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Effect of ultrasound on survival and growth of *Escherichia coli* in cactus pear juice during storage

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ABSTRACT

The aim of this study was to investigate the effectiveness of ultrasound as a conservation method for the inactivation of *Escherichia coli* inoculated into cactus pear juices (green and purple). Total soluble solids, pH, titratable acidity, and the kinetics of *E. coli* in cactus pear juices treated by ultrasound (60%, 70%, 80% and 90% amplitude levels for 1, 3 and 5 min) were evaluated over 5 days. Total inactivation was observed in both fruit juices after 5 min of ultrasound treatment at most amplitude levels (with the exception of 60% and 80%). After one and two days of storage, the recovery of bacteria counts was observed in all cactus pear juices. Ultrasound treatment at 90% amplitude for 5 min resulted in non-detectable levels of *E. coli* in cactus pear juice for 2 days. The parameters of pH, titratable acidity and soluble solids were unaffected.

Introduction

Health conscious consumers are demanding minimally processed foods, which has stimulated research on non-thermal processing technologies. Pulsed electric fields, high hydrostatic pressure, shortwave ultraviolet irradiation, and ultrasound, used alone or combined, are intended to achieve microbial and enzymatic inactivation with significantly less heat. Among these technologies, ultrasound processing for food preservation purposes has received increasing attention.[1]

Ultrasounds applied to a liquid medium induce cavitation bubbles, which lead to the disintegration and destruction of microorganisms. The collapse of bubbles results in an area of high temperature and pressure, called the "hot spot".[2] During ultrasound, two phases are distinguished: compression and rarefaction. In the first phase, wave microbubbles are formed at various nucleation sites in the fluid. In the second phase, these bubbles grow rapidly and implode and collapse with a new compression phase, releasing a shock wave that propagates through the liquid.[1] These effects disrupt microbial structures and inactivate and decompose toxic chemicals.[3]

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Various studies addressing the effect of ultrasound alone or combined with other treatments on microbial inactivation have been previously published.[1,4]

Ultrasound is used in a variety of applications, including food processing and food analysis. Two approaches are commonly used: low-intensity (high frequency of 100 kHz to 1 MHz and low power <1 W cm^{-2}) and high-intensity (low frequency of 16–100 kHz and high power of 10–1000 W cm^{-2}) ultrasound.[5] Low-intensity ultrasound generates low power levels such that the treated material is not physically or chemically altered. Generally, low-intensity ultrasound is a non-destructive treatment, which has been successfully used for non-invasive monitoring of food processes[6] and as an analytical technique for determining physicochemical food properties (e.g., texture, density, porosity, grain size, etc.). In contrast, high-intensity ultrasound generates physical disruptions and induces chemical reactions on the material to which it is applied.[7] This ultrasound approach has been used in food manufacturing for peeling, cell disintegration, extraction of intracellular components and enzymes, acceleration of enzyme reactions and microbial fermentation, dispersion of dry powders in liquids, emulsification, deactivation of enzymes and microorganisms, and other processes.[8,9]

Cactus pear (*Opuntia ficus indica*) is a common fruit in Mexico and various regions of Latin American, South Africa, and the Mediterranean[10] and is considered a nutraceutical and functional food[11] because of its high contents of vitamin C, flavonols, phenolic acids and betalains.[12,13] This fruit is classified as a low-acid food (pH > 4.5) and contains a high content of soluble-solids, making it suitable for juice production[14] but also susceptible to microbial spoilage and a short shelf life.[15]

Escherichia coli is a fecal coliform bacteria, commonly found in the intestines of animals and humans. *E. coli* in water and foods is a strong indication of recent fecal contamination, and recognized classes of enterovirulent *E. coli* cause gastroenteritis in humans.[16] *E. coli* cells subjected to heat treatments exhibit variable heat resistance[17] depending on the media, e.g., low pH and high acidity sensitizes cells to heat, whereas high sugar concentrations increase thermotolerance.[18,19] Previous studies have demonstrated that ultrasound can inactivate *E. coli* in water and apple cider[2,20] and that low pH can enhance this effect on the bacteria.[21] These studies have evaluated different ultrasound conditions but the behavior of *E. coli* previously inactivated by ultrasound has not been addressed for other fruit juices, such as cactus pear juice during storage. Therefore, our aim was to evaluate the effect of ultrasound treatment of inoculated cactus pear juices (green and purple) on the pH, soluble solids and survival of *E. coli* over five days of storage.

