

Full Length Research Paper

Sanitization protocols applied to commercial restaurants: Effects on natural contaminant microbiota and *Salmonella enterica* Enteritidis adhered on tomatoes

Maria Clara de Moraes Motta Machado¹, Grazielli Ramos de Lyra², Erika Madeira Moreira da Silva¹ and Jackline Freitas Brilhante de São José^{1*}

¹Department of Integrated Education in Health, Federal University of Espírito Santo, Avenida Marechal Campos, 1468, 29040-090 Vitória, Brazil.

²Federal University of Espírito Santo, Avenida Marechal Campos, 1468, 29040-090 Vitória, Brazil.

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Sanitization is considered as essential for the microbial control of vegetables. The aim of this study was to evaluate the sanitization procedures used in commercial restaurants located in Vitória, Brazil. The efficacy of these sanitization procedures in reducing the presence of natural microbiota and *Salmonella enterica* Enteritidis cells on tomatoes were evaluated. All the restaurants in this study applied the sanitization methods using containers for diluting the sanitization solution. After the sanitization treatments, a reduction in mesophilic aerobic counts, yeasts, moulds and *S. enterica* occurred in all the treatments. A higher reduction in microorganisms was observed after treatment with 2% acetic acid. There was no significant difference between tomatoes treated with a sodium dichloroisocyanurate solution and sodium hypochlorite for all microorganisms which were evaluated. Chlorinated compounds are the most used products but a limitation in microbial inactivation was observed in this study.

Key words: Disinfection, tomatoes, *Salmonella enterica* Enteritidis, acetic acid, quality control, sanitization protocols.

INTRODUCTION

Dietary consumption of vegetables and fruits has health benefits, including to avoid or decrease the possibility of developing several chronic diseases (Bang et al., 2017).

The benefits related to fresh cut products have contributed to an important increase in the consumption of ready-to-eat vegetables (Callejón et al., 2015; Bang et

*Correspondent author. E-mail: jackline.jose@ufes.br.

al., 2017). However, the consumption of raw or minimally processed fruits and vegetables can be a route for the transmission of foodborne illnesses (Park et al., 2013; Prado-Silva et al., 2015). Among the multiple failures in food services, the development of foodborne illness outbreaks may occur due to inadequate conservation, cross-contamination, improper hygiene, and use of leftovers and illegal products (Lima et al., 2013, Da Cunha et al., 2016). A restaurant employee may wash vegetables to remove the dirt, bacteria, and/or pesticides that may have accumulated during cultivation or processing, but it has demonstrated that multiple washes with plain water may not significantly reduce the bacterial concentration on contaminated produce (Jensen et al., 2015). These infectious diseases are caused by the consumption of food or water that is contaminated with pathogenic microorganisms or toxins that are produced by them. Outbreaks are a global reality, and considered a public health problem of wide scope and have negative impacts on the productivity, economy and consumer confidence. According to Rahman et al. (2016), the Centers for Disease Control and Prevention (CDC) have reported numerous different foodborne pathogens that can cause infections in humans. From 2013 to 2015, several *Salmonella* outbreaks were reported worldwide, with one outbreak per year attributed to the consumption of cucumbers contaminated with this pathogen in the USA (CDC, 2013; Angelo et al., 2015; CDC, 2015).

The microbiological quality of vegetables that are eaten raw is a relevant factor to health that should be controlled and should be guaranteed by sanitization with chemicals that are effective in inactivating contaminant microbiota (Poimenidou et al., 2016). Vegetables provided in restaurants or as minimally processed are often linked to the origin of disease outbreaks because they are ready for consumption and are not subjected to sufficient processes to reduce microbial contamination. Thus, the use of effective chemical agents during the washing and sanitizing steps to ensure the safety of the products has gained more interest in the scientific community (Petri et al., 2015). A washing operation associated with the use of sanitizing solutions is considered the only process that can effectively reduce the number of spoilage-inducing and pathogenic microorganisms and contribute to the safety of the product (São José et al., 2014). The chemicals most often used to sanitize vegetables are chlorinated compounds (Rosário et al., 2017). Chlorine and its diverse forms are the most frequently used disinfectants. Easily use, low cost, good antimicrobial activity and dissolution in water make chlorinated agents attractive for frequent use in sanitization step in industry and restaurants (Petri et al., 2015). The recommended total chlorine concentrations as a disinfectant agent range from 50 to 200 mg·L⁻¹ (Ali et al., 2017) In Brazil, it is recommended at a concentration of 100 to 250 mg·L⁻¹ for 15 min for the disinfection of vegetables (Oliveira et

