

# EFFECT OF ULTRASOUND AND CHEMICAL COMPOUNDS ON MICROBIAL CONTAMINATION, PHYSICOCHEMICAL PARAMETERS AND BIOACTIVE COMPOUNDS OF CHERRY TOMATOES

J.F.B. DE SÃO JOSÉ<sup>a</sup>, H.S. MEDEIROS<sup>b</sup>, N.J. DE ANDRADE<sup>b</sup>, A.M. RAMOS<sup>b</sup>  
and M.C.D. VANETTI<sup>c</sup>

<sup>a</sup>Federal University of Espírito Santo, Vitória, Espírito Santo, Brazil

<sup>b</sup>Food Technology Department, Federal of Viçosa University, Viçosa, Minas Gerais, Brazil

<sup>c</sup>Microbiology Department, Federal of Viçosa University, Viçosa, Minas Gerais, Brazil

\*E-mail address: jackline.jose@ufes.br

## ABSTRACT

In this study, different sanitization methods were evaluated to understand their effects on the microbiological and physicochemical characteristics of cherry tomatoes stored at 7°C. Microbiological analysis showed that treatment with silver nanoparticles at 6 mg/L in combination with ultrasound treatment demonstrated a significant reduction in the assessed microorganism content of the cherry tomatoes. There were no changes in titratable acidity, pH, soluble solids, instrumental color, firmness, lycopene or beta carotene content after treatment followed by 10 d at 7°C. It was observed that cherry tomatoes treated with ultrasound had lower firmness values. Treatment with sodium dichloroisocyanurate, detergent-based surfactants, lactic acid, ultrasound treatment combined with detergent and ultrasound treatment combined with silver nanoparticles all led to increased ascorbic acid content during storage. An application of the silver nanoparticles promoted the best results in terms of microbial count reduction without significantly affecting the quality characteristics of the cherry tomatoes.

*Keywords:* food quality, organic acids, sanitizers, silver nanoparticles, vegetables

## 1. INTRODUCTION

A tomato variety with great popularity worldwide is the cherry tomato (*Lycopersicon esculentum* var *cerasiforme*) (WANG *et al.*, 2008; ZHAO *et al.*, 2010). The cultivation of cherry tomatoes is gaining ground due to the interest in incorporating the plant into modern cuisine (LENUCCI *et al.*, 2006) as it is tasty and sweet and can be consumed either as a fruit or as an appetizer. The reduced size and great versatility of their applications have made cherry tomatoes very popular in diverse culinary preparations. The use of cherry tomatoes as adornments, appetizers and the main feature of several dishes has led to increased consumption of this vegetable. This vegetable is present in diets because it has many antioxidants, such as carotenoids, ascorbic acid and phenolic compounds that can promote beneficial effects (GUERREIRO *et al.*, 2016).

The microbiological quality of fruits and minimally processed vegetables is directly related to the presence of spoilage microorganisms, which cause changes in sensory characteristics. Additionally, the pathogenic micro-organisms in a given population can cause damage to consumer health (RICO *et al.* 2007; LETHO *et al.*, 2011). Fruits and vegetables often possess naturally high amounts of bacteria, yeast and mold (ROMEIO *et al.*, 2010). Microorganism contaminants come from various sources such as irrigation water, manure, cultivation, harvesting, postharvest processing and distribution handling (TORNUK *et al.*, 2011; GUO *et al.*, 2016).

Washing with sanitizing solutions is considered to reduce the number of spoilage and pathogenic microorganisms, thus contributing to product safety (ALLENDE *et al.*, 2008; RAHMAN *et al.*, 2011; SÃO JOSÉ & VANETTI, 2012). Among the sanitizers used in the food industry, especially to wash fresh produce, chlorine and chlorinated compounds are widely used (ALVARO *et al.*, 2009). The ease of use, low cost, high antimicrobial activity and complete dissolution in water make chlorinated agents a common choice for disinfectants in the fruit and vegetable industry (ALLENDE *et al.*, 2008; SANCHEZ *et al.* 2015; YAMANER *et al.*, 2016).

New sanitization treatments have also been evaluated. The choice of sanitizer is important for hygienic and sanitary requirements and to maintain and, where possible, improve the sensory and nutritional characteristics of fruit and vegetables.

Organic acids are generally recognized as safe (GRAS) and can inactivate pathogens involved in food contamination (AKBAS & OLMEZ, 2007; TIRAWAT *et al.*, 2016). Lactic acid is an organic acid that may be applied to fresh produce (SAGONG *et al.*, 2011). Ultrasound is a technology recommended for use in the food industry for different applications. Among these uses is the removal of particles adhering to surfaces and the inactivation of microorganisms (GOLMOHAMADI *et al.*, 2013; BEVILACQUA *et al.*, 2014; SÃO JOSÉ *et al.*, 2014). This inactivation is the result of a process called cavitation, which consists of the formation, growth and collapse of bubbles that generate mechanical energy where chemicals are located. When ultrasound treatment is used in conjunction with chemical agents, the intense pressure gradient allows for penetration of these agents into the cell membrane of microorganisms (PIYASENA *et al.*, 2003; SÃO JOSÉ *et al.*, 2014).

The use of detergents / surfactants in sanitizing solutions or washing water reduces the surface tension of the solution and thus improves contact with bacterial cells, causing a greater inactivation / removal of bacteria from vegetable surfaces (KESKINEN & ANNOUS, 2011). The combination of detergents with physical sanitizing agents may enhance their action (SAGONG *et al.*, 2013).

Silver has been extensively evaluated as a therapeutic agent and for its use in water disinfection processes. Moreover, the interest in the use of silver has increased, especially as a possible replacement for chlorine compounds. Other prospective silver applications are related to post-harvest processing of fruits and vegetables (GOPAL *et al.*, 2010).

It is known that fruits and vegetables undergo a series of changes after harvesting due to the environment, nutrient supplies and crop injury from processing. Sanitization treatments aim to eliminate spoilage and pathogenic microorganisms to provide consumers with a safe product with a longer shelf life. Sanitization should prevent undesired enzymatic reactions as well as microbial growth and multiplication to preserve the nutritional and sensorial aspects of the fruits and vegetables.

Growing consumer interest in food has led to looking beyond the basic functions of food for energy and nutrient supply and into purchasing products that have properties that promote health and prevent diseases. The quality of these food products is strongly related to their preparation, handling and storage. Some studies have shown that these operations have important effects on the content of bioactive compounds (SORIA & VILLAMIEL, 2010; RAWSON *et al.*, 2011a; RAWSON *et al.*, 2011b; PLAZA *et al.*, 2011; ZINOVIADOU *et al.*, 2015). In this context, this study was done to evaluate the effects of different methods of sanitization on the microbiological and physicochemical properties of cherry tomatoes stored at 7°C.

