chlorinated sanitizing agent with antimicrobial power similar to or greater than that of sodium hypochlorite. Peracetic acid acts fast at low concentrations through the oxidation of cellular components of a wide spectrum of microorganisms (Kitis, 2004). Commercial peracetic acid-based formulations also have hydrogen peroxide in their composition, which also shows antimicrobial activity (Kitis, 2004). Once dissolved in water, peracetic acid does not lead to the generation of toxic residual substances in the solution. Its decomposition only produces acetic acid and oxygen, dispensing with the need of rinsing produce after treatments (Kitis, 2004). Contrary to chlorine, peracetic acid is not susceptible to peroxidases and is capable of maintaining its antimicrobial activity in the presence of organic residues (Hilgren et al., 2007; Srebernich, 2007). The pH of the peracetic acid solution may vary from acid to slightly alkaline and does not usually need to be adjusted (Kitis, 2004).

In addition to the sanitizer formulation, exposure time for decontamination against microorganisms is also an important factor in the process of cleaning citrus fruit. The current legislation (Brasil, 2018) determines that citrus fruit be treated with a solution of sodium hypochlorite for at least 2 min. That time is a reason for dispute and is considered by many growers to be exaggerated for significantly increasing the costs of the fruit cleaning process. The longer the treatment time required, the larger the size of the fruit immersion tank and consequently the necessary investment in infrastructure. Moreover, there are previous studies showing the effectiveness of bactericides in decontaminating oranges and grapefruits exposed only for 45 s or 1 min (Gottwald et al., 2009; Narciso et al., 2005).

Due to there being bactericides with proven efficiency against diverse microorganisms and to the imminent need for regulation by MAPA (Brazilian Department of Agriculture, Livestock and Food Supply) of alternative products and treatment period, this study was intended to assess the efficiency of different sanitizers and exposure times in the post-harvest decontamination of citrus fruit against *X. citri*, the causal agent of citrus canker.

2. Materials and methods

2.1. Assessed bactericides and X. citri growing conditions

The selection of the bactericides assessed in this study was based on the demand of the fresh citrus producers from São Paulo state and on previous studies that have shown the potential of diverse sanitizers to clean fruit and vegetables at post-harvest. Rate of each product tested was recommended by the manufacturers (Table 1). The study comprised four types of experiments: (i) assessment of the bactericidal capacity of the sanitizing solution against *X. citri*, (ii) assessment of the efficiency of the sanitizers in decontaminating citrus fruit artificially contaminated with *X. citri*, (iii) assessment of the efficiency of the sanitizers in decontaminating citrus fruit naturally contaminated with *X. citri*, and (iv) infective potential of *X. citri* suspensions of different concentrations.

The type strain *X. citri* 306 (IBSBF 1594) (Schaad et al., 2006) was used in all experiments. The isolate was previously grown on nutrient agar medium (NA, 3 g meat extract, 5 g peptone, 15 g agar and 1000 mL distilled water at pH 7) for 24 h at 28 °C. The concentration of bacterial suspensions was pre-adjusted to 10^8 ufc/mL using a spectrophotometer (OD = 0.3 at 600 nm) before obtaining suspensions at lower concentrations through serial dilutions to be used in the experiments.

2.2. Bactericidal capacity against X. citri

This experiment was carried out to assess the bactericidal capacity of different products against *X. citri* in a sanitizing solution. For that purpose, a suspension of *X. citri* at the final concentration of 10^6 colony forming units/mL (cfu/mL) was exposed to a solution of 50 mL in a Falcon tube containing one of the different sanitizers mixed with autoclaved tap water at the concentration recommended by the manufacturers (Table 1). Tubes were tilted and kept shaking for 1 h. Aliquots of 100 μL of the original solution and of dilutions 10^{-1} and 10^{-2} were plated on nutrient agar medium at 0 h (immediately after adding the bacteria) and 1 h after exposing the bacteria to the product. Colony forming units on plates were counted after 96 h incubation at 28 °C and the viable bacterial concentration remaining after exposure to each bactericide was estimated in $\log_{10}$ cfu/mL. A suspension of *X. citri* at the same concentration not exposed to a sanitizer product was used as control. There were five replications per treatment (tubes) and this experiment was conducted three times. The average pH of the water used in the three experiments was 5.8 ± 0.2. For solutions of calcium oxychloride, chlorine dioxide, peracetic acid and sodium hypochlorite, the average pH were 7.2 ± 0.5, 8.9 ± 0.2, 4.2 ± 0.1 and 6.8 ± 0.2, respectively.

2.3. Sanitation of citrus fruit artificially contaminated with X. citri

This experiment was carried out to assess the potential of the products to sanitize citrus fruit artificially contaminated in the laboratory with a high load of *X. citri* inoculum. The following treatments were carried out to that aim: sanitation through the exposure of the fruit to the sanitizing solution for (i) 1 min (half the time required by the legislation in force), (ii) for 2 min (legal requirement), and (iii) control, comprised of fruit not exposed to a sanitizing agent. There were five replications per treatment with three fruits per replication. This experiment used Tahiti limes, considering that the production of this species is highly representative to the domestic and foreign fresh citrus fruit markets. Trials were carried out with fruit of uniform diameter of approximately 50 mm, obtained from commercial orchards with no occurrence of citrus canker.