al., 2012). Thus, the aim of this study was to survey and evaluate the procedures used in the sanitization step routinely applied for tomatoes in commercial restaurants in Vitória, Espírito Santo, Brazil.

MATERIALS AND METHODS

Experimental design

The first stage of the study was to survey the major sanitizing procedures of vegetables that are used in commercial restaurants located in Vitória, Espírito Santo, Brazil. The restaurants were contacted by an invitation letter that presented the research objectives and requested permission to visit. The responsible parties of each participating restaurant signed an authorization form to allow the research. For the sample definition, the total number of registered restaurants was defined as the number of restaurants in the Bars and Restaurants Union of Espírito Santo located in three districts which were selected for their proximity to the research institution. In 2014, 58 total commercial restaurants were registered and a final sample of 18 establishments was determined to be necessary to have a sampling error of 10% with a 90% confidence level.

The second stage of the study was conducted in a completely randomized manner, with each treatment subjected to three repetitions.

Survey of sanitization procedures

A checklist of sanitization procedures used by a previously trained researcher was used. The seventeen items in the list included the type of service, number of meals offered, sanitizers products used, duration of exposure, concentration of the food subjected to sanitization, containers used, training of the handlers to perform sanitation, presence of industry-specific tasks for pre-prepared salads, and use of instructional materials with guidance on how to sanitize properly and with which the protocol was developed.

Evaluation of sanitization protocols on natural contaminant microbiota

In the second phase of the study, the major sanitization methods used by the restaurants were used to analyse their efficiency to eliminate or reduce microorganisms. The sanitizers were sodium dichloroisocyanurate at a concentration of 200 mg·L⁻¹ (NippoClor, Nippon Chemical®, São Paulo, São Paulo, Brazil), sodium hypochlorite at 200 mg·L⁻¹ (Hidrosteril®, Itapevi, São Paulo, Brazil), 2% red vinegar (Toscano®, Várzea Paulista, São Paulo, Brazil), 2% acetic acid (Fmaia®, Belo Horizonte, Brazil) and running water. Acetic acid was studied as a sanitizing proposal, as it is currently observed with an interest in applying it for the sanitization of vegetables. For each treatment, approximately 250 g of tomato (*Solanum lycopersicum* L.) was immersed in one litre of sanitizing solution for 15 min. Tomatoes were acquired from local retailers and from a single producer to avoid variation. Tomatoes were stored under refrigeration at 7°C for a maximum of 24 h before processing, and damaged or rotten tomatoes were discarded. The tomato was chosen as a model system; it is widely consumed by the population in households and in commercial restaurants.

These sanitization methods were tested for their efficiency in reducing the count of natural contaminant microbiota (aerobic mesophiles, yeasts and moulds). Samples sanitized were subsequently subjected to microbiological analysis (Downes and

Ito, 2001). Samples of tomatoes were homogenized with 0.1% peptone water in a stomacher (Seward Medical Co., London, United Kingdom) for 2 min at normal speed. Appropriate decimal dilutions were prepared, and aliquots were transferred to growth media specific for the detection of each microbial group. To determine the number of aerobic mesophiles, inoculation was performed on standard agar plates for counting (Himedia®, São Paulo, Brazil) followed by incubation for 48 h at $35 \pm 1^\circ\text{C}$. Yeasts and moulds aliquots were inoculated on potato dextrose agar (Himedia®, Brazil) acidified with 10% tartaric acid and incubated at $25 \pm 2^\circ\text{C}$ for 5 to 7 days. Plating rate experiments were performed in duplicate, and the results were expressed in colony-forming units per gram ($\text{CFU}\cdot\text{g}^{-1}$).

