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Effect of ultrasound and commercial sanitizers in removing natural contaminants and *Salmonella enterica* Typhimurium on cherry tomatoes

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ABSTRACT

The aim of this study was to evaluate the effectiveness of ultrasound treatment combined with commercial sanitizers in the decontamination step of minimally processed cherry tomatoes. Previously selected cherry tomatoes were treated with ultrasound (45 kHz) for 10 min in the presence of 20 and 200 mg/L sodium dichloroisocyanurate, 5% hydrogen peroxide, 10 mg/L chlorine dioxide or 40 mg/L peracetic acid. The reduction of natural contaminant microbiota and inoculated *Salmonella* adhered to the surface of the tomatoes were evaluated. The aerobic mesophilic contamination on the cherry tomatoes was reduced by 0.7–4.4 $\log_{10}$ cfu/g while molds and yeasts were reduced by 1.1–3.4 $\log_{10}$ cfu/g after different sanitization treatments. The combined treatment of ultrasound and 40 mg/L peracetic acid resulted in the highest reduction of the natural contaminant population and a reduction of adherent *Salmonella* Typhimurium ATCC 14028 by 3.9 $\log_{10}$ cfu/g. These results indicate the potential of using ultrasound as auxiliary strategy in the sanitization of cherry tomatoes.

1. Introduction

The consumption of minimally processed fruits and vegetables has significantly increased over the last few decades as modern society seeks healthier lifestyles (López-Gálvez, Allende, Selma, & Gil, 2009). In addition to the increase in consumption of these foods, there has been an evident concern about high quality that involves nutritional, sensory and microbiological aspects of foods. To minimize the risk of food contain high population of microorganisms, potential sources of contamination in the production environment should be identified and specific preventive measures should be implemented.

In the production chain of minimally processes fruits and vegetables, washing with sanitizing solutions is usually, the only step by which the number of pathogenic and spoilage microorganisms can be reduced. Sanitizing with chemical, that generally include chlorine compounds, is the primary contribution to the safety and preservation of these products (Allende, Selma, López-Gálvez, & Gil, 2008; Artés & Allende, 2005; Ruíz-Cruz, Félix, Cinco, Osuna, & Aguilar, 2007). Moreover, chlorine-based compounds are corrosive and cause skin and respiratory tract irritation (Alvaro et al., 2009). The possibility of water hyperchlorination, which results in high concentrations of trihalomethanes (trichloromethane, bromodichloromethane, dibromochloromethane, tribromomethane and chloroform) and other disinfectant byproducts that are proven carcinogens should also be considered (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007; Ruíz-Cruz et al., 2007; Selma, Ibaneza, Allende, Cantwella, & Suslow, 2008). Chlorinated compounds have been the focus of environmental concern and some environmental groups have suggested ending the use of these products worldwide. In European countries, such as the Netherlands, Sweden, Germany and Belgium, the use of chlorine on minimally processed vegetable products is prohibited (Rico et al., 2007). There have also been studies on emerging pathogens that are more tolerant to chlorinated compounds, which raise further concerns about the effectiveness and use of chlorine in the minimally processed food industry (Allende et al., 2008; Alvaro et al., 2009).

The complete removal and/or inactivation of pathogens such as *Salmonella* from the surfaces of fresh products is considered a challenge for the food industry. Therefore, it is important to develop and evaluate new strategies that contribute to the microbial safety of the products that are available to consumers. These strategies should consider alternatives that do not use or result in toxic residues that can endanger human health and the environment, once according Sagong et al. (2011), consumers increasingly demand that food industries reduce their use of chemical additives.