Table 1
Formulations and rates of sanitizers assessed for post-harvest decontamination of citrus fruit against *Xanthomonas citri* susbp. *citri*.

Sanitizer	Commercial name	Rate	
		Commercial product	Active ingredient (ppm)
Calcium oxychloride	Calcium oxychloride	0.3 g/L	200
Chlorine dioxide	Dioxiplus	1.0 mL/L	70
Peracetic acid	Peracetic	0.666 mL/L	100
Sodium hypochlorite	Pluron 444 A	1.7 mL/L	200

After harvest, each fruit was manually washed in water with the Wash Fruit 33 detergent (Aruá, Matão, SP Brazil) for 30 s at the concentration recommended by the manufacturer, then rinsed in autoclaved tap water and dried at room temperature on brown paper sheets for approximately 2 h. After drying, fruit was contaminated by sprinkling (four sprays homogeneously distributed around each fruit) with a suspension of *X. citri* at 10^7 cfu/mL.

After being contaminated with *X. citri* and dried for approximately 2 h, the 3 fruits of each replication were immersed for 1 or 2 min in 2 L of the sanitizing solution prepared in tap water at the recommended rate (Table 1). A fresh solution was used for each replication. Fruit immersed only in autoclaved tap water for 2 min served as control. After treatment, fruit was placed on brown paper and let dry at room temperature for approximately 2 h. Next, fruit of each replication was washed by being shaken inside plastic bags containing 100 mL of 0.1 M phosphate buffer solution (pH 7.2) for 5 min. Fruit was disposed after being washed. The washing suspension was collected, placed inside two 50 mL Falcon tubes and subsequently centrifuged for 20 min at 10,000×*g*. The pellet of each replication was resuspended in 3 mL of 0.1 M phosphate buffer solution for further assessment of the viable *X. citri* population through plating. Plating was performed with the use of 100 μL of the original suspension and of dilutions of 10^{-1} and 10^{-2} on nutrient agar medium. After 96 h of incubation at 28 °C, *X. citri* colonies on plates were counted and the concentration of *X. citri* remaining on the fruit was estimated (Gottwald et al., 2009). This experiment was conducted three times.

2.4. Sanitation of fruit contaminated with X. citri harvested in orchards with citrus canker

This experiment was carried out to assess the potential of products to sanitize asymptomatic fruit naturally contaminated by *X. citri*. For that purpose, the same treatments of the previous experiment were performed. There were five replications per treatment with three fruits per replication. Tahiti limes of uniform diameter of approximately 50 mm obtained from commercial orchards with the occurrence of citrus canker were used.

In order to increase contamination with *X. citri*, fruits with and without symptoms of citrus canker were mixed together in plastic bags at a rate of 2:1 (2 fruits with symptoms to 1 fruit without symptoms), spray-moistened with autoclaved tap water and kept in a moist bag at room temperature for 24 h to favor bacterial exudation. Next, fruit with symptoms were disposed and asymptomatic fruit were placed on brown paper and let dry for 2 h. After drying, fruits were sanitized and the washing suspension was plated as described before for the previous experiment. This experiment was conducted three times.

2.5. Infective potential of X. citri suspensions at different concentrations

In order to assess the potential of the population of *X. citri* remaining on the fruit treated with bactericides to cause citrus canker, suspensions of *X. citri* were inoculated at concentrations of 10^0, 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6 cfu/mL. For each concentration, 30 young leaves (3/4 expansion) of three Hamlin sweet orange (highly susceptible to citrus canker) potted trees (10 leaves per tree) kept in greenhouse were inoculated. Inoculation was performed by the infiltration with the use of a needleless syringe of 500 μL of bacterial suspension into each leaf

through the abaxial surface (~250 μL/side of the midrib). Assessments were made 30 days after inoculation by counting the number of leaves with symptoms and the number of lesions within in a fixed area of 3.5 cm^2 per leaf (two fully infiltrated circular areas with 1.5 cm diameter centered at the infiltration sites, one in each side of the leaf), later converted into number of lesions/cm^2 leaf. This experiment was conducted three times.

2.6. Data analysis

For each bactericide assessed, the amounts of *X. citri* recovered on culture medium from artificially and naturally contaminated fruit after exposure times of 1 and 2 min and without exposure to the products were subjected to a one-way analysis of variance (ANOVA) using the treatment means from each of the three experiments. The mean bacterial amounts recovered were compared using Tukey's HSD test ($\alpha = 0.05$). Statistical analysis were performed using R version 4.0.2. For the experiment that assessed the infective potential of *X. citri* suspensions at different concentrations, the standard error of the mean among experiments of the incidence of leaves with citrus canker and the average number of lesions/cm^2 leaf were calculated.

3. Results

Solutions of all sanitizers studied were able to completely eliminate viable *X. citri* cells immediately after the contact with the bacterial suspension (0 h) or after 1 h exposure to the each product. No cfu of *X. citri* were recovered from solutions containing different bactericides after exposure for the two treatment times evaluated. On the other hand, the control, which contained only autoclaved tap water, resulted in the recovery of 6.4 ± 0.2 and 5.8 ± 0.5 $\log_{10}$ cfu/mL of *X. citri* after 0 or 1 h treatments, respectively.

