Organic acids and hydrogen peroxide can replace chlorinated compounds as sanitizers on strawberries, cucumbers and rocket leaves

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Abstract

This work evaluated the effectiveness of 1 and 2% acetic and lactic acid, 3% hydrogen peroxide, 200 mg/L sodium hypochlorite and 200 mg/L sodium dichloroisocyanurate to reducing natural contaminants as well as *Salmonella enterica* Enteritidis inoculated on the surface of strawberries, cucumbers, and rocket leaves. The reduction of aerobic mesophilic and molds and yeasts was between 1.67 – 2.73 and 0.61 – 1.46 log CFU/g in strawberries, 1.48 – 2.19 and 1.02 – 2.01 log CFU/g on rocket leaves, and 1.10 – 2.08 and 0.88 – 1.58 log CFU/g in cucumbers, respectively. The most effective sanitizers for reducing *Salmonella enterica* Enteritidis in strawberries were 1% and 2% lactic acid, while in cucumbers and rocket leaves was the 2% lactic acid as well as the 3% hydrogen peroxide, for both samples. The results of this study clearly show the potential of the proposed strategies to replace chlorinated compounds in the sanitization step of fresh produce.

Keywords: ready-to-eat food; non-chlorinated sanitizers; microbial load; food safety; quality.

Practical Application: Investigate alternatives in the sanitization step of fruits and vegetables.

1 Introduction

The industry of minimally processed fruits and vegetables, as well as the consumption of these foods, has grown significantly in recent years (Joshi et al., 2013; Rosário et al., 2017). Minimally processed foods, or ready-to-eat (RTE), are those that have undergone selection, washing, sanitizing, peeling and/or cutting, and subsequent appropriate packaging during processing, in order to obtain products ready for immediate consumption (Paula et al., 2009; Smanioto et al., 2009). However, the increase in the market and consumption of RTE has been correlated with an increase in the incidence of foodborne disease outbreaks due to the ingestion of fruits and vegetables contaminated by pathogens.

Among the microorganisms most involved in the occurrence of foodborne outbreaks involving fresh products are *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, and *Shigella* spp. *Salmonella* is a major foodborne bacterium that causes gastroenteritis and is estimated to be responsible for approximately 3 million cases of foodborne illness in China and 1 million cases in the United States of America (Guo et al., 2016). From 2013 to 2016, several *Salmonella* outbreaks have been reported worldwide, with one outbreak per year attributed to the consumption of cucumbers contaminated with this pathogen in the USA (Angelo et al., 2015; Bottichio et al., 2016; Centers for Disease Control and prevention, 2016). In Brazil, from 2000 to 2015, 11,241 outbreaks of foodborne illness were registered (Food Safety Brazil, 2016). According to the Epidemiological Surveillance of Foodborne Diseases in Brazil, the cases of outbreak increased by 10% from 2016 to 2017. *Salmonella* spp. accounted for the largest number of cases (35%) whose cause was identified (Food Safety Brazil, 2019).

The increase of new cases due to the inefficiency in the production and processing stages, as during sanitization, can result in complications in several countries (Fernandes et al., 2014). The efficiency of washing and sanitizing processes is directly related to the microbiological quality of the final product (Ramos et al., 2013; Rosário et al., 2017). Washing with potable water allows the removal of soil components and some of the pathogens but alone is not sufficient to significantly reduce the microbial load present in the food (Joshi et al., 2013; Gomes et al., 2014). The sanitization step is a key point for the microbiological control of foods (Joshi et al., 2013; São José et al., 2014) in order to ensure microbial control and food safety.