Materials and methods

Green and purple cactus pear juice preparation

Green and purple cactus pear fruits (*Opuntia ficus indica*) were provided by the Mexican association (CoMeNTuna, Actopan, Hidalgo, México) in the spring of 2012. Fruits free of external injuries were selected, washed and manually peeled. To extract the juices, the pulp was stirred using an industrial blender (38BL52 (LBC10), Waring Commercial®, USA) and then passed through a conventional strainer to remove seeds. Samples were centrifuged (Beckman Coulter, Inc., Allegra 25R, CA, USA) at 15,317 × *g*, 4 °C for 25 min to clarify the juices, and then pasteurized using a water-jacket (400 mL capacity) at a controlled temperature of 85 °C for 25 min to eliminate native microbiota. Juice samples (100 mL) were distributed aseptically into previously sterilized 250 mL glass bottles and then stored at 4 °C until subsequent inoculation and ultrasound treatment. After heat treatment, the juice was analyzed by plating serial dilutions to confirm the sterility of the juice.

Bacteria stock cultures, inoculation

The *E. coli* strain was obtained from the Culture Collection of the Laboratory of Nutrigenomics (Health Science Institute, Autonomous University of the State of Hidalgo, México) and maintained in LB-Glycerol (Sigma–Aldrich, St. Louis, MO, USA). Stock cultures were stored at −80 °C in 0.7 mL tryptic soy broth (TSB: Difco Becton Dickinson Sparks, MD, USA). Cultures were streaked onto tryptic soy agar (TSA; BD Difco™, USA), incubated at 37 °C for 24 h and stored at 4 °C. One colony was inoculated in TSB and incubated with shaking (S1600, Jeiotech, Co., Ltd., Korea) at 37 °C for 24 h. The final concentration of *E. coli* in the inoculum was determined by plating serial dilutions on TSB and incubating at 37 °C for 24 h. Pasteurized juice samples (100 mL) placed in the sterile glass bottles were inoculated with 100 μL of the inoculum to a final concentration of 7 log CFU/mL and allowed to adapt for 20 min prior to ultrasound treatment.

Ultrasound treatment

Inoculated juices were treated using an ultrasound generator (VCX-1500, Sonics & Materials, Inc. Newtown, CT, USA) at 1500 W and a constant frequency of 20 kHz, by applying amplitude levels of 60%, 70%, 80% and 90% for 1, 3 and 5 min with pulse durations of 2 s on and 4 s off. Aliquots of 1 mL of juice were distributed in 1.5 mL sterilized microtubes and analyzed for microbial survival immediately after ultrasound treatment (day 0). An untreated inoculated sample was used as a control. Samples were then stored at 4 °C until analysis after 1, 2, 3, 4 and 5 days of storage. Temperatures before and after the ultrasound treatment were also monitored (Table 1).

pH and total soluble solids (°Brix)

The pH was measured using a digital, calibrated pH-meter (Hanna Instruments, pH 210, USA) and the total soluble solids were measured using a refractometer (Brix/ATC FG-113, Hangzoung Chincan Trading Co., Ltd., China) immediately after ultrasound treatment (day 0) and at the end of storage (day 5).

Titratable acidity (TA)

Samples of 20 mL were placed in 250 mL glass beakers, and 80 mL of distilled water was added. This solution was titrated against standardized 0.1 N NaOH (Sigma–Aldrich, Dublin, Ireland) to the phenolphthalein end point (pH 8.2 ± 0.1). The volume of NaOH was converted to grams of citric acid per

Table 1 – Conditions of ultrasound treatment of green and purple cactus pear juices inoculated with *Escherichia coli*.