The bactericides assessed exhibited a similar performance and caused significant reduction in the *X. citri* population on fruit artificially contaminated after 1- or 2-min treatment. For the treated fruit, regardless of the exposure time, the amount of *X. citri* recovered varied from 0.5 to 1.0 $\log_{10}$ cfu/mL. Those figures represent a reduction of 2.0 to 3.0 $\log_{10}$ cfu/mL in the quantity of viable cfu as compared to the control (Fig. 1 a-d). There was no significant difference between the number of *X. citri* cells recovered after exposition to the sanitizers for 1 or 2 min (Fig. 1 a-d). The performance of the bactericides in cleaning fruit from orchards contaminated with citrus canker was similar to that observed for fruit contaminated in the laboratory. There was no discrepancy among the products, which promoted a decrease in the *X. citri* population from 1.3 to 1.8 to 0.1–0.3 $\log_{10}$ cfu/mL in fruit naturally contaminated, regardless of the time of exposure to the sanitizers (Fig. 2 a-d).

Inoculation of *X. citri* into orange leaves showed that concentrations below 10^3 cfu/mL are not capable of causing significant number of citrus canker lesions. Suspensions of *X. citri* at concentrations ranging from 10^3 to 10^6 cfu/mL caused lesions in 68.9–100% of inoculated leaves (Fig. 3a). Concentrations of 10^3 and 10^4 cfu/mL produced 2.3 and 10.4 lesions/cm^2, respectively (Fig. 3b). The inoculation of suspensions of 10^5 and 10^6 cfu/mL evolved into a countless number of lesions (Fig. 3b). On the other hand, no lesions were seen on leaves inoculated with *X. citri* suspensions at the concentrations of 10^0 and 10^2 cfu/mL (Fig. 3 a,b). Inoculations with an *X. citri* suspension at 10^1 cfu/mL produced only one lesion on a single leaf in one experiment, resulting in an average incidence of affected leaves of 1.1% and 0.1 lesion/cm^2 (Fig. 3 a,b).

4. Discussion

The present study showed that sanitizers based on chlorine dioxide, calcium oxychloride and peracetic acid, in addition to the benchmark sodium hypochlorite, were capable of completely eliminating viable *X. citri* immediately after the bacterial suspension was added to the solution. Moreover, the assessed bactericides were equally efficient in sanitizing post-harvest limes contaminated with *X. citri*. In general, the sanitizers tested were capable of reducing the recovered *X. citri* population by up to ~3.0 units of $\log_{10}$. Levels of reduction of *X. citri* populations upon post-harvest fruit decontamination were similar to those obtained in previous studies. Gottwald et al. (2009) showed a reduction of approximately 2.0–2.5 units of $\log_{10}$ cfu/mL in the *X. citri* population recovered from the surface of asymptomatic grapefruit harvested from orchards with citrus canker, when treated with sodium hypochlorite at 200 ppm for 45 s. In that case, and also as observed by Al-Saleh and

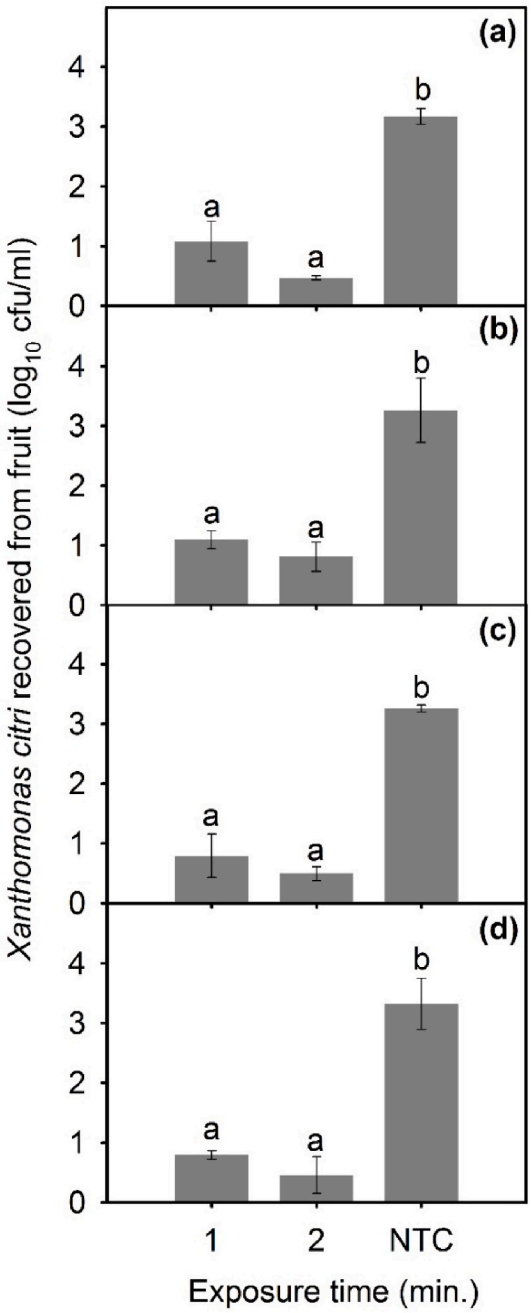


Fig. 1. Amount of *Xanthomonas citri* subsp. *citri* recovered from Tahiti limes artificially spray-contaminated in the laboratory followed by sanitation for 1 or 2 min with calcium oxychloride (**a**), chlorine dioxide (**b**), peracetic acid (**c**) or sodium hypochlorite (**d**). NTC, non-treated control. cfu, colony forming unit. For each bactericide, columns followed by the same letter did not differ statistically by the Tukey's test ($P = 0.05$). Error bars indicate the standard error of the mean of three independent experiments.