Usually, it is common to use chlorine-based compounds, especially sodium hypochlorite, during the sanitization of vegetables. These compounds are recommended in Brazil (Brasil, 1988; Brasil, 2007), but several European countries such as Germany, Belgium, Holland, and Sweden have prohibited the use of these compounds in the sanitization of fruits and vegetables (Rico et al., 2007). A concern about use of chlorine compounds is their potential for negative interactions with organic load contributed by plant tissue or other contaminants encountered during pre-harvest to post-harvest handling. The discussion on the use of chlorinated compounds is related to the possibility of generating highly carcinogenic by-products, which is of environmental problem (São José & Vanetti, 2012). In addition, the washing and use of chlorinated compounds may not be fully effective in the disinfection step of RTE (Huang et al., 2012; Rosário et al., 2017) because of the limited reduction of the contamination by 1 to 2 log CFU/g. Therefore, studies on

new disinfection strategies can contribute to the microbiological safety of the food (Huang & Chen, 2011; Sagong et al., 2011; Joshi et al., 2013; São José & Vanetti, 2015; Rosário et al., 2017; São José et al., 2018).

Lactic, acetic and citric acids are examples of organic acids that can be applied in the sanitization process. These substances are considered as generally recognized as safe (GRAS) and are acknowledged for their ability to act on a broad spectrum of bacteria with rapid inhibition of microbial cells present in food (Akbas & Ölmez, 2007; Huang & Chen, 2011; In et al., 2013; Sagong et al., 2011). Another alternative sanitizer is hydrogen peroxide due to its high oxidation power and highly bacteriostatic and bactericidal properties, including bacterial spore reduction. In addition, it does not react with organic components and does not form toxic waste. For these reasons, it has been gaining prominence in the minimum processing industry (Ölmez & Kretzschmar, 2009). These sanitizers can be used in the processing of RTE and can thus assist processers in adapting to new market trends. This work aimed to evaluate the application of organic acids and hydrogen peroxide in removing the natural contaminants microbiota and *Salmonella enterica* Enteritidis intentionally inoculated on the surface of strawberries, cucumbers, and rocket leaves.

2 Materials and methods

2.1 Obtaining the samples and sanitizers treatments

Samples of strawberries (*Fragaria x ananassa* Duch), cucumbers (*Cucumis sativus* L. var Aodai) and rocket leaves (*Eruca sativa* L.) were acquired from the local market of Vitória (Espírito Santo State, Brazil) and stored at 7 °C for a maximum of 24 h before processing. Before the analyses, the vegetables were selected and damaged or rotten ones were discarded. The stems were removed from yellowing rocket leaves. They were then washed in water to remove dirt that had adhered to the surface and were then drained for 10 minutes. The sanitization step consisted of the isolated immersion of approximately 200 g of strawberries, a unit of cucumber (approximately 250 g) and 50 g of rocket leaves in 1 L of sanitizing solution to analyze the efficiency against natural microbial contamination. In the analysis of *Salmonella* Enteritidis cell contamination, immersion occurred with approximately 100 g of strawberries, a unit of cucumber (approximately 250 g) and 25 g of rocket leaves in 500 mL of solution.

Sanitizing solutions were prepared immediately prior to use, and immersion occurred for 5 min at 20 ± 1 °C. The concentrations and time of exposure were based on previous experiments and scientific literature. The effects of the following sanitizing agents were evaluated: 1% and 2% lactic acid (Neon®, Suzano, São Paulo, Brazil), 1% and 2% acetic acid (Fmaia®, Belo Horizonte, Minas Gerais, Brazil) and 3% hydrogen peroxide (Scientific Exodus®, Hortolândia, São Paulo, Brazil). Sanitization treatments with chlorinated compounds (200 mg/L sodium dichloroisocyanurate (Nippoclor®, Indaiatuba, São Paulo, Brazil) and 200 mg/L sodium hypochlorite (Hidrosteril®, Itapevi, São Paulo, Brazil)) were carried out to compare the efficiency of the proposed sanitizers.

The control was the sample only washed in running water and did not undergo the sanitation stage.

The pH value of each sanitizer was measured in 50 mL of each prepared solution using a digital potentiometer (Tecnopon®, mPA210, Piracicaba, São Paulo, Brazil).

2.2 Evaluation of sanitation procedures to removal natural microbial contamination

This step was carried out in accordance with the American Public Health Association (APHA), as described in the Compendium of Methods for the Microbiological Examination of Foods (Downes & Ito, 2001).