Removal of *Salmonella* Enteritidis ATCC 13076 cells attached to the surface of tomatoes

S. Enteritidis ATCC 13076 was obtained from stock culture. The culture was kept in 1 mL microtubes containing Brain Heart Infusion (BHI) broth (Himedia®, Brazil) with activation by two consecutive replications and incubated at 37°C for 18-24 h until the concentration reached 10^6 to 10^7 $\text{CFU}\cdot\text{mL}^{-1}$.

Tomato samples were selected and then cleaned and washed in sterile distilled water in aseptic conditions. After this, 250 g of tomatoes were placed in previously sterilized plastic bags. For each treatment evaluated, were used six plastic bags to place the tomatoes separately. After this, the inoculum (10 mL) was added with 100 mL of 0.1% of peptone water in each plastic bag. The plastic bag containing the inoculum and vegetables was lightly stirred for 5 min. The tomatoes were kept in static contact with the cell suspension for 60 min at $24 \pm 1^\circ\text{C}$. Then, the cell suspension was drained, and the tomatoes that were contaminated with *S. Enteritidis* were placed in sterile plastic bags and incubated at 25°C for 24 h to allow bacterial adhesion.

Subsequently, the contaminated samples were subjected to the previously selected sanitization methods. As a control, inoculation without subsequent sanitization was performed. For each treatment, approximately 250 g of tomato were immersed in a litre of sanitizing solution for 15 min. After each treatment, 25 g of the tomatoes were transferred to sterile plastic bags containing 0.1% peptone water and then manually homogenized for 2 min. Then, 1 mL samples were removed to prepare serial dilutions that were plated by the surface spreading technique on *Salmonella Shigella* (Acumedia®, Indaiatuba, Brazil) agar. After incubation for 18 to 24 h at 37°C , colonies were counted (and recorded as $\text{CFU}\cdot\text{g}^{-1}$). To evaluate the effect of the sanitizing treatment, the units were converted from $\text{CFU}\cdot\text{g}^{-1}$ to $\log \text{CFU}\cdot\text{g}^{-1}$. Counts from inoculated tomatoes that were not sanitized were considered as the initial count. The effect of the sanitizing treatments was calculated according to the following formula: exponential reduction = \log (initial count with no sanitization) – \log (final count after treatment).

Analysis of the sanitizing effect of removing *S. Enteritidis* adhered to the surface of tomatoes by scanning electron microscopy

For this analysis, we chose the best and worst treatment applied in the previous step. Thus, evaluations were made of tomatoes cuts treated with 2% acetic acid, and 2% red vinegar and samples that did not undergo sanitization. Samples of tomato were selected and then cleaned and washed in sterile distilled water. After washing the tomatoes, the outermost layer of the fruits was aseptically removed, and 1.0 cm sections were cut with the aid of a sterile scalpel. The sections were placed in Petri dishes containing sterile water for

rinsing and removing waste from the plant tissue.

S. Enteritidis cells were grown in BHI broth (Acumedia® or Himedia, Brazil) for 16 h at 37°C . After this step, the broth was distributed onto the 13.5 cm diameter Petri dishes containing the tomato cuts. The cuts ($n = 10$) were then distributed into sterile plastic bags containing sterile distilled water for 1 min to remove planktonic cells and then subjected to the previously described sanitization methods. After sanitization, the sections were subjected to the microscopy preparation protocol.