Ultrasound was adopted by the electronics industry to decontaminate surfaces and its use has recently been recommended as an alternative sanitization step in the food industry (Adekunte, Tiwari, Scannell, Cullen, & O'Donnell, 2010; Cao et al., 2010; Knorr, Zenker, Heinz, & Lee, 2004; Lee, Heinz, & Knorr, 2003; Nascimento et al.,

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2008; Sagong et al., 2011). When applied to liquids, ultrasonic waves promote the occurrence of the cavitation phenomenon, which consists of the formation, growth and collapse of air bubbles. These bubbles generate localized mechanical and chemical energies that are capable of inactivating microorganisms (Adekunte et al., 2010; Gogate & Kabadi, 2009; Piyasena, Mohareb, & McKellar, 2003; Valero et al., 2007). When a bubble collapse occurs, pressure changes which is considered main cause of disruption of microbial cell (Patil, Bourke, Kelly, Frías, & Cullen, 2009; Piyasena et al., 2003).

Ultrasound has been used to disrupt biofilm or even inactivate microorganisms. This strategy may contribute to the processing of many products, including minimally processed fruits and vegetables, and thus help in adapting to new market trends. Therefore, considering the importance to evaluate the efficiency of ultrasonic waves isolated and in combination with sanitizers in reducing contamination on minimally processed foods, the aim of this work was to apply these processes in removing natural contaminants and *Salmonella* that were permitted to adhere to the surface of tomatoes.

2. Materials and methods

2.1. Sanitization of tomatoes

Cherry tomatoes (*Lycopersicon esculentum* var. cerasiforme) were acquired from local retailers and stored under refrigeration at 7 °C for a maximum of 24 h before processing. The cherry tomatoes were selected for firm texture and lack of splits or cracks on the surface. Samples of 100 g of tomatoes were sanitized in 300 mL of the following sanitizers: 50 or 200 mg/L sodium dichloroisocyanurate (Sumaveg®), 5% hydrogen peroxide (Perhydrol®), 40 mg/L peracetic acid (Proxitane®) or 10 mg/L chlorine dioxide (Veromax 80®). During sanitization for 10 min, the samples were treated, or no, with ultrasound at 45 kHz frequency (Ultrasonic Cleaner Model 08849-00, Cole Parmer®). Then, they were dried in a biosafety cabinet for 20 min at 20 ± 0.2 °C. All treatments were carried out at room temperature (20 ± 2 °C).

2.2. Microbiological analysis

After each sanitization treatment, a 25 g sample of cherry tomatoes was 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 and inoculated in standard Plate Count Agar (PCA) (Difco®) that were incubated for 48 h at 35 ± 1 °C to determine the number of aerobic mesophiles. Molds and yeasts were determined by inoculating appropriate aliquots on Potato Dextrose Agar (PDA) (Oxoid®), pH adjusted to 3.5 and, incubated at 25 ± 2 °C for 5 to 7 days. As a control, we examined the number of these microorganisms in tomatoes no submitted to sanitization treatment.

2.3. Contamination of cherry tomatoes with Salmonella

Salmonella enteritidis serovar Typhimurium ATCC 14028 were grown in 200 mL of Luria Bertani (LB) broth at 37 °C for 16 h until reached an approximate population of 6–7 $\log_{10}$ cfu/mL. The cherry tomatoes were washed in sterile distilled water, placed on Petri plates and exposed to ultraviolet light (254 nm) at a distance of 38 cm for 15 min to reduce the naturally-occurring contaminating microorganisms. Preliminary evaluations showed that this ultraviolet radiation treatment reduced de number of contaminants, on average 1.0 $\log_{10}$ of cfu/g on the cherry tomatoes. We placed 10 whole tomatoes in sterile plastic bags and then added 150 mL of 6–7 $\log_{10}$ cfu/mL *Salmonella* culture. After incubation for 48 h at 25 °C, they were removed with sterile tweezers, dried for 20 min in biosafety cabinet and, immersed in 300 mL of the sanitizers evaluated. Each treatment consisted of 50 g of tomatoes in solution of sodium dichloroisocyanurate at 200 mg/L or peracetic acid at 40 mg/L and a treatment of 45 kHz low frequency ultrasound (Ultrasonic Cleaner Model 08849-00, Cole Parmer®) during 10, 20 and 30 min. A combination of peracetic acid and ultrasound treatment for 10 min was also evaluated. Non-sanitized cherry tomatoes washed in sterile distilled water were used as a control.