Treatment		Temperature (°C)		
		T^1	T^2	
Amplitude	Time (min)		Green	Purple
60%	1	30	38.60 ± 1.69	40.65 ± 0.07
	3	30	50.25 ± 2.19	49.60 ± 0.14
	5	30	62.40 ± 1.97	53.95 ± 0.07
70%	1	30	40.25 ± 0.50	40.60 ± 2.97
	3	30	53.85 ± 0.50	55.05 ± 2.47
	5	30	66.10 ± 0.56	64.30 ± 1.13
80%	1	30	41.30 ± 0.70	38.35 ± 0.77
	3	30	55.25 ± 0.70	56.00 ± 0.00
	5	30	68.50 ± 0.34	65.80 ± 0.14
90%	1	30	34.75 ± 5.16	23.70 ± 4.95
	3	30	48.20 ± 0.57	34.20 ± 7.07
	5	30	62.55 ± 4.03	62.45 ± 4.31

T^1, inlet temperature.
T^2, outlet temperature.
Temperature in ultrasound treatment of green and purple cactus pear juice.

100 mL of juice.[22] TA was measured immediately after ultrasound treatment (day 0) and at the end of storage (day 5), which was calculated using the following formula:

$$\text{TA} = \frac{\text{mL base titrant} \times \text{Normality of base} \times \text{Acid factor} \times 100}{\text{Sample volume (mL)}}$$

Microbiological analysis

Serial dilutions of juices were performed in TSB and plated on TSA for bacteria counts and incubated at 37 °C for 24 h. The results were expressed as log colony forming units per milliliter (CFU/mL) of juice, where the limit of detection is 1 UFC/mL.

Statistical analysis

Data were obtained from three independent experiments. ANOVA was performed to determine significant differences at the 5% probability level using the SPSS® System for WIN™ (15.0.1 version) (SPSS Inc., Chicago, IL, USA). The Student–Neuman–Keuls (SNK) test was used for comparison of the data.

Results and discussion

pH, total soluble solids and titratable acidity

The mean values for pH, total soluble solids and TA in green and purple cactus pear juice are shown in Tables 2 and 3, respectively. Soluble solids and pH determine the degree of ripeness of the fruit and are influenced by physical factors, such as place of origin, species, maturity, and cultivar.[12] The results obtained show that the pH, soluble solids and TA differed significantly ($p < 0.05$) between treatments. Fresh juice (day 1) showed values of pH between 4.68 and 5.68, soluble solids content of 12.78–13.33 °Brix, and TA of 0.01, which are similar to values reported for ultrasound-treated cactus pear juices[23,24] and other fruit juices.[22,25] After 5 days of storage, the pH and soluble solids values changed to ranges of 4.90–5.50

Table 2 – pH, soluble solids and titratable acidity values of green cactus pear juice inoculated with *Escherichia coli* after ultrasound treatment and 5 days of storage.

Determination	Days	Treatment				
		Control	60% 5 min	70% 5 min	80% 5 min	90% 5 min
pH	0	5.68 ± 0.01^{a}	5.21 ± 0.03^{b}	4.68 ± 0.00^{c}	5.21 ± 0.03^{b}	5.28 ± 0.10^{b}
	5	5.52 ± 0.00^{a*}	5.42 ± 0.14^{a*}	4.90 ± 0.03^{d*}	5.12 ± 0.00^{b*}	5.19 ± 0.20^{b}
Soluble solids (°Brix)	0	12.90 ± 0.00^{b}	13.20 ± 0.00^{a}	12.78 ± 0.14^{c}	13.00 ± 0.00^{b}	12.95 ± 0.05^{b}
	5	13.81 ± 0.04^{c*}	16.30 ± 0.10^{a*}	12.93 ± 0.08^{d}	16.00 ± 0.00^{b*}	12.90 ± 0.32^{d}
Titratable acidity (g citric acid/mL)	0	0.01 ± 0.00^{d}	0.08 ± 0.00^{c}	0.15 ± 0.01^{a}	0.08 ± 0.00^{c}	0.14 ± 0.02^{b}
	5	0.01 ± 0.00^{c*}	0.09 ± 0.00^{c}	0.16 ± 0.01^{a}	0.09 ± 0.00^{c}	0.12 ± 0.04^{b}

^{a,b,c} Different letters in the same line indicate significant differences ($p < 0.05$).
* Significant differences between days 0 and 5 of storage for the same treatment ($p < 0.05$).

Table 3 – pH, soluble solids and titratable acidity values of purple cactus pear juice inoculated with *Escherichia coli* after ultrasound treatment and 5 days of storage.