The tomato cuts that were selected for observation in a scanning electron microscope were rinsed in phosphate buffered saline (PBS, 0.05 mol L^{-1} , pH 6.8 to 7.2) for removal of sanitizer residues and non-adherent cells. The fixation step consisted of a treatment in 5% glutaraldehyde in 0.1 M PBS buffer (v/v) for 1 h (25°C). The sections were then washed six times for 10 min in 0.05 M PBS buffer (pH 6.8 to 7.2). The dehydration step consisted of serial treatments in ethanol with 30, 50, 70, 80 and 95% ethanol for 10 min each and then three treatments of 100% ethanol for 15 min each. The samples were then transferred to a critical point drier (Critical Point Dryer – model CPD020, Balzers, Liechtenstein) for total dehydration. The samples were finally sputter coated (Denton Vacuum Desk II Sputtering, Denton Vacuum, Cherry Hill, N), and images were recorded using a scanning electron microscope, model JEOL JSM-6010LA (Jeol USA, Peabody, MA, USA). The analyses were performed in the Ultrastructure Cell Laboratory Carlos Alberto Redins (LUCCAR) of the Federal University of Espírito Santo.

Data analysis

The information collected in the first stage of the study were compiled into a Microsoft Excel spreadsheet for descriptive analysis of the data. Data were analysed with Genes® (Minas Gerais, Brazil) using the analysis of variance (ANOVA) method on the average of the logarithms of the number of colony forming units per gram ($\log \text{CFU}\cdot\text{g}^{-1}$); post-test analysis was performed with the Tukey test, with a p -value <0.05 determined to be statistically significant.

RESULTS AND DISCUSSION

Survey of sanitization procedures

Eighteen commercial restaurants were contacted, and twelve agreed to participate in the research. In the evaluated restaurants, 58.3% ($n = 7$) were self-service types and 41.7% ($n = 5$) were a la carte, serving approximately 500 meals/day. All restaurants performed some hygiene procedure for vegetables. Washing and sanitizing fruits and vegetables are essential to prevent foodborne diseases (Petri et al., 2015). In this study, 91.66% ($n = 11$) of the restaurants use chlorinated compounds for their sanitization step, using one of four different brands of chlorinated compounds and sanitary water with the addition of 2.5% of sodium hypochlorite. Among the sanitizers used in the food industry and restaurants, especially to wash fresh produce, chlorine and chlorinated compounds are often used (Rosário et al., 2017). Their ease of use, low cost, high antimicrobial activity and complete dissolution in water make chlorinated agents a common choice for a disinfectant in

Table 1. Sanitation procedures adopted in restaurants in Vitória-ES, 2014.

| Variables related of sanitation procedures | Yes (%) |
|--|----------------|
| Volume of sanitizing solutions | 0.00 |
| Technical manager | 33.33 |
| Sanitation | 100.00 |
| Use of registered product | 83.34 |
| Temperature control | 0.00 |
| Product reuse | 0.00 |
| Rinse after application of the product | 83.34 |
| Responsible and trained employee to carry out the procedure | 0.00 |
| Use of exclusive container for sanitation | 100.00 |
| Existence of instructional material to carry out the procedure | 25.00 |
| Existence of exclusive sanitation area | 92.66 |

the fruit and vegetable industry (Petri et al., 2015).

In only one of the restaurants, a vinegar-based solution was followed by washing with water. Nascimento and Silva (2010) observed that the vinegar solution had a 50% lower reduction in the microbial load of the plant as compared to what was obtained with sodium hypochlorite. It is worth noting that all establishments surveyed in this study conducted sanitization procedures (Table 1), demonstrating the concern for this contaminant reduction step.

As for the contact time with the sanitizing solution, 50% of the restaurants that were surveyed immersed the vegetables for 15 min. In the others establishments, there was no controlled soaking time because the vegetables were left immersed during the period in which the handlers performed another activity. The time that food stays in contact with the sanitizing solution is well established (Chen and Zhu, 2011) as the samples are immersed in a sanitizing solution for approximately 15 min.