Samples for microscopic observation were prepared from tomatoes washed and dried as previous described and, after this, the outermost layer was aseptically removed and 1.0 × 1.0 cm sections were made with a sterilized scalpel. The sections were placed in a Petri plate containing sterile water and exposed under ultraviolet light (254 nm) as described above. Approximately, 25 sections were distributed in a 13.5 cm-diameter sterile Petri plates containing LB broth with approximately, 6–7 $\log_{10}$ cfu/mL *Salmonella* Typhimurium ATCC 14028 cells. The sections were then incubated and at 48 and 96 h, five sections were removed with sterile tweezers. The sections underwent the sanitization treatments as described previously and were prepared for microscopic observation. Non-sanitized sections and sections washed only in distilled water were used as controls.

2.3.1. Quantification of adherent cells

After each sanitization treatment, whole tomatoes were washed in a 0.1% sterile peptone saline solution to remove the non-adherent cells. The number of adherent cells on the tomatoes surfaces was determined after homogenizing samples in a stomacher apparatus (Seward, United Kingdom) with sterile 0.1% peptone saline solution for 2 min. Subsequently, an aliquot was removed to prepare the serial dilutions and inoculation onto *Salmonella Shigella* agar (SSA). Colonies were counted after a period of incubation of 18–24 h at 37 °C.

2.3.2. Scanning electron microscopy

The sanitized tomato sections prepared for scanning electron microscopy (SEM) were rinsed in a 0.05 M PBS buffer (pH 6.8–7.2) to remove sanitizer residues and non-adherent cells. The fixation step consisted of a treatment in 1:1 5% glutaraldehyde: 0.1 M PBS buffer for 1 h at room temperature. The sections were then washed six times for 10 min in PBS buffer at 0.05 M (pH 6.8–7.2). The dehydration step consisted of serial treatments in ethanol, consisting of 30%, 50%, 70%, 80% and 95% ethanol for 10 min each and three treatments of 15 min in 100% ethanol. The samples were then transferred to a critical point drier (Critical Point Dryer-model CPD020, Balzers, Liechtenstein) for total dehydration. Finally, the samples were sputter coated (model FDU 010, Bal-Tec, Balzers, Liechtenstein) and the images were recorded on a scanning electron microscope (model LEO 1430 VP Zeiss, Cambridge, England).

2.4. Statistical analyzes

The experiment was conducted four times. The results were analyzed with the aid of the SAS version 9.1 software (Statistical Analysis-SAS Institute Inc., Cary, NC, USA) using analysis of variance (ANOVA). The averages of the logarithms of the number of colony forming units per gram ($\log_{10}$ cfu/g) for each treatment were compared using the Tukey test at 5% probability.

3. Results and discussion

Sanitization treatments evaluated in this study promoted the reduction of 0.7-4.4 $\log_{10}$ cfu/g in the population of mesophilic aerobic and 1.1–3.4 $\log_{10}$ cfu/g in the population of molds and yeasts naturally present in cherry tomatoes (Fig. 1). Treatments with 50 or 200 mg/L sodium dichloroisocyanurate or ultrasound individually, lead to reductions up 1.0 $\log_{10}$ cfu/g of aerobic mesophiles. Elimination of 1.0 $\log_{10}$ cfu/g of mesophilic microorganisms also be related when ultrasound was applied to decontaminate fresh *Tuber aetivum* truffles (Susana-Rivera, Venturini, Oria, & Blanco, 2011). The limitations in reducing microorganisms from the surface of vegetables may be related to the presence of the multilayered hydrophobic cuticle composed of cutin and wax molecules that cover the epidermis of fruits and vegetables, where this cuticle is highly repellent (Velázquez, Barbini, Escudero, & Estrada, 2009). Ultrasonication has potential to be used for the inactivation of bacteria but alone, it is not very effective in killing microorganisms in food at ambient or at sublethal temperatures (Sengül, Erkaya, Baslar, & Ertugay, 2011).