Determination	Days	Treatment				
		Control	60% 5 min	70% 5 min	80% 5 min	90% 5 min
pH	0	4.97 ± 0.00^{b}	5.50 ± 0.01^{a}	4.72 ± 0.01^{c}	5.50 ± 0.00^{a}	5.11 ± 0.27^{b}
	5	$5.52 \pm 0.00^{c*}$	$5.07 \pm 0.00^{a*}$	$4.90 \pm 0.10^{b*}$	$5.07 \pm 0.00^{a*}$	5.00 ± 0.12^{a}
Soluble solids (°Brix)	0	13.33 ± 0.00^{b}	13.33 ± 0.10^{a}	12.90 ± 0.24^{a}	13.00 ± 0.00^{a}	13.33 ± 0.73^{a}
	5	$13.80 \pm 0.21^{b*}$	$14.40 \pm 0.00^{a*}$	12.95 ± 0.05^{c}	$14.00 \pm 0.00^{b*}$	12.90 ± 0.32^{c}
Titratable acidity (g citric acid/mL)	0	0.04 ± 0.00^{d}	0.10 ± 0.00^{c}	0.17 ± 0.01^{a}	0.10 ± 0.00^{c}	0.16 ± 0.01^{b}
	5	$0.01 \pm 0.00^{a*}$	0.10 ± 0.00^{d}	0.17 ± 0.00^{b}	0.10 ± 0.00^{d}	0.14 ± 0.01^{c}

[a,b,c] Different letters in the same line indicate significant differences ($p < 0.05$).

* Significant differences between days 0 and 5 of storage for the same treatment ($p < 0.05$).

and 12.90–16.40 °Brix, respectively, and the TA increased to 0.09–0.17.

Ultrasound treatment causes the release of specific compounds, such as sugars, phenolic compounds and organic acids.[26–28] For instance, release of citric acid may explain the increase in TA after treatment and storage. Ultrasound also exerts a mechanical effect that increases the contact surface between the solid and liquid, allowing for greater penetration of solvent into the matrix and thus greater diffusion of material into the medium.[29]

During storage, significant differences were observed between ultrasound juices and the control ($p < 0.05$) for all of these parameters, whereas experimental samples exhibited differences in pH and soluble solids, except at 90%, which remained unchanged.

Survival and growth of Escherichia coli

E. coli counts after ultrasound treatment and over 5 days of juice storage are shown in Figs. 1 and 2. The initial inoculum was 7 log CFU/mL. Counts increased in the control from day 1 to values >11 log CFU/mL at the end of storage, whereas ultrasound treatment reduced bacteria counts, particularly when higher amplitudes and longer times (3 and 5 min) were applied. It is possible that ultrasound applied to the microbial suspensions disperses microorganism clumps, disrupts cells and modifies cellular activity from the outside to the inside of the structures.[30] These effects result from the combined physical and chemical mechanisms that occur during the collapse of cavitation bubbles, the formation of free radicals (e.g., OH–), and the generation of hydrogen peroxide.[31,32] In addition, during ultrasound treatment, microorganisms are also subjected to mild temperatures (>50 °C), which increase the weakening of the bacteria membrane and possibly further lysis attributed to cavitation.[33–35] In our study, the temperature increased with treatment time (>3 min), and most ultrasonicated juices reached temperatures >50 °C (Table 1). Although all samples subjected to treatment for 5 min reached high temperatures, treated juice at 90% for 5 min showed the total inactivation reaching temperatures of 62.5 °C. We performed additional experiments to prove the bactericidal effect at 62.5 °C to determine the microbial inactivation at this temperature. The result showed reduction only of 4.5 ± 0.4 log CFU/mL and 4.6 ± 0.2 log CFU/mL for green and purple cactus pear juices, respectively, on day 0 (data not showed). Therefore, the combined effects of ultrasound treatment and temperature may explain these results. Similar observations were reported by Herceg et al.[36] who found that amplitude, time, and temperature during ultrasound treatment of milk substantially affected the inactivation of *E. coli*. These findings reinforce the suitability of this type of emerging technology to process liquids without affecting their quality.[23,24]

For both green and purple cactus pear juice, ultrasound applied for 1 min reduced bacteria counts by 1 and 3 log CFU/mL, which increased (3–4 log CFU/mL) when treated for 3 min (Figs. 1 and 2). Inactivation of *E. coli* under the detection limit was observed only in juices treated for 5 min and 90% amplitude (Figs. 1D and 2D); therefore, the high amplitudes and longer treatment times were more effective for microbial inactivation.