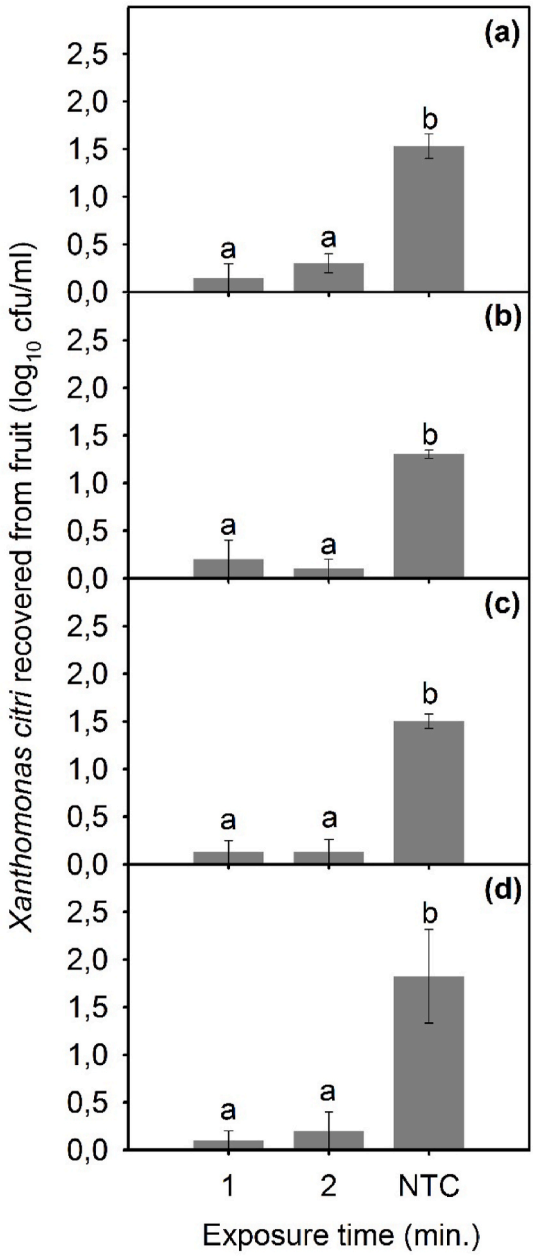


Fig. 2. Amount of *Xanthomonas citri* subsp. *citri* recovered from Tahiti limes harvested from orchards where citrus canker is endemic followed by sanitation for 1 or 2 min with calcium oxychloride (**a**), chlorine dioxide (**b**), peracetic acid (**c**), or sodium hypochloride (**d**). NTC, non-treated control. cfu, colony forming unit. For each bactericide, columns followed by the same letter did not differ statistically by the Tukey's test ($P = 0.05$). Error bars indicate the standard error of the mean of three independent experiments.

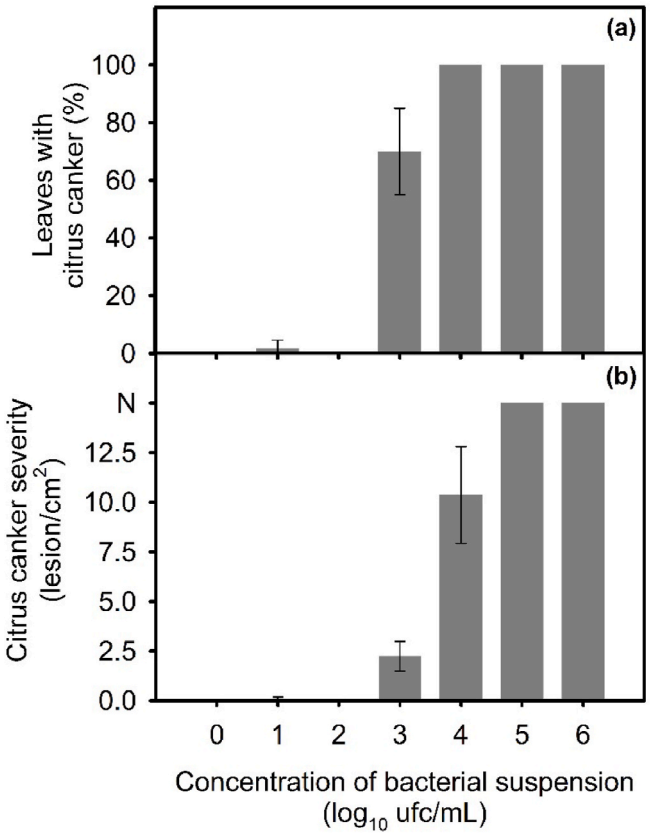


Fig. 3. Incidence of leaves of Hamlin sweet orange with citrus canker (**a**) and number of citrus canker lesions/cm^2 leaf (**b**) 30 days post infiltration-inoculation of *Xanthomonas citri* subsp. *citri* suspensions at different concentrations in the greenhouse. Error bars indicate the standard error of the mean of three experiments. N, uncountable number of lesions. Each leaf was inoculated with 500 μL of bacterial suspension (250 μL/side of the midrib) and a fixed area of 3.5 cm^2 per leaf (two fully infiltrated circular areas with 1.5 cm diameter centered at the infiltration sites, one in each side of the leaf) was used to estimate the number of lesions per leaf area.