## 2. MATERIALS AND METHODS

### 2.1. Cherry tomato samples

Cherry tomatoes (*Lycopersicon esculentum* var. *cerasiforme*) 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. Tomatoes were washed in water to remove dirt adhered to the surface and were then drained for 30 min in a laminar flow hood.

### 2.2. Preparation of sanitizers

The effects of the following sanitization methods were evaluated: sodium dichloroisocyanurate (Nippon®, Indaiatuba, São Paulo, Brazil) at a concentration of 200 mg/L (SD), 1 % lactic acid (95 %; Vetec®, São Paulo, Brazil) (LA), a solution of silver nanoparticles at 6 mg/L (NP), and detergent Nitrol WV 2640 (Nippon®, Indaiatuba, São Paulo, Brazil) (DET) with or without ultrasound treatment (US) at a low frequency of 40 kHz, 300 W, with a tank size of 6"L x 5.5"W x 4"D (Ultrasonic Cleaner Branson® 1510, St. Louis, USA). A 1 % solution of lactic acid (v/v) was prepared. The distilled water used to prepare the solution was previously sterilized and kept under refrigeration. The colloidal dispersions of silver nanoparticles were obtained according to the methodology developed by FERNANDES (2010).

To prepare the sanitizing solutions that were applied in conjunction with ultrasound, we used water at 5°C. Previously, it was found that the ultrasonic sanitization time implied a 2 degree increase in water temperature. The next sanitization step consisted of immersing the cherry tomatoes in the sanitizing solution for 5 min at a temperature of 7±1°C. A group of tomatoes exposed to treatment with sterile distilled water and a group of tomatoes exposed to no sanitization treatment were used as controls.

For each treatment, 2 kg of cherry tomatoes were sanitized in containers made of polyethylene terephthalate that had previously undergone exposure to ultraviolet light (254 nm) for 30 min in a laminar flow hood. Cherry tomatoes were distributed with 250 g in each container and stored at 7°C for 10 d and evaluated at days 1 and 10 for microbiological qualities and on days 1, 2, 4, 6, 8 and 10 for physicochemical and nutritional qualities.

### 2.3. Microbiological analysis

The procedures used in this step were performed according to the methods of the American Public Health Association (APHA) as described in the Compendium of Methods for the Microbiological Examination of Foods (DOWNES & ITO, 2001).

After each sanitization treatment, 25 g of cherry tomatoes were homogenized separately in 0.1% peptone water at a dilution of  $10^{-1}$ . Homogenization was conducted in a stomacher (Seward Medical Co., London, United Kingdom) for 2 min at the normal speed. Appropriate dilutions were prepared, and aliquots of these dilutions were transferred to growth media specific for the detection of each microbial group. Plating rate experiments were performed in duplicate, and the results were expressed in colony-forming units per gram (CFU/g). To determine the number of aerobic mesophiles, inoculation was performed in standard plate count agar (PCA) (Difco®), followed by incubation for 48 h at  $35 \pm 1^\circ\text{C}$ . Mold and yeast aliquots were inoculated on potato dextrose agar (PDA) (Oxoid®) at a pH of 3.5 and incubated at  $25 \pm 2^\circ\text{C}$  for 5 to 7 d. Coliform counts were carried out at  $35^\circ\text{C}$  using the Petrifilm technique (3M®) according to the recommendations of the Association of Official Analytical Chemists (AOAC, 2005). Plates were incubated at  $35^\circ\text{C}$  for 48 h, enumerating *E. coli* as blue colonies entrapped with gas, and other coliform colonies as those colored red and associated with gas. Standard plate counts were used for aerobic psychrotrophics on Plate Count Agar (Himedia®), with plates incubated at  $7^\circ\text{C} \pm 1^\circ\text{C}$  for 7-10 d. Lactic acid bacteria counts were performed using the method of plating in depth using 1 mL of the sample and a spilling-out quantity of MRS agar (Himedia®) in Petri dishes followed by incubation at  $30^\circ\text{C}$  for 5 d.

### 2.4. Physicochemical analysis

Tomato samples were evaluated for their physical and chemical characteristics. These analyses were intended to identify possible changes in the quality of the cherry tomatoes after sanitization treatments compared to non-sanitized samples (controls).

The determination of titratable acidity (TA) was performed using titration according to the technique described by AOAC (2005). The titration was performed with 0.1 mol/L NaOH, and the results were expressed as % citric acid.

For pH determination, 10 g of tomato samples were randomly collected and homogenized together with 100 mL of distilled water (IAL, 2004). Readings were taken using a Digimed DM 20 pH meter (São Paulo, Brazil).

Total Soluble Solids (TSS) content was determined by refractometry using a portable digital ABBE refractometer at  $25^\circ\text{C}$ . Readings were taken using three drops of pulp juice made with 10 g of tomato. The results were expressed as % (IAL, 2004).

Color change was measured objectively by colorimetry using a Minolta colorimeter Color Reader CR 10 CIELAB system, calibrated with a direct reflectance reading of  $L^*$  coordinates (lightness),  $a^*$  (relative green to red) and  $b^*$  (relative yellow to blue). For each sample, the average of three measurements in five random cherry tomato areas was used for each repetition of treatments in each evaluation day. The parameters  $L^*$ ,  $a^*$  and  $b^*$  were determined using the Hue angle ( $H^\circ$ ) and chroma through the following equations:

$$^\circ\text{H} = \tan^{-1} \cdot (b^*/a^*)$$

$$\text{Chroma} = [(a^*)^2 + (b^*)^2]^{1/2}$$

The firmness of the cherry tomatoes was determined using an Instron Test Apparatus (Series 3367) with a 3-mm diameter probe, a speed of 5 mm/s and a penetration distance of 19 cm before contact with the sample. Five cherry tomatoes were analyzed for each treatment, with two measurements taken per tomato. The results of the firmness analysis were expressed as the maximum force (N).

## 2.5. Extraction and analysis of ascorbic acid

Ascorbic acid (AA) extraction conditions were optimized by CAMPOS *et al.*, (2009). A 5-g sample of the cherry tomatoes and 15 mL of extraction solution (3 % metaphosphoric acid, 8 % acetic acid, 0.3 mol/L sulfuric acid and  $1 \times 10^{-6}$  mol/L EDTA) were mixed using microtrituration for 5 min, centrifuged at 1789 g for 30 min, and the supernatant was adjusted up to 25 mL with ultrapure water. AA was determined according to CAMPOS *et al.*, (2009) using a high-performance liquid chromatograph (Shimadzu) equipped with a LiChrospher 100 RP-18, 250 x 4 mm, 5  $\mu$ m chromatographic column. The flow of the mobile phase (1  $\mu$ mol/L  $\text{NaH}_2\text{PO}_4$ , 1  $\mu$ mol/L EDTA, pH 3.0) was 1.0 mL/min, and the run time was 5.0 min. Elution was detected using a photodiode array detector (Shimadzu SOD-M10 AVP) with the wavelength set to 245 nm. AA identification was done by comparing the retention times obtained for the standard and for samples analyzed under the same conditions. Furthermore, the authors compared the absorption spectra of the standard and the peaks of interest in the samples using a diode array detector. The standard used was L-ascorbate (Vetec, Brazil). Quantification of the compound in the samples was taken from the analytical regression curve equation of ascorbic acid ( $y = 3,870,141.085x + 883,647.051$ ;  $R^2=0.997$ ), and results were expressed in mg of fresh matter.