During storage, juice subjected to 5 min and 60%, 70% and 80% amplitudes exhibited bacterial growth after treatment (1 day) (Figs. 1A–C and 2A–C), whereas growth was observed after 2 days (Figs. 1D and 2D) at 90% amplitude. This delayed regrowth may result from the disruption of the lipid membrane occurring at higher ultrasound amplitudes, which impairs bacteria growth and may induce artificial competence.[30] The lethal and sublethal effects of ultrasound treatments on microbial cells are strongly influenced by time. Sublethal effects refer to a stage previous to cell death where reversible damage occurs and the cell can recover if the effect ceases under appropriate physical parameters.[37] Certain ultrasound processing conditions seem to be selective in terms of exclusively destabilizing the outer membrane of *E. coli* without severely affecting the cytoplasmic membrane.[38] The effects of increasing intensities of ultrasound on eukaryotic cell viability are well documented.[39,40] Studies performed by Yeo and Liong[38] with gram-negative bacteria, such as *E. coli* and *Haemophilus influenza*, showed that after 5 min of sonication at 40 kHz, the bacteria were nearly eliminated. However, Allison et al.[41] reduced the viability without cell death by applying 20 kHz sonication, which suggests that increasing the power output level of ultrasound results in a faster cell death rate.

The results observed for ultrasound-treated cactus pear juice suggest that regrowth of *E. coli* occurred during storage,

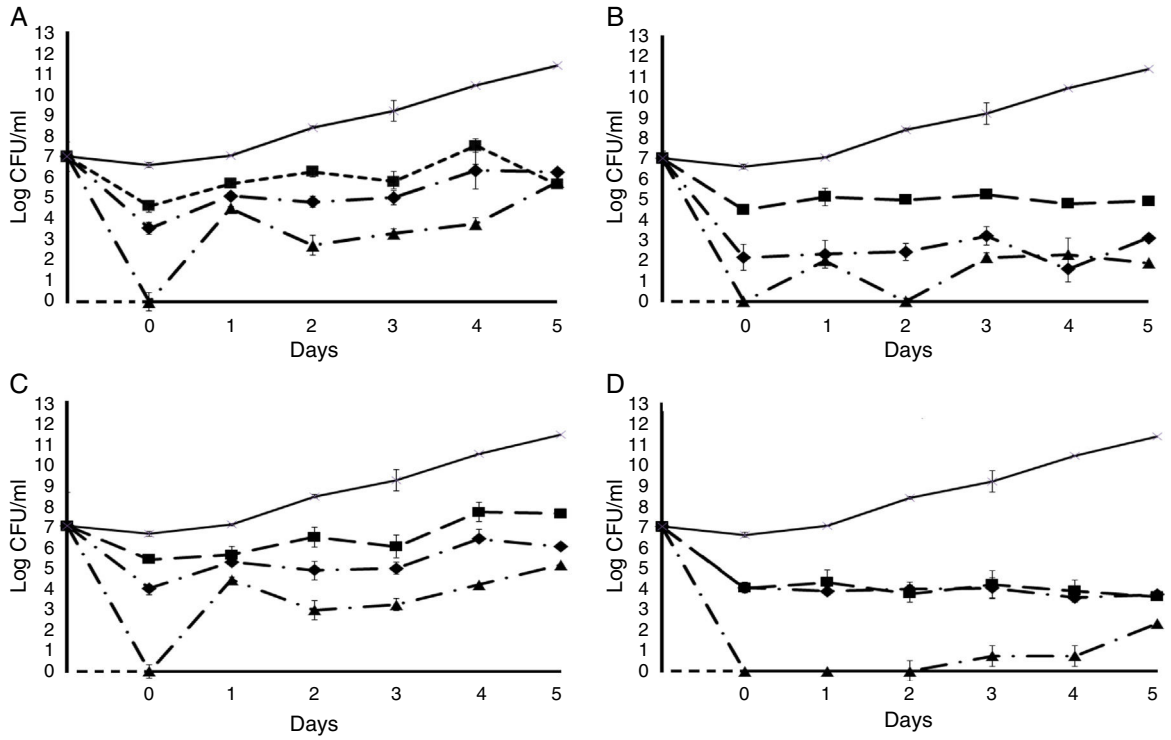

Fig. 1 – Survival and growth of *Escherichia coli* during storage of green cactus pear juice treated by ultrasound at (A) 60%; (B) 70%; (C) 80% and (D) 90% amplitude levels for 1 (■), 3 (♦) and 5 (▲) min and control (×), results of microbiological analysis realized immediately after performing ultrasound treatment (- - -).