Ibrahim (2010), best results were obtained when the treatment with sodium hypochlorite was preceded by washing the fruit to remove impurities.

Sodium hypochlorite is historically the most used substance in the world for the decontamination of fruit and vegetables and the only regulated sanitizer in Brazil for cleaning fresh citrus fruit at post-harvest (Behlau, 2021; Brasil, 2018). In addition to the present study, several others have shown the efficiency of sodium hypochlorite in the decontamination of citrus fruit against *X. citri* and other contaminating microorganisms (Al-Saleh and Ibrahim, 2010; Gottwald et al., 2009; Narciso, 2005; Martinez et al., 2014). Despite being efficient, important disadvantages such as active chlorine easily complexing with organic molecules, the constant need to adjust the solution pH, the corrosive capacity and resulting equipment depreciation, and secondary toxic substances forming in the presence of organic matter (Chen and Hung, 2017; López-Gálvez et al., 2010; Mcneal et al., 1995; Ritenour et al., 2014) have encouraged the assessment of alternative sanitizers to be used in cleaning fresh citrus fruit throughout the country.

As an alternative to liquid formulations, calcium oxychloride as a sanitizer was assessed in this study which resulted in a reduction of up to 2.7 $\log_{10}$ cfu/mL in the viable population of *X. citri* on Tahiti limes. Among the assessed bactericides, calcium oxychloride is the least researched as a fruit and vegetable cleaning agent. There are no reports on the efficiency of calcium oxychloride to decontaminate fruit against *X. citri*. In the US, this sanitizer has been used to treat seeds of alfalfa and mung bean used to produce edible sprouts against *E. coli* and *Salmonella* (Fett, 2002; Suslow et al., 2002). There are also studies, though scarce, on the use of calcium oxychloride in other production systems, such as treating fresh-cut lettuce and diced tomatoes (Simmons and Beuchat, 2006). Although calcium oxychloride and sodium hypochlorite have the same disadvantages, these chlorinated sanitizers are still an important option in cleaning fresh food all over the world especially due to their low cost and to the lack of definite evidence on the potential risk that residual substances they produce represent to health when they are used according to recommended concentrations and conditions.

Driven by its high biocide activity and the fact of being a safe and residue-free food disinfectant and preservative, chlorine dioxide has

been increasingly applied in sanitizing fresh food in the last decades. At the same rate, the volume of information on the use of that sanitizer in different production systems has become more widely available. Particularly in the present study, chlorine dioxide was able to reduce the viable population of *X. citri* by up to 2.4 $\log_{10}$ cfu/mL. Such reduction is similar to the results of this study on sodium hypochlorite and to that observed by Sun et al. (2016) in grapefruit treated with 20 ppm of chlorine dioxide. That same study, which is the only one available in the literature on the use of chlorine dioxide against *X. citri* on citrus fruit, also showed that this sanitizer was able to completely eliminate natural contamination by *X. citri* on fruit when used at the concentration of 60 ppm. Chlorine dioxide was also shown to be effective against other common contaminating microorganisms on citrus fruit, such as *Geotrichum candidium* (Brown and Wardowski, 1986) and *Penicillium digitatum* (Liu et al., 2020; Sun et al., 2016).

Peracetic acid is a non-chlorinated sanitizer that just like chlorine dioxide has been increasingly more used in decontaminating diverse fresh fruit and vegetables at post-harvest (Zoellner et al., 2008). In the current study, that sanitizer proved efficient in the decontamination of citrus fruit with the elimination of the viable population of *X. citri* in up to 2.8 $\log_{10}$ cfu/mL. However, despite being regulated and routinely used in some countries in the decontamination of citrus fruit against citrus canker causing bacteria at post-harvest, there are no previous studies describing the efficacy of peracetic acid for that purpose. Only technical documents (Ritenour et al., 2008; USDA, 2021b) and assessments of other microorganisms are available. Brown and Shubert (1987) inferred on the potential of that acid against citrus canker causing bacteria in tests carried out with bacteria of the same genus *X. campestris* pv. *vesicatoria*. Narciso (2005) reported that the treatment of oranges with peracetic acid at 100 ppm for 1 min was able to reduce the load of important fungi at post-harvest, namely *P. digitatum*, *Geotrichum citri-aurantii* and *Colletotrichum gloeosporioides* by up to 2.1 units of $\log_{10}$ cfu/cm^2. Besides reporting efficiency, the study mentioned above also showed an additional reduction of 0.9 $\log_{10}$ cfu/cm^2 in the contaminating microorganism population on fruit treated with peracetic acid in relation to treatment with sodium hypochlorite. That trend was also observed by Alvaro et al. (2009), who reported that peracetic acid was more efficient to prolong shelf-life of green peppers as compared to sodium hypochlorite.