## 2.6. Extraction and analysis of carotenoids

Carotenoid extraction was performed according to RODRIGUEZ *et al.* (1976) with modifications. Approximately 7 g of tomato were triturated in 60 mL of acetone (divided into three volumes of 20 mL), vacuum filtered on a Buchner funnel and transferred to 50 mL of cold petroleum ether. Extracts obtained from the samples were then concentrated in a rotary evaporator at a temperature between 35 and 37°C. Then, carotenoids were dissolved in 25 mL of petroleum ether and stored in amber glass vials in a freezer (at approximately -5°C) for chromatographic analysis.

Carotenoids were determined according to PINHEIRO-SANT'ANA *et al.* (1998) using a high-performance liquid chromatograph (Shimadzu) equipped with a Phenomenex C18 RP-18 chromatographic column, 4.6 x 250 mm, 5  $\mu$ m. The mobile phase was methanol:ethyl acetate:acetonitrile (50:40:10). Flow of the mobile phase was 2.0 mL/min, and running time was 10 min. Elution was detected using a photodiode array detector (Shimadzu SOD-M10 AVP) with the wavelength set to 450 nm. Identification of carotenoids was done by comparing retention times obtained for standards and samples analyzed under the same conditions. Furthermore, the authors compared the absorption spectra of the standard and the peaks of interest in the samples, using a diode array detector.

The standards used were  $\beta$ -carotene (Sigma Aldrich, USA) and lycopene (Sigma Aldrich, USA). Quantification of the compounds in samples was taken from calibration curves and regression equations for lycopene ( $y = 6.844.138,9670x - 13.153,9821$   $R^2 = 0.9996$ ) and  $\beta$ -carotene ( $y = 7.994.514x + 1.277,3727$ ;  $R^2 = 0.999$ ). The results were expressed in  $\mu$ g/100 g of fresh matter.

## 2.7. Statistical analysis

A completely randomized design was used, with eight treatment groups and a control group (no sanitizing) with tests run in triplicate. To evaluate the efficiency of sanitization treatments on microbial load at the beginning and end of the storage period, Student's t test was used to compare each treatment. To compare the treatments with each other within each period, the results of the counts (log CFU/g) were analyzed with an analysis of variance (ANOVA), with means compared statistically using a Duncan's test with a 5 % significance threshold.

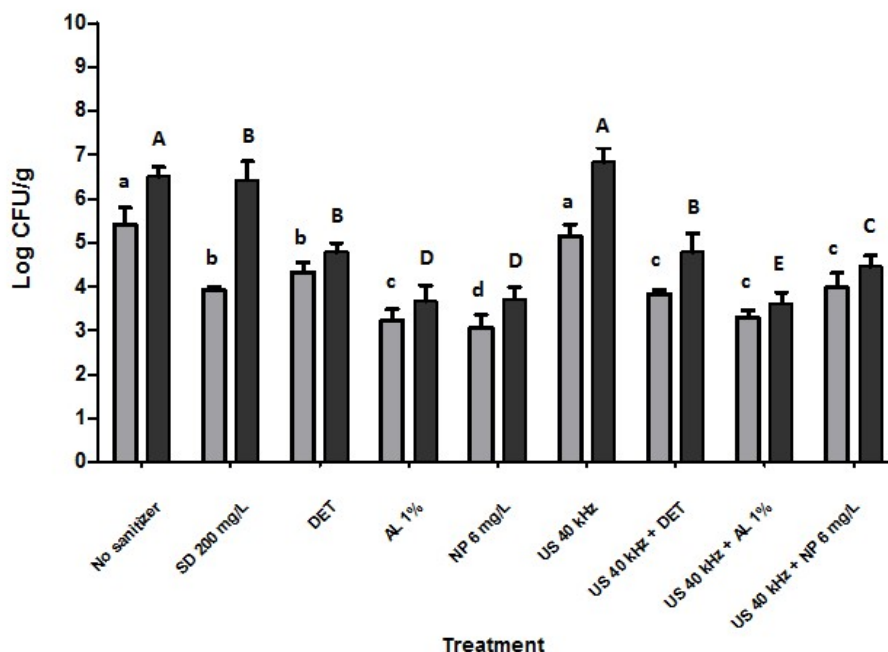
A completely randomized design was used for physicalchemical and nutritional quality with split plots, where treatments were in the plots and time in the sub-plots, with three replicates. The results of the parameters were analyzed using ANOVA, and the means were compared statistically using Duncan's test for qualitative variables (treatment) and regression analysis was performed for quantitative variables (storage time). The significance level used was 5 %.

All statistical analyzes were performed using the Statistical Analysis System (SAS Institute, North Carolina, USA), version 9.1, licensed for use by the Federal University of Viçosa, Minas Gerais, Brazil.

## 3. RESULTS AND DISCUSSION

### 3.1. Microbiological analyses

After sanitization treatments, the mesophilic aerobic bacteria count was reduced between 0.27 and 2.33 log CFU/g compared to cherry tomatoes that were not sanitized (Fig. 1).