After each treatment, 25 g of each sample were rinsed and homogenized in 225 mL of 0.1% peptone water using a stomacher (Seward Medical Co., London, United Kingdom). Appropriate serial dilutions were prepared for inoculation in standard plate count agar (PCA) (Difco®) that was incubated for 48 h at 35 ± 1 °C to determine the number of aerobic mesophilic bacteria. Mold and yeast aliquots were inoculated on potato dextrose agar (PDA) (Oxoid®) at a pH of 3.5 and incubated at 25 ± 2 °C for 5 to 7 days. For both analyses, plots of the two dilutions were made in duplicate. The result was expressed in log of colony forming units per gram (log CFU/g).

2.3 Challenge test to evaluate the efficiency of sanitizers in the removal of Salmonella enterica Enteritidis ATCC 13076

This test evaluated the potential of the different sanitizers for the inactivation of *Salmonella enterica* Enteritidis (ATCC 13076) intentionally inoculated on the samples. Vegetables were selected, cleaned and rinsed with sterile distilled water. A suspension of *Salmonella enterica* serovar Enteritidis (ATCC 13076) vegetative cells was utilized. From a pure culture of bacteria maintained at -80 °C in Eppendorf® tubes containing Brain Heart Infusion (BHI) and glycerol (80:20, v:v), we prepared suspensions containing approximately 1.0×10^7 CFU/mL of bacteria. For use in trials, suspensions of vegetative cells were produced in two consecutive subcultures in BHI broth by incubation at 35 °C for 24 h. The culture was preserved in 1 mL microtubes containing BHI (Himedia®), activated by two consecutive BHI replications, and incubated at 37 °C for 24 h until reaching a population of 10^6 to 10^7 CFU/mL. The number of microorganisms in the suspensions was determined by plating on *Salmonella-Shigella* agar (Himedia®, Brazil) and incubating the plates at 35 °C for 24 h.

Inoculation procedures were conducted according to São José (2013) with approximately 100 g of strawberries, 250 g of cucumbers and 25 g of rocket leaves that were placed separately in a previously sterilized plastic bags. The inoculum (10 mL) was added along with 500 mL of 0.1% peptone water. The bag containing the inoculum and the vegetable was gently shaken for 5 minutes. The samples were kept in contact with the cell suspension for 60 minutes at 25 ± 1 °C. The cell suspension was drained, and the strawberries and cucumbers contaminated with *Salmonella* were placed in sterile plastic bags and incubated at 25 °C for 24 h to allow for better adherence of the bacteria. Nevertheless, the rocket leaves were kept at the same temperature

for 1 hour, due to the characteristic of the sample. Afterward, contaminated samples were immersed in 500 mL of sanitizing solution for 5 minutes at 7 ± 1°C.

After each treatment, 10 g samples of each vegetable were transferred to sterilized plastic bags containing 90 mL of 0.1% peptone water and were then homogenized in a stomacher (Marconi®, MA440/CF, Piracicaba, São Paulo, Brazil) for 2 min at normal speed. Then, 1 mL of the sample was used to prepare decimal dilutions plated on the surface of *Salmonella-Shigella* agar (Himedia®, Brazil). After incubation for 18 to 24 h at 37 °C, the colonies were counted, and the results were expressed as log CFU/g.

2.4 Statistical analyses

Three experimental repetitions were conducted for each sanitizing treatment. The results were analyzed with the InfoStat Statistical Software (version 2012, Cordoba National University, Argentina). The analysis of variance (ANOVA) was applied. The average logarithms of the number of colonies forming units per gram (log10 CFU/g) for each treatment were compared using Tukey's test at a 5% probability.

3 Results and discussion

3.1 Ph evaluation of sanitizing solutions

The 2% acetic acid solution had the lowest pH value among the sanitizers evaluated (Table 1). pH control in the sanitization solution is fundamental to the efficiency of the operation because can contributed to bactericidal effect.

Table 1. Values of pH averages of sanitization treatments at a temperature of 24 ± 1 °C.

Treatments	pH
1% Lactic Acid	2.51[d] ± 0.05
2% Lactic Acid	2.33[d] ± 0.06
1% Acetic Acid	1.89[e] ± 0.04
2% Acetic Acid	1.72[f] ± 0.05
3% Hydrogen Peroxide	3.98[b] ± 0.16
200 mg/L Sodium Dichloroisocyanurate	6.55[a] ± 0.06
200mg/L Sodium Hypochlorite	6.50[a] ± 0.22

Different letters in the same column indicate statistically significantly different at p ≤ 0.05 by Tukey Test.