Ultrasound combined with 200 mg/L sodium dichloroisocyanurate or chlorine dioxide reduced respectively, 2.1 $\log_{10}$ cfu/g and 1.9 $\log_{10}$ cfu/g in of aerobic mesophiles (Fig. 1). This value is greater than the sum of the reductions promoted by each individual treatment, indicating a synergistic effect.

Hydrogen peroxide, when applied separately or in combination with ultrasound results, in approximately, 2.1–2.6 $\log_{10}$ cfu/g reduction in contamination. Using a combination of ultrasonic waves and 40 mg/L peracetic acid treatment largest reduction in the population aerobic mesophiles occurred corresponding to 4.4 $\log_{10}$ cfu/g and 3.4 $\log_{10}$ cfu/g in molds and yeasts. One important advantage of the use of peracetic acid is the fact that this compound does not present a risk to the environment and does not produce toxic compounds after reaction with organic materials (Rossini & Gaylarde, 2000). Ultrasound is also considered a clean technology, as are other recently suggested sanitizer alternatives, such as electrolyzed water (Rahman, Ding, & Oh, 2010). Considering that the US Environmental Protection Agency (EPA) Scientific Advisory panel has stated that any treatment which can reduce microbial contamination by 2 logs would be significant and that any new sanitizer or test method presented should be compared to 200 mg/L chlorine as a standard for efficacy (Michaels, Gangar, Schattenberg, Blevins, & Ayers, 2003), we can conclude that the combination of peracetic acid and ultrasound has great potential to replace chlorine in the sanitization step of fresh fruits and vegetables. However, peracetic acid should always be handled in a dry and ventilated and avoided the incidence of direct sunlight. It is recommended the use of personal protective equipment (apron, gloves, protective eye or facial) during handling.

The population of *Salmonella* Typhimurium adhered to the surface of the whole cherry tomato was 7.7 $\log_{10}$ cfu/g and the effects of the different sanitization treatments are shown in Table 1. The presence of sodium dichloroisocyanurate at a concentration of 200 mg/L for 10 min reduced the population of *Salmonella*

Table 1
Effect of sanitization treatments in reducing the population of *Salmonella* Typhimurium ATCC 14028 adhered on surfaces of whole cherry tomatoes.

Treatment	Time (min)	Reductions log (N/N_o)[a]
Sodium dichloroisocyanurate 200 mg L^{-1}	10	0.41 a
Peracetic acid 40 mg L^{-1}	10	2.73 c
Ultrasound 45 kHz	10	0.83 b
Ultrasound 45 kHz	20	1.22 b
Ultrasound 45 kHz	30	1.73 b
Ultrasound 45 kHz + Peracetic acid 40 mg L^{-1}	10	3.88 c

Different letters in the same column indicate statistically significantly different at $p < 0.05$.
[a] N = final number (count of cfu/g in sanitized tomatoes); N_o = initial number (count of cfu/g in water-wash tomatoes).