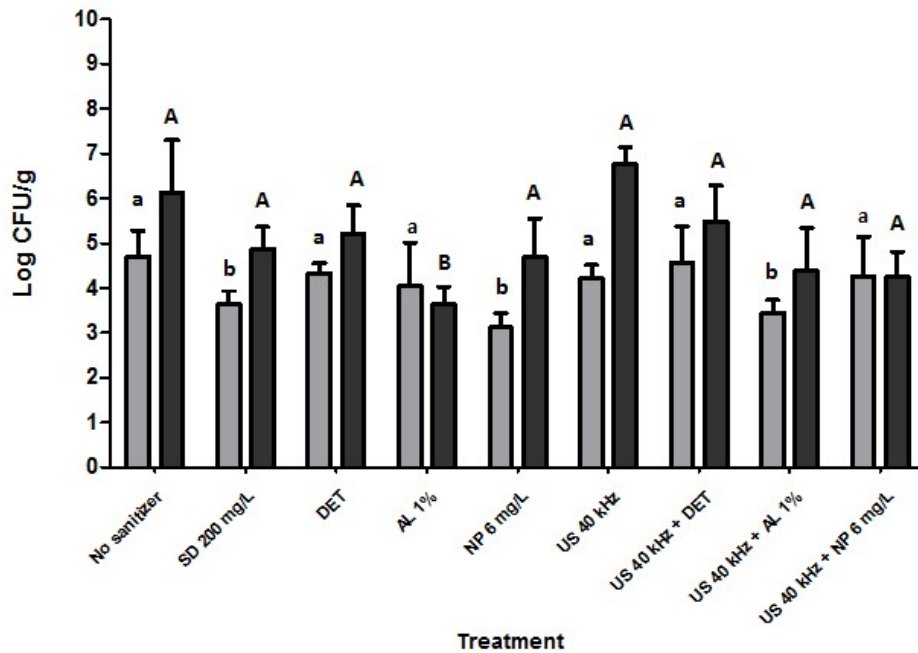
**Figure 1.** Mesophilic aerobic bacteria count and standard deviation after sanitization treatments (□) and the tenth day (■) of storage at 7°C. Treatments indicated with the same lowercase letter did not differ ( $p > 0.05$ ) between one another after sanitization treatments. Treatments indicated with the same capital letter did not differ ( $p > 0.05$ ) between one another on the tenth day.

On the tenth day of storage at 7°C, counts were 6.83 log CFU/g of aerobic mesophilic bacteria on tomatoes sanitized by ultrasound at 40 kHz. Treatment with ultrasound did not achieve reduction of 1.0 log CFU/g, a result that was similar to that observed by SÃO JOSÉ & VANETTI (2012) using ultrasound with a frequency of 45 kHz for 10 min. Susana-Rivera, Venturini, Oria & Blanco (2011) observed a reduction of 1.0 log CFU/g of aerobic mesophilic bacteria by applying ultrasound to decontaminate fresh truffles (*Tuber aestivum*). The limitations for the reduction of microorganisms from the vegetable surface can be related to the presence of a hydrophobic cuticle composed of several layers and cutin wax molecules covering the skin of the fruits and vegetables (VELÁZQUEZ *et al.*, 2009).

The combination of chemical agents with ultrasound promoted reductions between 1.73 and 2.11 log CFU/g. Immediately after sanitization, treatment with detergent promoted a reduction similar to that of sodium dichloroisocyanurate treatment ( $p > 0.05$ ). SÃO JOSÉ *et al.* (2014) and ROSÁRIO *et al.* (2017) mentioned that ultrasound has a potential to be used to inactivate bacteria but must be applied in combination with other sanitizing agents. Treatments with 1 % lactic acid, silver nanoparticles and both of these sanitizing agents combined with ultrasound demonstrated a superior and significant reduction in aerobic mesophilic bacteria counts when compared to the application of sodium dichloroisocyanurate ( $p < 0.05$ ). However, when lactic acid 1 % was combined with ultrasound treatment, there was no significant difference ( $p > 0.05$ ), indicating that in this combination of treatments, a greater reduction in bacteria was not found. When detergent was combined with ultrasound, we found a significant reduction in bacteria when compared to the reduction promoted by the application of only detergent solution ( $p < 0.05$ ). Lactic acid and 1 % silver nanoparticles at 6 mg/L reduced contamination with aerobic mesophilic bacteria by 2.18 and 2.33 log CFU/g, respectively. GOPAL *et al.* (2010) investigated the use of silver (as silver nitrate) and electrochemically generated silver in minimally processed lettuce and found that sanitization with silver was more effective than chlorinated solutions for different micro-organisms, such as mold, yeast, *Enterobacteriaceae* and *Pseudomonas*.

At the end of the storage period, the counts of mesophilic aerobic bacteria in the cherry tomatoes treated with lactic acid, detergent, silver nanoparticles and lactic acid + ultrasound and silver nanoparticles + ultrasound were significantly lower than in the sodium dichloroisocyanurate treatment group ( $p < 0.05$ ).

Fruits and vegetables can also be contaminated by mold and yeast. After harvest, fungal contamination may deteriorate plant material and produce toxic metabolites. Some molds and yeasts can also produce mycotoxins as they grow on these products and others, which present pathogenic risks to consumer health (TOURNAS, 2005). Untreated cherry tomatoes showed 4.70 and 6.13 log CFU/g of mold and yeast after sanitization and at the end of the storage period, respectively (Fig. 2). Treatments promoted reductions between 0.37 and 1.58 log CFU/g. At the initial time of storage, the greatest reductions observed in mold and yeast counts were 1.58 and 1.26 log CFU/g, as registered in samples of cherry tomatoes treated with silver nanoparticles and ultrasound combined with 1 % lactic acid, respectively. A smaller reduction was observed and recorded for mold and yeast counts after treatment with ultrasound applied by itself.



**Figure 2.** Mold and yeast counts and standard deviation after sanitization treatments (□) and the tenth day (■) of storage at 7°C. Treatments indicated with the same lowercase letter did not differ ( $p > 0.05$ ) between one another after sanitization treatments. Treatments indicated with the same capital letter did not differ ( $p > 0.05$ ) between one another on the tenth day.

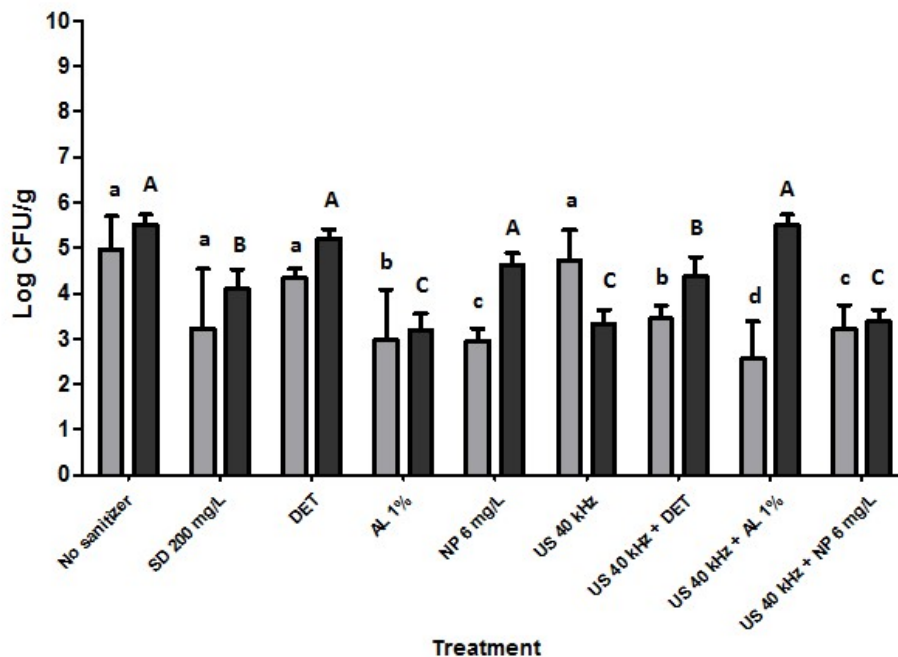
Of the treatments proposed in this work, only sanitization with silver nanoparticles and ultrasound combined with lactic acid promoted reductions statistically equal to sodium dichloroisocyanurate ( $p > 0.05$ ). This result indicates that these treatments had similar effects in reducing mold and yeast counts. A loss of efficiency in the reduction of the mold and yeast counts was observed when detergent was applied to the silver nanoparticles in combination with the ultrasound. In evaluating the effect of ultrasound on the structure of the nanoparticles (data not shown) it was observed that the action of the ultrasound may have contributed to the removal of the surfactant present in the structure of the silver nanoparticle in order to facilitate the approximation of the silver particles. This aggregation of the particles possibly culminated in an increase in diameter and consequently a lower antimicrobial effect.