Regarding the concentration of chlorinated products used in the commercial restaurants, it was observed that all establishments used dilution metres and followed the manufacturer's recommendation. Oliveira et al. (2012) noted that 88% of visited restaurants did not use the sanitizer in pre-defined concentration. The concentration of sanitizer must be strictly controlled because it may lead to unacceptable sensory impact on the food. There was no temperature control for sanitizing in all the evaluated establishments. The best activity of chlorinated compounds is at a pH range between 6.0 and 7.5 and at low temperature (Banach et al., 2015). All commercial products used at participating restaurants were approved by the Ministry of Health, under the Brazilian regulations that are described in the DRC 216/2004. The sanitizing products should be identified and stored in a place reserved for this purpose (Brazil, 2004).

Regarding the volume of sanitizing solutions and the quantities of food sanitized at a time, there were no pre-

established values in the surveyed establishments (Table 1). The use of large amounts of food in a low volume of sanitizing solution may cause a reduction in antimicrobial efficiency. Products that are used for sanitization must be applied properly in order to avoid residues on prepared food (Brazil, 2004; Oliveira et al., 2012).

All establishments that were surveyed use unique containers for the sanitization of vegetables, which corroborates with Oliveira et al. (2012), which found that most studied restaurants used unique tools for sanitization. It was observed that in 33.3% of the studied restaurants, the protocols were drafted by a technical manager of the establishment, a nutritionist.

In the establishments that were surveyed, only 75% were not observed in the presence of posters and instructional materials related to the execution of vegetable sanitization procedure near the area of pre-preparation. The presence of these materials facilitates the understanding of the manipulator, clarifying any doubts that arise during the execution of their functions in the pre-prepared vegetable area.

Efficiency of sanitization treatments on natural contaminant microbiota

After sanitization, a reduction in mesophilic aerobic count with all treatments was observed, with the greatest reduction occurring after treatment with acetic acid 2% ($p < 0.05$) (Table 2). There was no significant difference in the score between tomatoes without sanitization and tomatoes immersed in running water ($p > 0.05$). This result demonstrated the importance of the application of sanitizing compounds to inactivate microorganisms and guarantee food safety. Regarding the aerobic mesophilic count, Brazilian legislation (Brazil, 2004) does not provide a limit to the maximum count allowed on fresh vegetables, so the maximum count was considered based on the recommendation of a maximum value of

Table 2. Effect of sanitizing treatments for 15 min on reduction of natural microbiota on tomatoes (*Solanum lycopersicum* L).

| Treatments | Aerobic mesophiles (Log CFU·g ⁻¹) | Reduction (Log CFU·g ⁻¹) | Mould and yeasts (Log CFU·g ⁻¹) | Reduction (Log CFU·g ⁻¹) |
|--|--|---|--|---|
| No sanitizer | 4.82 ^a ± 0.44 | - | 5.02 ^a ± 0.22 | - |
| Running water | 4.50 ^a ± 0.45 | 0.32 | 4.34 ^{ab} ± 0.18 | 0.68 |
| Sodium dichloroisocyanurate 200 mg·L ⁻¹ | 4.08 ^{ab} ± 0.88 | 0.74 | 4.08 ^{ab} ± 0.94 | 0.94 |
| Sodium hypochlorite 200 mg·L ⁻¹ | 3.41 ^{ab} ± 0.18 | 1.09 | 3.81 ^{ab} ± 0.72 | 1.21 |
| 2% acetic acid | 2.93 ^c ± 0.20 | 1.86 | 3.23 ^b ± 0.25 | 1.79 |
| 2% red vinegar | 3.31 ^{bc} ± 0.31 | 1.51 | 3.76 ^{ab} ± 0.52 | 1.26 |

*The values presented are means followed by standard deviation (mean ±SD). Means marked with same letter in the same column do not differ ($p > 0.05$) between themselves.

10^5 to 10^6 CFU·g⁻¹ (Morton, 2001). Comparing the results shown in Table 1 with this limit, after sanitization, all the tomatoes were suitable for consumption. Fantuzzi et al. (2004) obtained similar results when assessing the immersion of cabbage in sanitizing solutions and verified a significant decrease of up to 1.8 log CFU·g⁻¹ of mesophilic aerobic bacteria as compared to samples that were washed only in water.