The current study also showed that for both fruit contaminated with *X. citri* in the laboratory and fruit contaminated naturally there was no significant difference in the efficiency of the bactericides assessed for decontamination against *X. citri* at exposure times of 1 or 2 min. Satisfactory results were also obtained in previous studies on fruit decontamination against *X. citri* with exposure times of 45 s and 1 min to a solution containing peracetic acid and sodium hypochlorite (Gottwald et al., 2009; Narciso, 2005). Likewise, Brown and Shubert (1987) showed that the exposure time of 1 min of treatment of citrus fruit in a chlorine dioxide solution was enough to completely eliminate viable spores of *P. digitatum* and *G. candidium*. In some countries, such as the United States, immersion of citrus fruit in a sanitizing solution for a period shorter than 2 min is already regulated and in use (USDA, 2021b). Results from the present study corroborated previous research and evidenced the possibility of reducing the treatment time of citrus fruit against *X. citri* for all sanitizers assessed. This reduction will result in the possibility of use of a more compact and lower cost infrastructure by citrus growers at the packing house without risking treatment quality.

The efficiency of sanitizers against *X. citri* on citrus fruit should not necessarily be measured by the capacity of totally eliminating the bacteria, but rather by the pathogen population reduction on the treated surface to levels of no epidemiological importance. Similarly to previous research (Gottwald et al., 2009; Sun et al., 2016), this study has shown that, although the assessed bactericides are not able to completely eliminate the viable population of *X. citri* present on citrus fruit surface, the infective potential of the remaining bacteria was insignificant. Suspensions of *X. citri* at concentrations of 10^0 to 10^2 cfu/mL were not capable of leading to the development of citrus canker lesions. One exception was observed on leaves inoculated with a suspension of *X. citri* at 10^1 cfu/mL that resulted in only one symptomatic leaf with one single disease lesion in three experiments. It is important to emphasize that the population of *X. citri* remaining on the treated fruit was always lower than that concentration and the inoculation was performed by infiltration of the bacterial suspension directly in the foliar mesophyll, which is a more aggressive method than natural infections in the field. Other important factors to be considered are that the *X. citri* population recovered from cleaned fruit do not necessarily correspond to the *X. citri* population capable of causing disease (Gottwald et al., 2009) and that there is a natural decline in the bacterial population during storage and transportation. Martinez et al. (2014) showed that shipping time of a container from South America to Europe, where the disease is not present, is also capable of diminishing the viable *X. citri* population on treated asymptomatic lemons.

According to the IN 21 in force in Brazil, post-harvest cleaning of citrus fruit against *X. citri* can only be performed by "immersion in sodium hypochlorite solution at two hundred ppm, pH 7, for 2 min" (MAPA, 2018). However, the same legislation allows for the regulation of "other cleaning methods or products approved by research and recognized by the Department of Agriculture" (MAPA, 2018). In view of such concession and of the results obtained in this study, which corroborate previous studies referred to throughout this text, both the regulation of the assessed sanitizers and the reduction of the exposure time to decontaminate citrus fruit in post-harvest against *X. citri*, causal agent of citrus canker, are plausible. It is also noteworthy to highlight that the results of the present study do not apply only to limes. Taking into account the mode of action of the sanitizers and the similarity of both the symptomatology of the disease (Gottwald et al., 2002) and the morphology of the fruit peel among the different citrus species (Ladaniya, 2008), the use of the assessed products may be extended to other citrus fruits, which are important to the fresh market such as oranges, lemons, mandarins, grapefruits and tangors. More importantly, this study supports the use of the assessed sanitizers not only in Brazil, but also in other fresh citrus fruit producing countries.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to the Brazilian Association of Fresh Fruit (*Associação Brasileira de Citros de Mesa - ABCM)* for providing relevant information for the conduction of the study, to Fundecitrus for funding this study (project #031619) and to the National Council for Scientific and Technological Development - CNPq for the research productivity fellowships of F. Behlau (grant #309261/2016-3). The authors also knowledge the co-author Dr. Lenice M. Nascimento, who passed away recently, for her devoted and remarkable carrier as a researcher and consultant in post-harvest of fresh citrus fruit.

References

Abrafrutas, 2021. Estatística de exportações de frutas em 2019. associação Brasileira dos Produtores Exportadores de Frutas e Derivados, Brasília, DF. https://abrafrutas.org/2020/01/28/8825/. (Accessed 11 January 2021).

Aday, M.S., Caner, C., 2011. The applications of active packaging and chlorine dioxide for extended shelf life of fresh strawberries. Packag. Technol. Sci. 24, 123–136.

Al-Saleh, M.A., Ibrahim, Y.E., 2010. Population dynamics of *Xanthomonas citri* subsp. *citri* on symptomless citrus fruits under Saudi Arabia conditions and effect of post-harvest treatments on survival of the bacteria. J. Plant Pathol. 92, 601–605.

Alvaro, J.E., Moreno, S., Dianez, F., Santos, M., Carrasco, G., Urrestarazu, M., 2009. Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. J. Food Eng. 95, 11–15.

Behlau, F., 2021. An overview of citrus canker in Brazil. Trop. Plant Pathol. 46, 1–12. https://doi.org/10.1007/s40858-020-00377-2.

Behlau, F., Belasque Jr., J., 2014. Cancro cítrico: a doença e seu controle. Araraquara, São Paulo, Brazil, Fundecitrus.

Beuchat, L.R., 1992. Surface disinfecting of raw produce. Dairy. Food Environ. Sanitat. 12, 6–9.