3.2 Evaluation of the efficiency of sanitizers to removal natural microbial contamination

Strawberries

All evaluated treatments significantly reduced the initial strawberry contamination (p ≤ 0.05). The reduction of aerobic mesophilic bacteria contamination ranged from 1.67 to 2.73 log CFU/g. There was no significant difference (p > 0.05) between the proposed treatments and the chlorinated compounds (Table 2). The results indicate that the treatments with organic acids and hydrogen peroxide were compatible with treatments with chlorinated compounds and could be an alternative. The reduction values for aerobic mesophilic bacteria by acetic acid at 1% and 2% were equal to 2.59 and 2.73 log CFU/g, respectively.

Nascimento & Silva (2010) found that 2% acetic acid reduced 1.90 log CFU/g for aerobic mesophilic bacteria in strawberries. However, sodium dichloroisocyanurate and sodium hypochlorite, sanitizers based on chlorine, reduced 1.55 and 1.25 log CFU/g, respectively. Moreover, the authors verified that 4% acetic acid sanitization reduced 2.43 log CFU/g, which indicates that despite the use of a higher concentration of active ingredient there was no further reduction in the count of aerobic mesophilic bacteria. This result may occur due to factors that indirectly interfere with the effectiveness of sanitizers, such as the anatomy, contact area, and structure of plant tissues (São José & Vanetti, 2015).

The treatment with hydrogen peroxide reduced 1.85 log CFU/g, and the result is similar to that found by Alexandre et al. (2012), who sanitized strawberry samples with the same sanitizer at a concentration of 1% and 5% for 2 minutes and obtained a reduction of 1.46 log CFU/g and 2.25 log CFU/g, respectively. The efficiency of sanitization involves other factors in addition to concentration and time of exposition, such as the surface characteristics of the food, formation of resistant biofilms, temperature, and pH (São José et al., 2014).

Treatments with 1% and 2% lactic acid, 2% acetic acid and with hydrogen peroxide promoted a reduction of aerobic mesophilic bacteria as well as the molds and yeasts statistically equal to chlorinated compounds (p > 0.05). In this study, both concentrations of organic acids applied were able to reduce the natural contamination of aerobic mesophilic bacteria and yeasts and molds in a similar proportion, which suggests the possibility of using the lowest concentration. Some factors

Table 2. Averages (log CFU/g) of the counts of the contaminating natural microbiota and *Salmonella* Enteritidis cells adhered to strawberries without sanitizing and submitted to different sanitization treatments for 5 minutes.

Treatments	Log CFU/g ± Standard Deviation		
	Aerobic mesophiles	Molds and Yeats	*Salmonella* Enteritidis
No Sanitizer	5.88[a] ± 0.20	6.04[a] ± 0.23	5.13[a] ± 0.41
1% Lactic Acid	3.87[b] ± 1.41	4.73[c] ± 0.32	3.22[c] ± 0.39
2% Lactic Acid	3.57[b] ± 1.18	4.64[c] ± 0.18	3.05[c] ± 0.04
1% Acetic Acid	3.29[b] ± 1.07	5.43[b] ± 0.38	4.38[b] ± 0.11
2% Acetic Acid	3.15[b] ± 0.85	4.58[c] ± 0.40	4.24[b] ± 0.10
3% Hydrogen Peroxide	4.03[b] ± 0.76	4.74[c] ± 0.23	4.38[b] ± 0.10
200 mg/L Sodium Dichloroisocyanurate	4.21[b] ± 0.76	4.64[c] ± 0.37	4.23[b] ± 0.14
200mg/L Sodium Hypochlorite	4.01[b] ± 0.71	4.65[c] ± 0.50	4.59[b] ± 0.12

Different letters in the same column indicate statistically significantly different at p ≤ 0.05 by Tukey Test.

should be evaluated when considering a sanitizer as ideal, such as being able to destroy microorganisms quickly and having a broad spectrum of antimicrobial action, in addition to presenting low toxicity and corrosivity and being stable under several conditions of use (Andrade et al., 2008). Nascimento & Silva (2010) subjected strawberries to sanitizing procedures with 2% and 4% of acetic acid, and 50% vinegar solutions and obtained results that demonstrated that the use of this sanitizer was effective under various conditions of use, thus fulfilling one of the aforementioned criteria.