Oliveira et al. (2012) found different levels of effectiveness of the sanitization processes in lettuce than was found in this study with tomatoes; specifically, a 200 mg L⁻¹ hypochlorite solution with 30 min of exposure promoted a 2.5 log CFU·g⁻¹ reduction in bacteria. These results promoted better reduction probably because of the higher time of contact and the particular features of the surface of the sanitized vegetable that are studied. According to Yuk et al. (2006), the microstructures of the plant tissue, such as gouges, cracks, cavities and other irregularities of the surface of the vegetable, can alter the contact of the sanitizing solution with the microorganisms and consequently affect the sanitization efficiency.

After sanitization, a decrease in moulds and yeasts was observed, with the greatest reduction occurring after treatment with 2% acetic acid ($p < 0.05$). However, Fantuzzi et al. (2004) found no significant difference in the reduction of microbial contaminants in cabbage samples treated with 1% acetic acid as compared to washing only with water. However, the present study showed that a higher concentration of acetic acid was more effective than water. Poimenidou et al. (2016) showed that vinegar was effective against *E. coli* O157:H7 with a 2.0 to 2.4 log CFU·g⁻¹ reduction on spinach samples and a 1.8 to 2.3 log CFU·g⁻¹ reduction on rinsed lettuce and vinegar-treated samples maintained the total viable cell counts at low levels during storage. The impact of vinegar on lettuce samples was not significant when the treatment was applied for only 2 min. Organic acid solutions and plant-derived compounds have gained attention due to their antimicrobial activity and their consumer-friendly nature. Organic acids are generally recognized as safe (GRAS) and their

bactericidal efficacy against *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* on fresh produce has been previously investigated (Huang and Chen, 2011; Sagong et al., 2011; Poimenidou et al., 2016). Nascimento and Silva (2010) treated strawberries with different chemical products and observed greater reductions with 4% acetic acid, specifically with reductions of 1.18 and 1.34 log CFU·g⁻¹ for mesophilic aerobic and moulds and yeast. Park et al. (2011) observed that after 10 min of treatment in apples, 1 and 2% acid acetic promoted 0.52 to 2.78 log reduction and exhibited significant ($p < 0.05$) antibacterial effects against *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* as compared to the control treatment.

There were no significant differences between the tomatoes treated with a solution of sodium dichloroisocyanurate and those treated with sodium hypochlorite ($p > 0.05$) for both groups of microorganisms. According to São José and Vanetti (2012), under the typical wash conditions of vegetables, the efficiency of chlorine compounds in reducing microbial contamination is limited, achieving a two-logarithmic reduction in the population of microorganisms. Thus, the results of treatment with 2% acetic acid, which promoted greater reduction in contaminant natural microbiota, is noteworthy. Additionally, it is worth noting that vinegar contains acetic acid, a commonly used ingredient in vegetable salads, and is considered an alternative sanitizing agent for the inactivation of pathogens (Sengun and Karapinar, 2005). However, the concentration of acetic acid in vinegar solutions is low, which may contribute to their reduced efficiency in microbial inactivation.

Evaluation of sanitization treatments on *S. Enteritidis* ATCC 13076 cells attached to the surface of tomatoes

After sanitization with 2% acetic acid, a reduction in *S. Enteritidis* counts was observed, and this reduction was

Table 3. Effect of sanitizing treatments for 15 min to inactivate *S. Enteritidis* ATCC 13076 cells intentionally inoculated in tomatoes (*Solanum lycopersicum* L).