Brasil, 2018. de maio de 2018. No. 90, Seção. Ministério da Agricultura, do Abastecimento e da Reforma Agrária (MAPA), Secretaria de Defesa Agropecuária. Instrução Normativa No. 21, de 25 de abril de 2018. Diário Oficial da União, Brasília, 11, vol. 1, pp. 9–12.

Brown, G.E., Wardowski, W.F., 1986. Use of chlorine dioxide in Florida citrus packing houses to reduce inoculum of decay pathogens. Citrus Ind. 67, 48–56.

Chen, X., Hung, Y.C., 2017. Effects of organic load, sanitizer pH and initial chlorine concentration of chlorine-based sanitizers on chlorine demand of fresh produce wash waters. Food Contr. 77, 96–101.

Chen, Z., 2017. A focus on chlorine dioxide: the promising food preservative. J. Exp. Food Chem. 3, e107.

FAO, 2021. Top 10 Country, Export Quantity of Lemons and Limes 2019. Internet Resource. The Food and Agriculture Organization. http://www.fao.org/faostat/en/#rankings/countries_by_commodity_exports/. (Accessed 11 January 2021).

Fett, W.F., 2002. Reduction of *Escherichia coli* O157:H7 and *Salmonella* spp. on laboratory-inoculated mung bean seed by chlorine treatment. J. Food Protect. 65, 848–852.

Gottwald, T., Graham, J.H., Bock, C., Bonn, G., Civerolo, E., Irey, M., Leite, R., McCollum, G., Parker, P., Ramallo, J., Riley, T., Schubert, T., Stein, B., Taylor, E., 2009. The epidemiological significance of post-packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit. Crop Protect. 28, 508–524.

Gottwald, T.R., Graham, J.H., Schubert, T.S., 2002. Citrus canker: the pathogen and its impact. Plant Health Prog. https://doi.org/10.1094/PHP-2002-0812-01-RV.

Gottwald, T.R., Taylora, E.L., Amorim, L., Bergamin Filho, A., Bassanezi, R.B., Silva Jr., G.J., Fogliata, G., Fouriee, P.H., Grahamg, J.H., Hattingheh, V., Krissi, A.B., Luoaj, W., Magareyj, R.D., Schuttee, G.C., Spósito, M.B., 2020. Probabilistic risk-based model for the assessment of *Phyllosticta citricarpa*-infected citrus fruit and illicit plant material as pathways for pathogen introduction and establishment. Crop Protect. 142, 10552.

Gómez-López, V.M., Rajkovic, A., Ragaert, P., Smigic, N., Devlieghere, F., 2009. Chlorine dioxide for minimally processed produce preservation: a review. Trends Food Sci. Technol. 20, 17–26.

Hilgren, J., Swanson, K.M., Diez-Gonzalez, F., Cords, B., 2007. Inactivation of *Bacillus anthracis* spores by liquid biocides in the presence of food residue. Appl. Environ. Microbiol. 73, 6370–6377.

Hua, G., Reckhow, D.A., 2007. Comparison of disinfection byproduct formation from chlorine and alternative disinfectants. Water Res. 41, 1667–1678.

Kang, Z., Wang, K., Zhao, H., Wu, H., Lu, N., Sun, Y., 2012. Corrosion of metal in chlorine dioxide disinfection solution. Corrosion Protect 12, 92–93.

Kaur, S., Smith, D.J., Morgan, M.T., 2015. Chloroxyanion residue quantification in cantaloupes treated with chlorine dioxide gas. J. Food Protect. 78, 1708–1718.

Kitis, M., 2004. Disinfection of wastewater with peracetic acid: a review. Environ. Int. 30, 47–55.

Ladaniya, M.S., 2008. Citrus Fruit: Biology, Technology and Evaluation. Academic Press, San Diego, CA.

Liu, X., Jiao, W., Du, Y., Chen, Q., Su, Z., Fu, M., 2020. Chlorine dioxide controls green mold caused by *Penicillium digitatum* in citrus fruits and the mechanism involved. J. Agric. Food Chem. 68, 13897–13905.

López-Gálvez, G., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J.A., Selma, M.V., Gil, M.I., 2010. Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. Postharvest Biol. Technol. 55, 53–60.

Martin-Diana, A.B., Rico, D., Frias, J.M., Barat, J.M., Henehan, G.T.M., Barry-Ryan, C., 2007. Calcium for extending the shelf life of fresh whole and minimally processed fruits and vegetables: a review. Trends Food Sci. Technol. 18, 210–218.

Martinez, V., Acosta, E., Rojas, A., Stein, B., Ramalho, J., 2014. Estudios de sobrevivencia de *Xanthomonas citri* subsp. *citri* en frutos de limoneros y su dispersión a partir de frutos cosechados y severamente infectados. Rev. Ind. Agric. Tucuman 2, 17–24.

Mathew, E.N., Muyyarikkandy, M.S., Bedell, C.A., Malaradjou, M.A., 2018. Efficacy of chlorine, chlorine dioxide, and peroxyacetic acid in reducing *Salmonella* contamination in wash water and on mangoes under simulated mango packinghouse washing operations. Front. Sustain. Food Syst. 2, 18.

Mcneal, T.P., Hollifield, H.C., Diachenko, G.W., 1995. Survey of trihalomethanes and other volatile chemical contaminants in processed foods by purge-and-trap capillary gas chromatography with mass selective detection. J. AOAC Int. 78, 391–397.