For coliforms at 35°C, non-sanitized samples showed 5.03 log CFU/g. Among the treatments that stood out, 1 % lactic acid, 6 mg/L silver nanoparticles and ultrasound combined with these two sanitizers promoted, respectively, reductions of 2.06, 2.34, 2.86 and 2.59 log CFU/g after the sanitization processes (Fig. 3). Oliveira *et al.* (2011) detected coliform bacteria in most samples of minimally processed vegetables, with populations of 3 log MNP/g. The presence of these microorganisms can also contribute to reducing the useful life of products (BERBARI *et al.*, 2001). Brazilian law RDC No. 12 of January 2001 (BRAZIL, 2001) provides a value of 10<sup>5</sup> MNP/g for testing sanitized vegetable samples. HUANG and CHEN (2011) studied different sanitizing treatments in spinach and noted that 1 % lactic acid reduced *E. coli* O157:H7 to 1.9 log CFU/g.

The application of an isolated form of ultrasound was inefficient for inactivating coliform, yielding a reduction of 0.27 log CFU/g. Detergent alone promoted a coliform reduction of 0.64 log CFU/g. However, a combination of detergent with ultrasound increased inactivation, resulting in a reduction of 1.53 log CFU/g, and a synergistic effect could be



observed. The intense pressure generated by ultrasound can contribute to the penetration of chemical oxidants through the cell membrane, and the cavitation process assists in the breakdown of microorganisms that culminates in increased efficiency of the sanitizer (GOGATE and KABADI, 2009). It was observed that, at the end of the storage period, the cherry tomatoes treated with lactic acid, ultrasound and ultrasound combined with silver nanoparticles presented lower counts than the tomatoes treated with sodium dichloroisocyanurate.

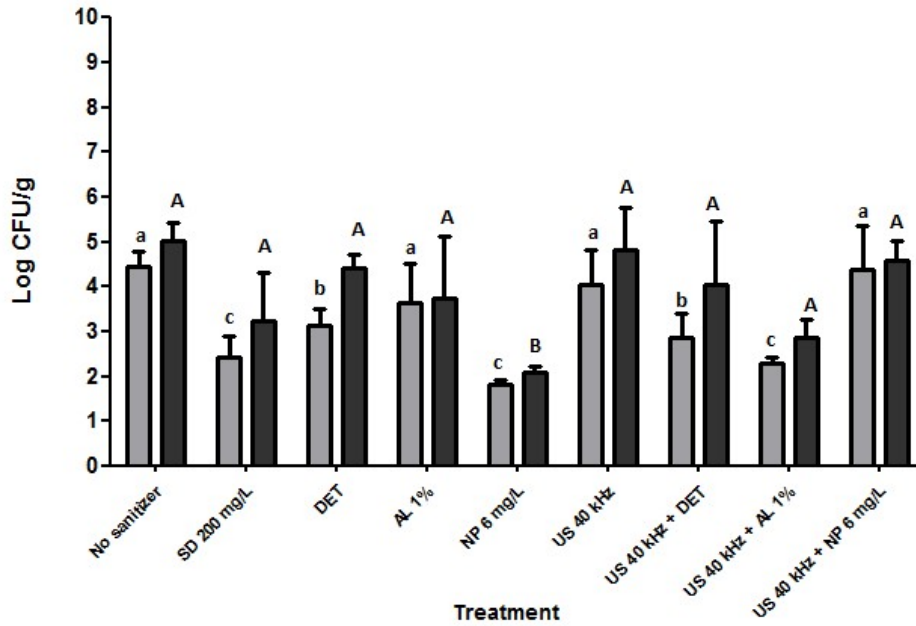


**Figure 3.** Coliform count and standard deviation after sanitization treatments (□) and the tenth day (■) of storage at 7°C. Treatments indicated with the same lowercase letter did not differ ( $p > 0.05$ ) between one another after sanitization treatments. Treatments indicated with the same capital letter did not differ ( $p > 0.05$ ) between one another on tenth day.

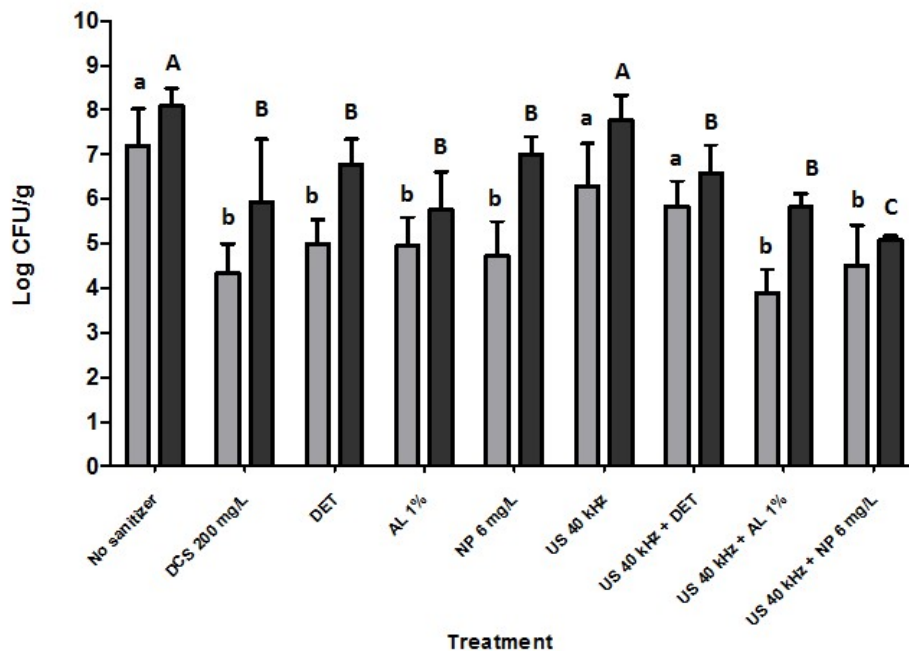
Treatments promoted a reduction of between 0.41 and 2.64 log CFU/g of lactic acid bacteria (Fig. 4).