| Treatments | <i>S. Enteritidis</i> (Log CFU·g ⁻¹) | Reduction (Log CFU·g ⁻¹) |
|--|--|--------------------------------------|
| No sanitizer | 6.11 ^a ± 0.43 | - |
| Running water | 5.55 ^{ab} ± 0.56 | 0.55 |
| Sodium dichloroisocyanurate 200 mg·L ⁻¹ | 4.87 ^{ab} ± 0.10 | 1.23 |
| Sodium hypochlorite 200 mg·L ⁻¹ | 4.84 ^{ab} ± 0.57 | 1.26 |
| 2% acetic acid | 4.07 ^b ± 0.83 | 2.04 |
| 2% red vinegar | 5.63 ^a ± 0.54 | 0.47 |

*The values presented are means followed by standard deviation (Mean ±SD). Means that are marked with the same letter in the same column do not differ ($p > 0.05$) between themselves.

significantly higher than the other methods that were applied ($p < 0.05$) (Table 3). In the study by São José et al. (2014), green peppers sanitized with 1% acetic acid for 2 min resulted in a reduction of 1.6 log CFU·g⁻¹. According to Nastou et al. (2012), the efficiency of acetic acid can be limited and vary with the treated vegetable. There was no significant difference between treatments with chlorine compounds and running water ($p > 0.05$); both treatments did not show satisfactory results in the reduction of *Salmonella* cells that were adhered to the surface of the tomato. The discussion on the use of chlorinated compounds is related to the possibility of generating highly carcinogenic by-products such as trihalomethanes, trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane (São José and Vanetti, 2012). This reinforces the need to apply appropriate methods of sanitization to fruits and vegetables.

Yang et al. (2009) evaluated the inactivation of *L. monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium with compounds available in households and observed that after 1 min at 25°C, 3% hydrogen peroxide achieved a >5 log CFU·g⁻¹ reduction of both *S. Typhimurium* and *E. coli* O157:H7, whereas undiluted vinegar had a similar effect only against *S. Typhimurium*. In a study by Sengun and Karapinar (2005), it was observed that 30 min of treatment with 50% vinegar on rocket leaves resulted in a reduction of 2.81 log CFU·g⁻¹. In the same study, scallion samples treated for 60 min had a reduction of 2.1 log CFU·g⁻¹ of the initial population of *Salmonella*. According to Machado et al. (2012), different types of microorganisms may have varying responses to action of antiseptics and disinfectants.

Analysis of the effect of sanitizers on the removal of *Salmonella* adhered to the surface of tomatoes by scanning electron microscopy

The images confirm bacterial adhesion to the surface of

tomatoes without sanitization (Figure 1). The fact that *Salmonella* grows and forms biofilms on the surface of tomatoes and other foods can hinder the action of sanitizers. The ability to strongly adhere to the plant epidermis may reduce the efficiency of the decontamination treatments and complete microbial inactivation might not be possible (Costa et al., 2012). In image B, the removal of attached *Salmonella* cells after treatment with 2% vinegar can be observed. In image C, it can be seen that 2% acetic acid promoted considerable removal of surface-adherent cells, and the tomato had a neater appearance than the other treatments. The inability of sanitizers to remove all microorganisms from the tomato surface suggests a potential for microbial growth in the event of post-sanitization storage and also suggests a chance that pathogenic cells remain on parts of the plant surface or as pre-existing biofilms.

Most of the results presented in the literature are of studies carried out evaluating treatments applied at industrial level but present compatible results. It is known that sanitizing treatments with chlorinated compounds are applied in both food industry and food services. However, caution should be applied on sanitizer application to avoid chemical residues generation that can influence the flavor and aroma of vegetables.

Conclusion

All the studied establishments sanitized vegetables with solutions based on chlorinated compounds or vinegar. These treatments promoted an average reduction of 1 log CFU·g⁻¹, less than the proposed treatment of 2% acetic acid, which was more effective in both reducing contaminants in natural microbiota as well as in tomatoes inoculated with *S. Enteritidis*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