Medir, M., Giralt, F., 1982. Stability of chlorine dioxide in aqueous solution. Water Res. 16, 1379–1382.

Mishra, V., Abrol, G.S., Dubey, N., 2018. Sodium and calcium hypochlorite as postharvest disinfectants for fruits and vegetables. In: Siddiqui, M.W. (Ed.), Postharvest Disinfection of Fruits and Vegetables. Academic Press, London, pp. 253–272.

Moretti, C.L., 2007. Manual de processamento mínimo de frutas e hortaliças. Brasília, DF, Brazil. Embrapa.

Narciso, J.A., 2005. An assessment of methods to clean citrus fruit surfaces. Proc. Fla. State Hortic. Soc. 118, 437–440.

Neves, M.F., Trombin, V.G., Marques, V.N., Martinez, L.F., 2020. Global orange juice market: a 16-year summary and opportunities for creating value. Trop. Plant Pathol. 45, 166–174.

Neves, M.F., Trombin, V.G., Milan, P., Lopes, F.F., Cressoni, F., Kalaki, R., 2010. O Retrato da Citricultura Brasileira. Ribeirão Preto, São Paulo, Brazil, Markestrat.

Pao, S., Kelsey, D.F., Khalid, M.F., Ettinger, M.R., 2007. Using aqueous chlorine dioxide to prevent contamination of tomatoes with *Salmonella enterica* and *Erwinia carotovora* during fruit washing. J. Food Protect. 70, 629–634.

Poovaiah, B.W., 1986. Role of calcium in prolonging storage life of fruits and vegetables. Food Technol. 40, 86–89.

Richardson, S.D., Thruston Jr., A.D., Caughran, T.V., Chen, P.H., Collette, T.W., Schenck, K.M., Lykins Jr., B.W., Rav-Acha, C., Glezer, V., 2000. Identification of new drinking water disinfection byproducts from ozone, chlorine dioxide, chloramine, and chlorine. Water Air Soil Pollut. 123, 95–102.

Ritenour, M., Graham, J.H., Narciso, J., 2008. Managing citrus canker for the fresh fruit industry. Citrus Ind. 89, 20–22.

Ritenour, M.A., Sargent, S.A., Bartz, J.A., 2014. Chlorine Use in Produce Packing Lines. University of Florida, Institute of Food and Agricultural Sciences. https://edis.ifas.ufl.edu/ch160/. (Accessed 11 January 2021).

Schaad, N.W., Postnikova, E., Lacy, G., Sechler, A., Agarkova, I., Stromberg, P.E., Stromberg, V.K., Vidaver, A.K., 2006. Emended classification of xanthomonad pathogens on citrus. Syst. Appl. Microbiol. 29, 690–695.

Simmons, J.L., Ryu, J., Beuchat, L.R., 2006. Comparison of treatment of fresh-cut lettuce and diced tomatoes with sodium hypochlorite and calcium hypochlorite for effects on microbiological and sensory qualities. Food Protect. Trends 26, 662–667.

Simons, L.K., Sanguansri, P., 1997. Advances in the washing of minimally processed vegetables. Food Aust. 49, 75–80.

Srebernich, S.M., 2007. Utilização do dióxido de cloro e do ácido peracético como substitutos do hipoclorito de sódio na sanitização do cheiro-verde minimamente processado. Cienc. Tecnol. Aliment. 27, 744–750.

Sun, X., Baldwin, E., Bai, J., 2019. Applications of gaseous chlorine dioxide on postharvest handling and storage of fruits and vegetables – a review. Food Contr. 95, 18–26.

Sun, X., Bai, J.H., Ference, C., Wang, Z., Zhang, Y.F., Narciso, J., Zhou, K.Q., 2014. Antimicrobial activity of controlled-release chlorine dioxide gas on fresh blueberries. J. Food Protect. 77, 1127–1132.

Suslow, T.V., Wu, J., Fett, W.F., Harris, L.J., 2002. Detection and elimination of *Salmonella* Mbandaka from naturally contaminated alfalfa seed by treatment with heat or calcium hypochlorite. J. Food Protect. 65, 452–458.

USDA, 2021a. Citrus: World Markets and Trade. United States Department of Agriculture, Foreign Agricultural Service, Washington, DC. https://apps.fas.usda.gov/psdonline/circulars/citrus.pdf/. (Accessed 11 January 2021).

USDA, 2021b. Treatment manual for fresh fruit. https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf/. (Accessed 11 January 2021).

Venkobachar, C., Iyengar, L., Rao, A.V.S.P., 1977. Mechanism of disinfection: effect of chlorine on cell membrane functions. Water Res. 11, 727–729.

Zamuner, C.F.C., Dilarri, G., Cavalca, L.B., Behlau, F., Silva, T.G., Bacci Jr., M., Ferreira, H., 2020. A cinnamaldehyde-based formulation as an alternative to sodium hypochlorite for post-harvest decontamination of citrus fruit. Trop. Plant Pathol. 45, 701–709.

Zoellner, C., Aguayo-Acosta, A., Siddiqui, M.W., Dávila-Aviña, J.E., 2008. Peracetic acid in disinfection of fruits and vegetables. In: Siddiqui, M.W. (Ed.), Postharvest Disinfection of Fruits and Vegetables. Elsevier, Amsterdam, pp. 53–66.