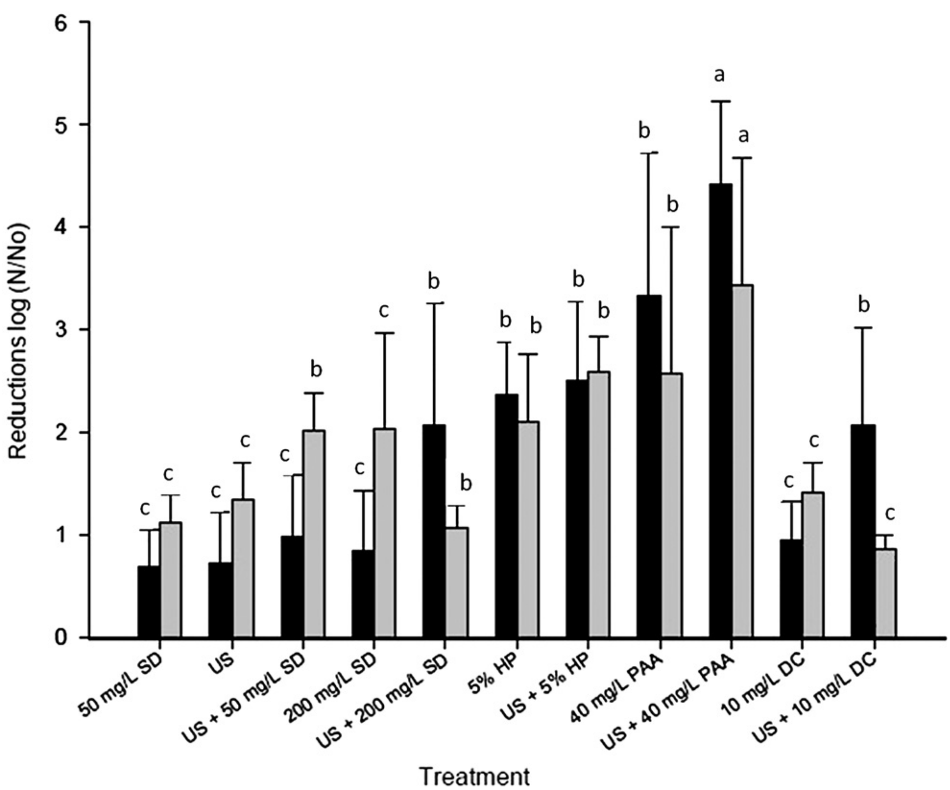

Fig. 1. Number of logarithmic cycles reduced in the initial count of aerobic mesophiles (▭) and molds and yeast (■) contaminants on cherry tomatoes sanitized using different methods. SD, sodium dichloroisocyanurate; US, ultrasound; HP, hydrogen peroxide; PAA, peracetic acid; DC, chlorine dioxide. Treatments indicated with same letter did not differ ($p > 0.05$) between themselves.

Typhimurium ATCC 14028 on the surface of the product by only 0.4 log_{10} cfu/g. In apples, lettuce, strawberry and melons, a 5-min exposure to 200 mg/L chlorine reduced the initial number of 6 log_{10} cfu/mL *Escherichia coli* O157:H7 to undetectable levels (Rodgers, Cash, Siddiq, & Ryser, 2004). Using sanitizers with the same active ingredient, Velázquez et al. (2009) verified a reduction in the number of *Yersinia enterocolitica* on tomatoes by 4.77 log_{10} cfu/g. In fact, other studies have shown that chemical treatments with chlorinated solutions, hydrogen peroxide, alcohol and detergents partially reduce the population of pathogens found on fruits and vegetables (Lapidot, Romling, & Yaron, 2006; Rayner, Veeh, & Flood, 2004).