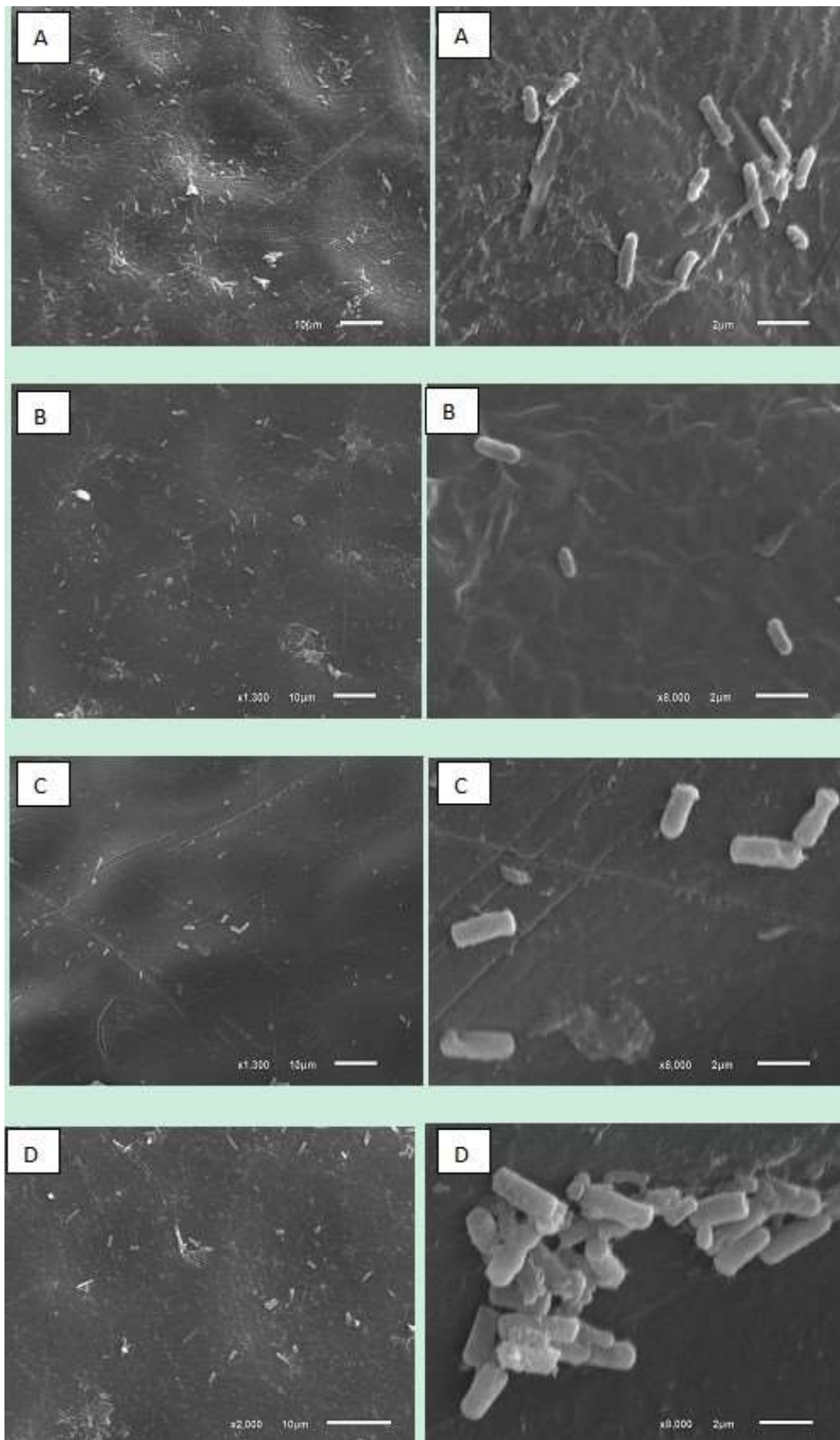


Figure 1. Images obtained by scanning electron microscopy. Photomicrographs of tomatoes cuts intentionally contaminated with *S. Enteritidis*: A) no sanitizing, B) treatment with 2% vinegar, C) treatment with 2% acetic acid, D) dichloroisocyanurate sodium 200 mg/L.

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