Cucumbers

All treatments significantly reduced the initial contamination (p ≤ 0.05). However, there was no significant difference (p > 0.05) for the contaminating natural microbiota among the evaluated treatments. The reduction of aerobic mesophilic bacteria contamination ranged from 1.10 to 2.08 log CFU/g (Table 3).

As with the strawberry samples, the reductions of molds and yeasts by 1% and 2% acetic acid had similar values, 1.32 log CFU/g, and 1.33 log CFU/g, respectively, which demonstrates that the lowest concentration is feasible. Ukuku (2006) obtained better results in the sanitation of melons using 2.5% hydrogen peroxide and 200 mg/L sodium hypochlorite. The difficulty in promoting the reduction of microorganisms of some foods can be explained by the characteristic repellency of their surface due to the presence of hydrophobic multilayers (São José et al., 2014).

3.2.3 Rocket leaves

All treatments were able to significantly reduce the initial contamination (p ≤ 0.05) (Table 4). The reduction of aerobic mesophilic bacteria contamination in rocket leaves ranged from 1.27 to 2.19 log CFU/g. In addition, for aerobic mesophilic bacteria, all alternative treatments proposed were statistically the same as the chlorinated compounds (p > 0.05). The present study showed that both concentrations of acetic acid tested were effective in removing aerobic mesophiles. High initial counts of microorganisms may be related to the way the plants are produced; for example, in the case of leaves, growing near the soil makes them prone to contamination. The presence of microorganisms on the leaves at the time of harvesting is one of the main challenges in the post-harvest handling of fresh products because that can initiate the process of deterioration during storage (Wieczyńska et al., 2016).

Zhang & Yang (2017), when evaluating potential sanitizers in organic and conventional lettuce, found that aerobic mesophilic populations in samples treated with 0.6% citric acid and 1% hydrogen peroxide had counts equal to 5.58 and 4.61 log CFU/g, respectively, while the population of molds and yeast were 4.74 and 3.78 log CFU/g, respectively. Fantuzzi et al. (2004) found no significant difference in the reduction of the contaminant microbiota in cabbage samples treated with 1% acetic acid when compared to the microbiota reduction with the water wash process only.

Table 3. Averages (log CFU/g) of the counts of the contaminating natural microbiota and *Salmonella* Enteritidis cells adhered to cucumbers without sanitizing and submitted to different sanitization treatments for 5 minutes.

Treatments	Log CFU/g ± Standard Deviation		
	Aerobic mesophiles	Molds and Yeats	*Salmonella* Enteritidis
No Sanitizer	6.19[a] ± 0.16	6.54[a] ± 0.20	5.07[a] ± 0.04
1% Lactic Acid	4.47[b] ± 0.73	4.96[b] ± 1.06	3.66[b] ± 0.14
2% Lactic Acid	4.11[b] ± 0.81	5.09[b] ± 0.53	2.72[de] ± 0.36
1% Acetic Acid	4.45[b] ± 1.11	5.22[b] ± 0.12	3.69[b] ± 0.31
2% Acetic Acid	4.35[b] ± 0.38	5.21[b] ± 0.15	2.99[cd] ± 0.24
3% Hydrogen Peroxide	5.09[b] ± 0.29	5.13[b] ± 0.15	2.36[e] ± 0.22
200 mg/L Sodium Dichloroisocyanurate	4.81[b] ± 0.69	5.66[b] ± 0.49	3.47[bc] ± 0.51
200mg/L Sodium Hypochlorite	5.13[b] ± 0.35	5.51[b] ± 0.48	3.45[bc] ± 0.16

Different letters in the same column indicate statistically significantly different at p ≤ 0.05 by Tukey Test.