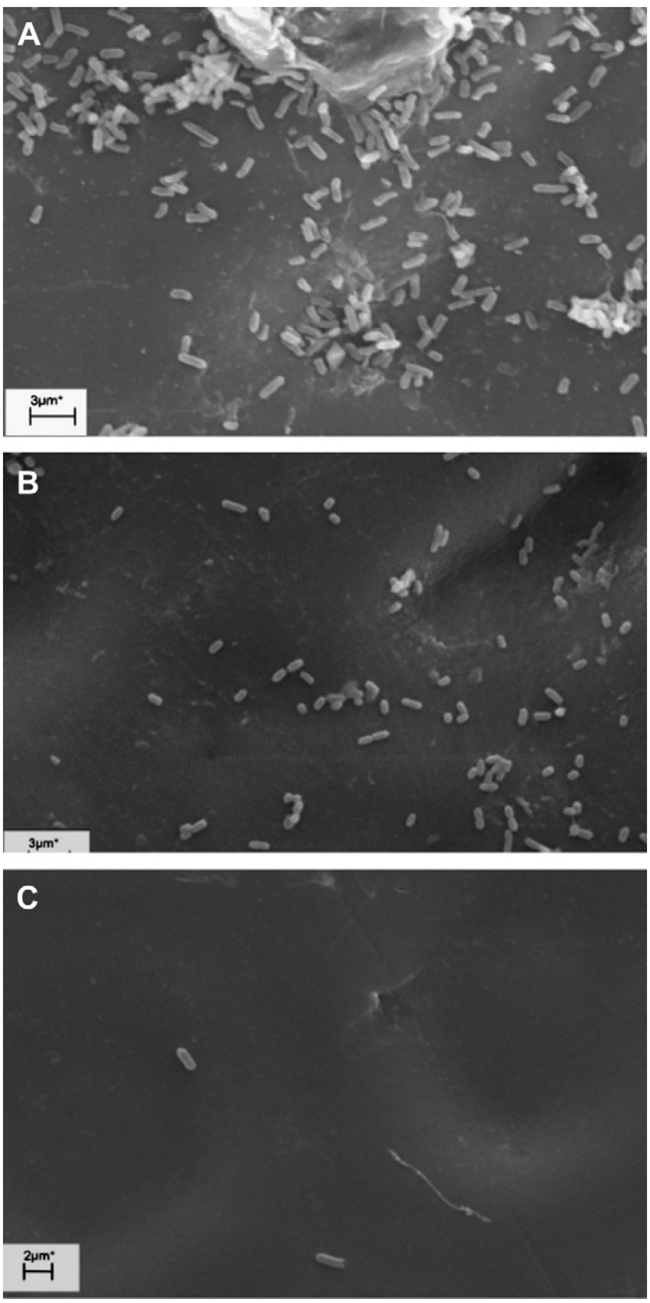

Fig. 2. Photomicrographs of *Salmonella* Typhimurium ATCC 14028 cells adhered to the surface of cherry tomatoes after 48 h, imaged using scanning electron microscopy: non-sanitized (A), after sanitization with 40 mg/L peracetic acid combined with ultrasound for 10 min (B), 45 kHz after ultrasound treatment for 30 min (C).

Ultrasound treatment removed an average of 0.83 log_{10} cfu/g of the *Salmonella* population and the increase in contact time from 10 to 30 min of treatment reduced contamination even further (Table 1). The effectiveness of a 40 kHz ultrasound applied for 10 min was demonstrated for strawberries, where treatments led to a large reduction in the microbial contamination while maintaining the quality indices during storage of the fruits (Cao et al., 2010).

The combined treatment of ultrasound for 10 min and peracetic acid resulted in greater removal of microorganisms from the surface of the tomatoes. Huang et al. (2006) observed that 170 kHz ultrasound removed more efficiency *Salmonella* than *E. coli* O157:H7 from apples surface. These authors also observed that the combination of chemical treatments and ultrasounds resulted in a greater reduction of the contaminant population on fruits and vegetables. Gogate and Kabadi (2009) also concluded that when ultrasounds are used in combination with chemicals, they could increase the efficiency of sanitizing agents. Sagong et al. (2011) found a synergistic effect in the use of organic acids combined with ultrasound (40 kHz) in the inactivation of *E. coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* inoculated on lettuce organic, without significantly affecting the color and texture. The intense pressure generated during the use of ultrasound may contribute to the penetration of the chemical oxidants