The counts obtained after treatment with 1 % lactic acid, ultrasound and ultrasound combined with silver nanoparticles did not differ statistically compared to the non-sanitized samples ( $p > 0.05$ ). Higher inactivation was achieved by treatment with sodium dichloroisocyanurate 200 mg/L, ultrasound combined with 1 % lactic acid and silver nanoparticles, which caused reductions of 2.02, 2.14 and 2.64 log CFU/g, respectively. As these treatments showed statistically similar reductions ( $p > 0.05$ ) in lactic acid bacteria count, this may suggest the possible replacement of sodium dichloroisocyanurate treatment with ultrasound combined with lactic acid or silver nanoparticles. Only treatment with silver nanoparticles was efficient in the microbiological control at the end of the storage period.

Psychotropic count after sanitization was between 3.90 and 6.29 log CFU/g, and at the end of storage, sanitized cherry tomatoes achieved scores of approximately 5 log CFU/g (Fig. 5).



**Figure 4.** Lactic acid bacteria counts and standard deviations after sanitization treatments (□) and the tenth day (■) of storage at 7°C. Treatments indicated with the same lowercase letter did not differ ( $p > 0.05$ ) between one another after sanitization treatments. Treatments indicated with the same capital letter did not differ ( $p > 0.05$ ) between one another on the tenth day.



**Figure 5.** Psychrotrophic aerobic counts and standard deviations after sanitization treatments (□) and the tenth day (■) of storage at 7°C. Treatments indicated with the same lowercase letter did not differ ( $p > 0.05$ ) between one another after sanitization treatments. Treatments indicated with the same capital letter did not differ ( $p > 0.05$ ) between one another on the tenth day.

OLIVEIRA *et al.* (2011) evaluated the contaminant microbiota of minimally processed vegetables and observed large populations of psychrotrophic aerobic bacteria in the samples, suggesting a short shelf life of the products. These counts can be related to the

storage condition of the product that favored this group of bacteria. Ultrasound, and ultrasound combined with detergent did not lead to significant reductions in bacterial counts ( $p > 0.05$ ). It is worth mentioning that the ultrasound treatment combined with silver nanoparticles promoted a significant reduction of the psychrotrophic aerobic count after the sanitization process and maintained the lower count at the end of the storage period.

### 3.2. Physicalchemical and bioactive compound analysis

Measurements of pH showed no significant differences ( $p > 0.05$ ) resulting from sanitization treatments, time or interaction between time and treatments. Quality monitoring of this parameter after sanitization and during the storage period was important because sanitizers may influence the pH values of the vegetables, causing changes that may accelerate the deterioration of the product (RICO *et al.*, 2007; SÃO JOSÉ and VANETTI, 2015).

Values of titratable acidity (TA), total soluble solids (TSS), and the ratio of TSS/TA had a significant association with storage time. TA values varied significantly with time, which is best represented by linear regression ( $y = 0,01965x^2 + 0,57070$ ,  $R^2 = 0,87$ ). For TSS, linear and quadratic models were not significant. For TSS/TA, linear regression ( $y = -0,41861x^2 + 12,42302$ ,  $R^2 = 0,99$ ) best represented the data. ADEKUNTE *et al.* (2010) evaluated the effect of ultrasound on tomato juice in different intensities and time and found no change in pH, TA or TSS.

Table 1 shows pH, TA, TSS values and the TSS/TA ratio for the different treatments. TA values ranged from 0.60 to 0.68 % citric acid. This parameter is involved in the assessment of food preservation, since, in most cases, the decomposition of food alters the concentration of hydrogen ions (IAL, 2004) in addition to influencing the sensory characteristics of food. This parameter can vary depending on the degree of maturation and growth conditions (CHITARRA and CHITARRA, 2005). Cao *et al.* (2010) treated strawberries with ultrasound at different frequencies and found that TA content decreased with storage time and that fruit treated with ultrasound at 40 kHz tended to have higher values of TA compared to the control fruit.

Average values of TSS for cherry tomatoes corroborated the mean value observed by PINHO (2011), who recorded 6.1°Brix in cherry tomatoes grown in conventional systems. TSS is a refractive index that indicates the ratio (%) of dissolved solids in solution. It is the sum of sugars (sucrose and hexose), acids (citrate and malate) and other components (phenols, amino acids, soluble pectins, ascorbic acid and mineral salts) present in minor proportions in tomato fruit pulp (BECKLES, 2012). According to the ABHorticultura (2011), the tomato has between 9 and 12 %, which features the highest concentration of soluble solids, especially sugars. CAO *et al.* (2010) evaluated the effect of ultrasound on TSS values and found a decrease in all treatments after strawberry harvest. These authors observed that treatment with 40 kHz ultrasound significantly inhibited the decline of TSS after six days of storage.

In this study, the TSS/TA ratio of cherry tomatoes tended to be lower after treatment with ultrasound combined with chemical sanitizers. This relationship is one of the indices most often used to determine the maturation and palatability of fruit and corresponds to fruit sugar and acid content and is thus an appropriate parameter to measure flavor perception by the consumer (SUAREZ *et al.*, 2008; BECKLES, 2012). JAVANMARDI and KUBOTA (2006) found that the average TSS of tomatoes stored at an ambient temperature and at a low temperature ranged from 5.0 to 5.1 %.

In this study, treatment with sanitizers, storage time and the interaction between storage time and treatments were not found to significantly affect color parameters (Table 1). The color of the food is one of the more attractive attributes for consumers and may vary

between species and even between cultivars. The color of a food product is an important freshness-related attribute for consumers when evaluating quality (SÃO JOSÉ & VANETTI, 2015). GANI *et al.* (2016) observed that ultrasound treatment applied in 10, 20, 30 and 40 min lengths resulted in better retention of color during storage of strawberry samples. MUZAFFAR *et al.* (2016) observed that ultrasound treatment between 30 and 40 min showed better retention of color of cherries (*Prunus avium*) during the storage period at 4°C.

ADEKUNTE *et al.* (2010) observed a decrease in the values of L\* and color parameters a\* and b\* in tomato juice treated with ultrasound under different conditions. They suggested that the color changes observed may have been caused by cavitation, which involves different physical, chemical and biological reactions.