Table 4. Averages (log CFU / g) of the counts of the contaminating natural microbiota and *Salmonella* Enteritidis cells adhered to rocket leaves without sanitizing and submitted to different sanitization treatments for 5 minutes.

Treatments	Log CFU/g ± Standard Deviation		
	Aerobic mesophiles	Molds and Yeats	*Salmonella* Enteritidis
No Sanitizer	7.01[a] ± 0.35	6.43[a] ± 0.06	5.26[a] ± 0.09
1% Lactic Acid	5.74[b] ± 0.47	5.43[b] ± 0.17	3.55[d] ± 0.12
2% Lactic Acid	4.97[b] ± 0.50	4.42[c] ± 0.22	2.15[e] ± 0.21
1% Acetic Acid	5.53[b] ± 0.89	5.41[b] ± 0.11	4.04[c] ± 0.04
2% Acetic Acid	5.21[b] ± 1.09	4.63[c] ± 0.62	3.37[d] ± 0.12
3% Hydrogen Peroxide	4.82[b] ± 0.36	4.53[c] ± 0.02	2.36[e] ± 0.26
200 mg/L Sodium Dichloroisocyanurate	5.03[b] ± 0.97	5.44[b] ± 0.40	3.22[d] ± 0.11
200mg/L Sodium Hypochlorite	5.36[b] ± 0.84	5.29[b] ± 0.52	4.50[b] ± 0.02

Different letters in the same column indicate statistically significantly different at p ≤ 0.05 by Tukey Test.

However, it is worth mentioning that each plant has different roughness and hydrophobicity, which may influence the sanitizing antimicrobial efficiency (Data not shown). The increase in the mean roughness value of the surfaces may favor higher retention of microorganisms on the surface (Lima et al., 2013).

According to Yuk et al. (2006), the microstructures of plant tissues, grooves, cracks, cavities and other irregularities of the vegetable surface can affect the contact of the sanitizing solution with the target microorganisms and, consequently, the efficiency of the process.

When evaluating the use of sanitizers in the present study against molds and yeasts, it was verified that 2% acetic acid, 2% lactic acid and hydrogen peroxide were statistically better than the other treatments, reducing 1.80, 2.01 and 1.90 log CFU/g, respectively. It is noteworthy that sodium dichloroisocyanurate obtained a smaller reduction than these procedures, reducing less than 1 log CFU/g.

Evaluation of the efficiency of sanitizers in Salmonella enterica Enteritidis cells ATCC 13076

The sanitization treatments that showed the greatest effect on the reduction of *Salmonella* Enteritidis cells in strawberries were 1% and 2% lactic acid, with a reduction of 1.91 log CFU/g and 2.08 log CFU/g, respectively (Table 2). These results are similar to the findings of Park et al. (2011), in which 1% lactic acid reduced 1.78 log CFU/g and 2% lactic acid reduced 2.53 log CFU/g in apple samples contaminated with *Salmonella* Typhimurium. Fernandes (2013) confirmed that solutions of 1% and 2% lactic acid promoted a higher reduction, compared to other treatments in strawberries contaminated with *E. coli* cells. Gurtler et al.(2014) evaluated different sanitizers for 2 minutes to remove four serovars of *Salmonella enterica* from strawberries and found higher reductions from treatment with 3% hydrogen peroxide compared to lactic and acetic acids, both 1%.

The cucumber samples had initial contamination of *Salmonella* Enteritidis cells of 5.07 ± 0.04 log CFU/g and after the treatments with hydrogen peroxide and 2% lactic acid reductions of 2.71 log CFU/g and 2.34 log CFU/g, respectively, were achieved (Table 3). Different from the results of natural microbial counts after sanitization treatments, the reduction of the pathogen in the cucumber samples was higher when the concentration of organic acids increased. Guo et al. (2016) evaluated the action of hydrogen peroxide at 2.5 and 5% for one minute on slices of cucumber intentionally contaminated with *Salmonella* Choleraesuis and observed a reduction of 0.9 and 1.6 log CFU/g, respectively. Amrutha et al. (2017) obtained a lower reduction (0.17 log CFU/g) of *Salmonella* spp. when compared to the present study after sanitizing cucumber with 2% lactic acid. Ukuku (2004) investigated the action of the hydrogen peroxide at 2.5% and 5% in the reduction of *Salmonella* spp. cells on the surface of cantaloupe and honeydew melons. In the first melon variety, the lowest concentration promoted a reduction of 2.56 log CFU/g. However, an increase in concentration did not enhance the reduction. In honeydew melons, both hydrogen peroxide concentrations reduced 3.00 log CFU/g from initial contamination. The increase of the concentration of hydrogen