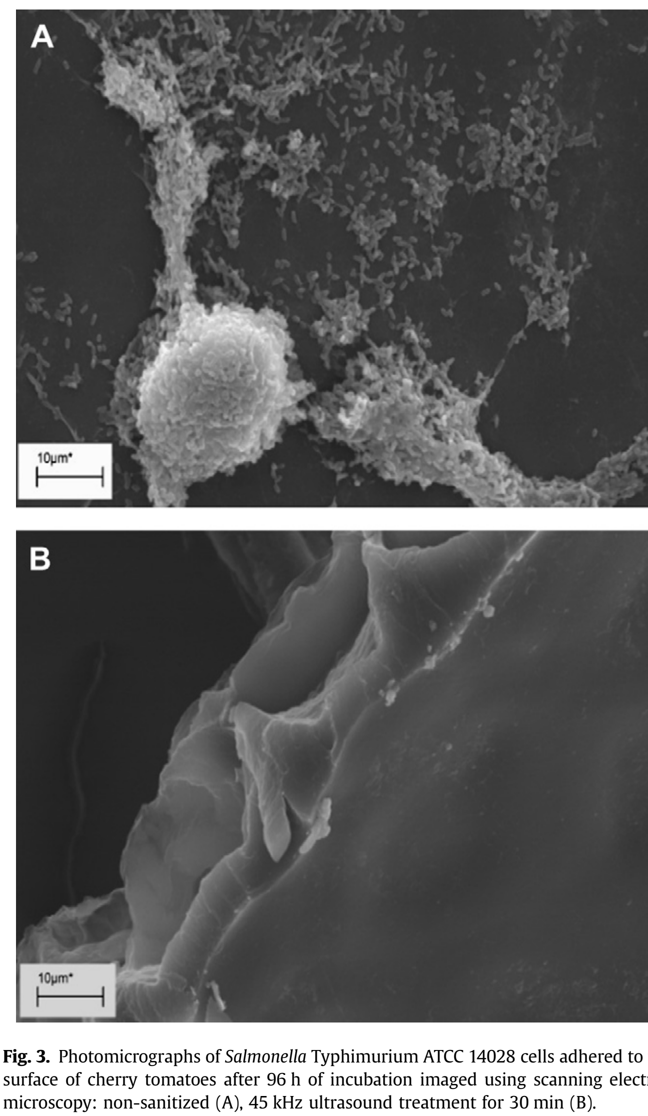

Fig. 3. Photomicrographs of *Salmonella* Typhimurium ATCC 14028 cells adhered to the surface of cherry tomatoes after 96 h of incubation imaged using scanning electron microscopy: non-sanitized (A), 45 kHz ultrasound treatment for 30 min (B).

through the cellular membrane and the cavitation process may assist in the disaggregation of the microorganisms, which culminates in an increased efficiency of the sanitization treatment (Gogate & Kabadi, 2009).

Images obtained by SEM indicated the adherence and biofilm formation by *Salmonella* on the surface of the cherry tomato sections after 48 h incubation (Fig. 2 A). Sanitization treatments with 40 mg/L peracetic acid and a 45 kHz ultrasound reduced this contamination (Fig. 2 B). The inability of sanitization treatments to remove and/or completely inactivate the microorganisms on the surface of fruits and vegetables indicates the possibility for microbial growth during storage or serve as a contamination source. This also suggests that pathogens can persist in protected sites on the surface or within preexisting biofilm on fruits and vegetables. The images showed that prolongation of ultrasound treatment alone to 30 min results in a larger removal of adherent cells (Fig. 2 C). Despite of this decontamination, evaluations should be performed in order to verify whether the increase in the sonication time would damage the quality of the sanitized product during the storage period.

The population of *Salmonella* Typhimurium ATCC 14028 adhered to the surface of cherry tomatoes after 96 h incubation was reduced by almost 4 $\log_{10}$ cfu/g when the combination of 40 kHz ultrasound and peracetic acid was used (Table 1). In microscopic observations of cherry tomato sections incubated in *Salmonella* Typhimurium culture for 96 h we observed large populations of adherent cells at certain points on the product surface in addition to compact clusters of cells, which indicated the presence of biofilm (Fig. 3 A). Even with the large population of adherent cells and the indications of biofilm formation, the 30 min ultrasound treatment was able to significantly remove the cells from the surface of the cherry tomatoes (Fig. 3B). This result confirms that ultrasound, an emerging technology for food processing applications, could enhance a microbial safety of fresh products.

4. Conclusion

The results of this study show that the use of ultrasounds at a frequency of 40 kHz to remove adherent cells is a valid approach. In combination with peracetic acid, sonication results in a greater reduction in the number of cells on the surface of cherry tomatoes, including *Salmonella*. This information is of special importance to the minimally processed industry in developing sanitizer treatments to improve the efficacy on pathogen inactivation on fruit and vegetable and thus, to improve produce safety.

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