In this study, because it involved the sanitization of intact cherry tomatoes, cavitation may have had less of an effect on color. The color of the tomatoes is mainly related to the presence of carotenoid pigments such as lycopene and is influenced by processing (LIANFU and ZELONG, 2008). SAGONG *et al.* (2011) evaluated the effects of 1 % lactic acid combined with ultrasound on other vegetables, including fresh lettuce, and observed no significant change in L\*, a\* or b\* after 7 days of storage at 4°C.

Firmness values did not change significantly ( $p>0.05$ ) due to the treatment, time or interaction between treatment and time (Table 2). The lack of significant changes in the firmness of the cherry tomatoes after the different sanitization treatments during the storage period indicates a preservation of the tomato structure. Firmness is a critical attribute of quality in the acceptability of fruit and vegetables by the consumer (CAO *et al.*, 2010) and may decrease due to loss of turgor cells due to water loss during storage (AKBAS and ÖLMEZ, 2007).

Despite the changes in firmness between the different treatments not being statistically significant, it was observed that firmness values were lower after treatment with ultrasound compared to after the application of chemical agents. CAO *et al.* (2010) observed that the application of 40 kHz ultrasound markedly inhibited the softening of strawberries, which maintained high levels of firmness during storage through inactivation of polygalacturonase and pectin methyl esterase enzymes. YANG *et al.* (2011) evaluated the effects of ultrasound both as an individual treatment and combined with salicylic acid for 10 min on peaches and observed no change in firmness after 6 days of storage at 20°C. GANI *et al.* (2016) observed that samples treated for 20 and 30 min with ultrasound showed a maximum retention of firmness and the decrease observed at 40 and 60 min could be attributed to the prolonged exposure resulting in cell injury and loss of water.

Other studies evaluating the influence of chemical sanitizers on the firmness of fruits and vegetables (AKBAS and ÖLMEZ, 2007, SAGONG *et al.*, 2011; ALEXANDRE *et al.*, 2012) have similarly observed maintenance of firmness during storage.

Fruits and vegetables lose firmness and freshness characteristics when they are kept in refrigerated storage, even for short periods. In this work, maintaining firmness may have been favored by the intact sanitization of cherry tomatoes. This is an important finding for the processing of this product. An ideal sanitization treatment should be simple and easy to apply without causing physical damage and maintain the sensory characteristics of the food (SUSANA-RIVERA ET AL., 2011).

In addition to the quality features already discussed, the assessment of losses in carotenoid and ascorbic acid contents after sanitization treatments is also important. The content of lycopene and  $\beta$ -carotene in sanitized cherry tomatoes did not change significantly due to sanitization treatments, time, or the interaction of treatment and time (Table 3).

**Table 1.** Mean values\* and standard deviations of pH, TA, TSS, ratio TSS/TA and color parameters of cherry tomatoes under different sanitization treatments stored for 10 d at 7°C.

Treatment	pH	TA	TSS	TSS/TA	<i>L</i> *	<i>a</i> *	<i>b</i> *	Hue	Croma
No sanitizer	4.30±0.33	0.64±0.23	6.59±1.57	10.30±1.36	29.19±1.04	19.41±2.66	27.80±1.66	55.17±4.00	33.98±2.07
SD 200 mg L <sup>-1</sup>	4.27±0.29	0.60±0.22	6.35±1.11	10.58±1.37	29.29±1.01	17.93±2.71	27.40±1.15	56.95±3.94	32.82±1.85
DET	4.30 ±0.37	0.63±0.13	6.22±1.33	9.87±1.44	29.78±1.19	16.78±2.80	26.94±3.94	57.95±5.20	31.88±3.76
1 % AL	4.29 ±0.35	0.62±0.17	6.05±1.04	9.75±1.95	29.80±1.12	18.10±3.69	27.38±2.02	56.78±5.16	32.95±2.95
NP 6 mg L <sup>-1</sup>	4.31 ±0.34	0.63±0.18	5.83±0.84	9.25±1.64	29.59±1.10	17.71± 3.15	27.11±1.94	57.01±4.75	32.49±2.56
US 40 kHz	4.30 ±0.35	0.66±0.18	6.23±1.11	9.43±1.12	28.99±0.76	17.12±2.93	27.07±1.22	57.89±4.10	32.10±2.15
US 40 kHz+DET	4.33 ± 0.32	0.68 ±0.20	5.77±0.89	8.48±2.98	28.97±1.08	16.50±3.09	25.42±0.72	57.27 ±4.74	30.41±1.95
US 40 kHz+1 % AL	4.26±0.33	0.60±0.17	6.46±1.24	10.67±2.95	28.95±0.73	17.77±5.73	26.45±1.53	56.82±6.86	32.13±4.25
US 40 kHz+NP 6 mg L <sup>-1</sup>	4.31 ±0.35	0.62±0.21	5.66±0.79	9.13±3.11	29.10±0.86	16.90±3.09	25.93±1.12	57.13±4.54	31.05±2.14

\*Interaction (treatment × storage time) not significant ( $p > 0.05$ ). Effect of the treatments were not significant ( $p > 0.05$ ).

**Table 2.** Mean values\* and standard deviations of firmness of cherry tomatoes under different sanitization treatments stored for 10 d at 7°C.

Treatment	Firmness (N)
No sanitizer	2.63±0.43
SD 200 mg L <sup>-1</sup>	2.32±0.72
DET	2.33±0.60
1 % AL	2.21±0.58
NP 6 mg L <sup>-1</sup>	2.56±0.75
US 40 kHz	2.07±0.75
US 40 kHz + DET	1.70±0.66
US 40 kHz + 1 % AL	1.75±0.55
US 40 kHz + NP 6 mg L <sup>-1</sup>	1.72±0.63

\*Interaction (treatment × storage time) not significant ( $p > 0.05$ ). Effect of the treatments were not significant ( $p > 0.05$ ).

**Table 3.** Mean\* values and standard deviations of lycopene and β-carotene contents of cherry tomatoes subjected to different treatments sanitization stored for 10 d at 7°C.

Treatment	Lycopene (µg/100 g FM)	β-carotene (µg/100 g FM)
No sanitizer	771.82±180.47	646.43±102.72
SD 200 mg L <sup>-1</sup>	769.24±221.42	704.94±122.04
DET	615.26±186.47	634.61±118.59
1 % AL	737.31± 216.39	635.95±99.93
NP 6 mg L <sup>-1</sup>	682.81±207.61	643.81±119.75
US 40 kHz	797.44±196.56	636.47±120.09
US 40 kHz + DET	653.77±233.90	651.64±132.59
US 40 kHz + 1 % AL	730.44±205.15	630.11±182.98
US 40 kHz + NP 6 mg L <sup>-1</sup>	831.54±207.54	675.78± 92.56

\*Interaction (treatment × storage time) not significant ( $p > 0.05$ ). Effect of the treatments were not significant ( $p > 0.05$ ). FM = Fresh matter

Lycopene degradation can occur due to processing and storage conditions, which cause isomerization and oxidation of this compound. Environmental factors such as oxygen, light and temperature have an important role in this process (DEMIRAY *et al.*, 2013). Free radicals, mechanical energy and hot spots (regions of high temperature and pressure) generated by ultrasonic cavitation can contribute to reducing the contents of nutrients in treated cherry tomatoes. However, this change was not observed, indicating that high retention values were obtained and that the applied sanitizing treatments seemed to have preserved the contents of lycopene and β-carotene.