peroxide (0.5, 1, and 2%) also did not cause statistical differences in the counts of *E. coli* ATCC 25922 and *Salmonella* Enteritidis after sanitization of fresh-cut snap bean (Palharini et al., 2017). In a study on the sanitization of melons and green peppers contaminated with *Salmonella* Enteritidis, São José et al. (2014) applied the same sanitizer at 1% concentration and obtained reduction of 1.80 log CFU/g and 2.00 log CFU/g, respectively.

The reductions in rocket leaves varied from 0.76 to 3.11 log CFU/g. All evaluated treatments significantly decreased the counts of *S. enterica* Enteritidis (p ≤ 0.05). The highest reductions were from sanitization with 2% lactic acid and hydrogen peroxide, which reduced the counts by 3.11 and 2.90 log CFU/g, respectively (Table 4). Similar to the cucumber results, the increase of the organic acids concentration enhanced the reduction of *Salmonella* Enteritidis cells in the rocket leaves. Lactic acid is an organic acid considered as GRAS and can be used for the removal of surface contamination or as a preservative and flavoring agent in foods (Poimenidou et al., 2016). Neal et al. (2012) obtained a reduction of 2.3 log CFU/g of *Salmonella* spp. when using lactic acid at 2% in spinach leaves. Zhang et al. (2015) used 3% hydrogen peroxide and found a smaller reduction to that found in this study, 1.5 log CFU/g of *Salmonella* Typhimurium cells in spinach leaves. Poimenidou et al. (2016) found that the addition of 300 mg/L of active chlorine in the treatment solution promoted a reduction of 1.7 log CFU/g of *E. coli* O157:H7 in spinach leaves. Valiolahi et al. (2019) tested different treatments with 2% of organic acids (acetic, lactic, and citric), and found that basil leaves samples treated with acetic acid presented the highest inhibition effects of *Escherichia coli* O157:H7 growth at the first day of storage. An antimicrobial formulation (3% hydrogen peroxide, 0.02 mM ethylenediaminetetraacetic acid (EDTA), and 20 mg/ml Nisin) tested by Mukhopadhyay et al. (2019) reduced 1.8 log CFU/g the count of *E. coli* O157:H7 of spinach leaves.

The microbial inactivation of organic acids can occur due to the damage and break in the function of the cell wall. The undissociated form of organic acid diffuses through freely the microbial membrane and inhibits enzymatic reactions as well as modifies proteins and DNA structure (Mani-López et al., 2012; Wang et al., 2015). The efficiency of hydrogen peroxide is related to damages caused in DNA of the microorganisms due to its high oxidation power (Linley et al., 2012).

4 Conclusion

Based on their antimicrobial action, the proposed sanitizers were as efficient as the chlorinated compounds in reducing the contamination of aerobic mesophilic bacteria in strawberries, cucumbers and rocket leaves. In the cucumber samples, there was a higher reduction in the contamination of molds and yeasts when 1% and 2% lactic acid was applied. The treatments that demonstrated the greatest effect on the reduction of *Salmonella enterica* Enteritidis cells in intentionally contaminated strawberries were 1% and 2% lactic acid. However, in the cucumber and rocket samples, 2% lactic acid and 3% hydrogen peroxide treatments promoted a greater reduction of the pathogen. All the evaluated treatments had a similar or better performance than the chlorinated compounds; therefore, they have a potential for use as alternatives in the sanitization of fresh fruits and

vegetables. Nevertheless, complementary studies are needed to evaluate the impact of these sanitizers on sensory qualities and economic viability.

Acknowledgements

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