Studies with other foods that evaluated other bioactive compounds (ALEXANDRE *et al.*, 2012, TIWARI *et al.*, 2010) showed that ultrasound allows for greater retention of anthocyanin content, with the authors suggesting that degradation may occur due to oxidation reactions promoted by the interaction of free radicals formed during sonication. ANESE *et al.* (2013) observed that the total concentration of lycopene from tomato pulp was unaffected by processing with ultrasound, and total contents ranged between

5.10±0.80 and 6.60±1.10 mg/g of dry mass in untreated and ultrasound-treated tomato samples, respectively. Mild heat treatment in tomatoes during food preparation, such as heating, boiling and cooking does not significantly alter carotenoid levels in tomatoes or other vegetables (ANESE *et al.*, 2013).

Storage at low temperatures inhibits lycopene formation (JAVANMARDI and KUBOTA, 2006); thus, the maintenance of the values of lycopene, as observed in this study, may also be associated with the storage conditions used here (7°C). It is important to emphasize the maintenance of lycopene and β-carotene content after sanitization using different treatments, because it is known that these carotenoids are directly related to the color displayed by tomatoes. This confirms the results obtained in the analysis of color parameters, which showed no significant changes due to sanitization treatments during storage. Although several studies (SASS-KISS *et al.*, 2005; KOTÍKOVÁ *et al.*, 2011; ILAHY *et al.*, 2011) on tomatoes have indicated a higher proportion of lycopene to β-carotene, this study indicated similar concentrations of these two carotenoids. This result may be related to the differences between varieties and/or geographical locations and climates that can promote lycopene production to a greater or lesser extent than the other carotenoids (KOTÍKOVÁ *et al.*, 2011).

Regarding ascorbic acid contents, the interaction between treatment and storage time was significant ( $p < 0.05$ ) (Table 4). With regards to this interaction, control treatments, ultrasound and silver nanoparticles showed no significant change over time. The ultrasound treatment combined with 1 % lactic acid exhibited non-significant regression coefficients, which suggests the unsuitability of linear and quadratic regression models to predict the behavior of ascorbic acid contents with respect to time. For the other treatments, regression equations were significant. Although vitamin C is the least stable of all vitamins and is easily destroyed during processing and storage, the present study indicated either maintenance or increased ascorbic acid content for some sanitizing treatments. CRUZ *et al.* (2008) additionally found that the rate of destruction is increased by the action of metals, particularly copper and iron, enzymes, oxygen availability, prolonged heating in the presence of oxygen and exposure to light. GANI *et al.* (2016) observed a decrease in vitamin C content in strawberries when ultrasound was applied for 10, 20, and 30 min.

**Table 4.** Regression equation models for ascorbic acid content in cherry tomatoes submitted to different sanitization treatments and stored.

Treatment	Regression model	R <sup>2</sup>	p > F
No sanitizer	$\bar{Y}=16.80$	-	-
SD 200 mg L <sup>-1</sup>	$0.3674x+12.8796$	0.42	0.0191
DET	$0.8653x+12.2803$	0.81	0.0024
1 % AL	$0.5169x+14.2298$	0.88	0.0017
NP 6 mg L <sup>-1</sup>	$\bar{Y}=15.18$	-	-
US 40 kHz	$\bar{Y}=15.84$	-	-
US 40 kHz + DET	$0.4562x+12.6830$	0.46	0.0155
US 40 kHz + 1 % AL	$Y=21.54$	-	-
US 40 kHz + NP 6 mg L <sup>-1</sup>	$0.4955x+12.9953$	0.75	0.0031

Y= mg of ascorbic acid. Function of storage time (x), determination coefficients (R<sup>2</sup>) and probability levels (p).

During cavitation, free radicals may be produced, and these compounds may react with food. This interaction may be beneficial or not, depending on the process and the food matrix (SORIA and VILLAMIEL, 2010). Rawson *et al.* (2011a) studied the effect of ultrasound on carrot disks and observed retention of vitamin C content results similar to those observed in the present study, which found that ascorbic acid content was preserved in samples that were not sanitized, treated with ultrasound at 40 kHz and treated with silver nanoparticles at 6 mg/L during the storage period. For the other treatments, ascorbic acid contents increased, which may be related to the maturation of the fruit during storage. YAHIA *et al.* (2001) stated that ascorbic acid is subjected to oxidation and reduction reactions during tomato ripening. Oxidation products are free acid radicals that can be processed again to form ascorbic acid, which allows for the increase of the substance as the fruit ripens. ERCAN and SOYSAL (2011) observed no significant difference ( $p > 0.05$ ) in the content of ascorbic acid of tomato extracts after treatment with ultrasound. CAO *et al.* (2010) found higher levels of vitamin C in strawberries treated with ultrasound at 40 and 59 kHz compared to controls. The differences observed in research reported earlier might be due to difference in food samples and treatment parameters.

#### 4. CONCLUSIONS

An application of silver nanoparticles promoted the best results in terms of microbial count reduction. In the case of the mesophilic aerobic bacteria, the application of the silver nanoparticles contributed to the control after the sanitization process and until the end of the storage period. For mold and yeast, silver nanoparticle treatment and a combination of ultrasound and lactic acid were also effective. For coliforms, ultrasound associated with nanoparticles promoted the best effect. For control of lactic bacteria and psychrotrophic aerobic counts, silver nanoparticle treatment was more efficient.

All of the sanitizing treatments maintained pH values, TA, TSS, color parameters, firmness, and lycopene and beta-carotene contents throughout the storage period at 7°C. Ascorbic acid content ranged between treatments and during storage and showed an increase for several treatments, including sodium dichloroisocyanurate, detergent-based surfactants, lactic acid, ultrasound combined with detergent and ultrasound combined with silver nanoparticles. These results indicate that some proposed treatments have the potential for use in sanitizing cherry tomatoes and showed greater effects on reducing microbiological contamination without affecting other quality characteristics of the vegetable. It is worth noting that the proposed treatments presented similar or superior results than those promoted by sodium dichloroisocyanurate. This indicates a possibility of replacement. However, when choosing a sanitization method, it is necessary to evaluate not only the efficiency but also the cost benefit of each one.

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