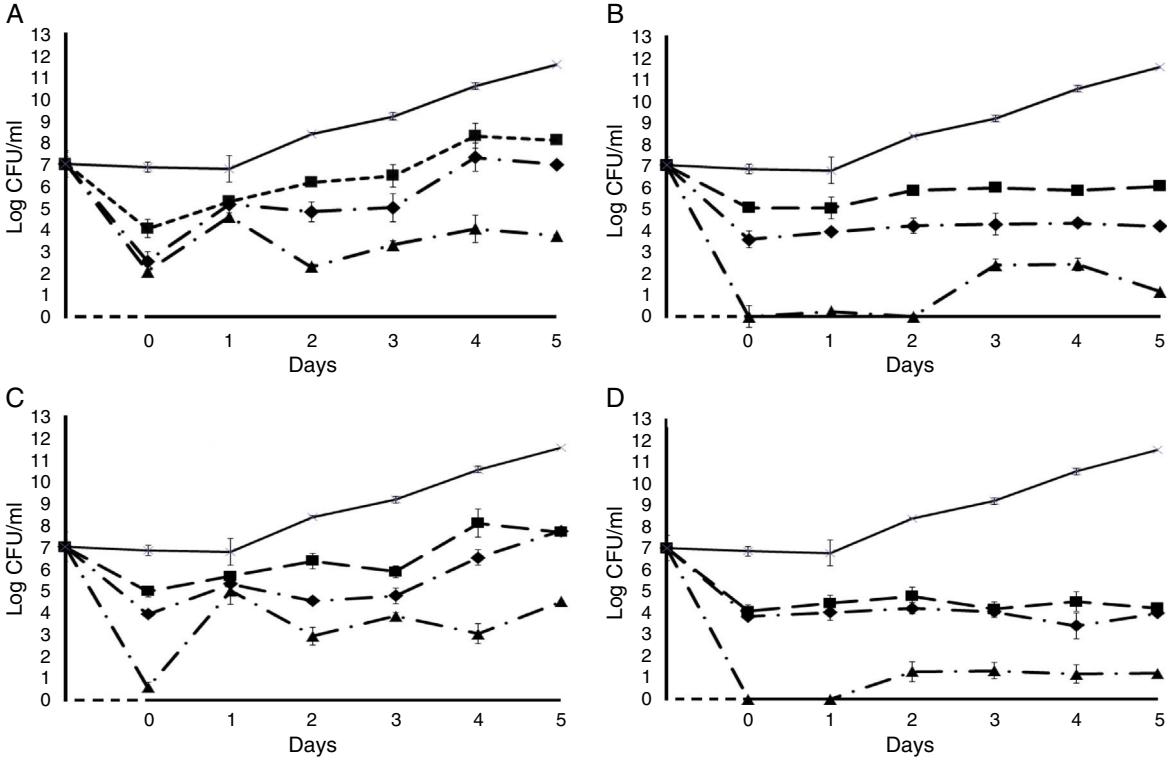

Fig. 2 – Survival and growth of *Escherichia coli* during storage of purple cactus pear juice treated by ultrasound at (A) 60%; (B) 70%; (C) 80% and (D) 90% amplitude levels for 1 (■), 3 (♦) and 5 (▲) min and control (×), results of microbiological analysis realized immediately after performing ultrasound treatment (- - -).

which may be attributed to reversible membrane permeabilization formed upon treatment at low intensities. The permeability may have increased the transport of nutrients and other substances into the cells, alleviating cell metabolism and subsequently enhancing bacterial viability.[42] Ultrasound can also enhance the disruption of cell walls and thus the release of their contents,[43] making them available for bacterial growth. For instance, polysaccharides can be released because of cavitation[44,45] and carbohydrates are used preferentially by *E. coli*.[46] Once the stress over the cells is removed (e.g., ultrasound), respiration and biosynthesis of carbohydrates, membranes, lipids, and proteins can recover, allowing for the regeneration of the cell membrane and bacteria physiology and structural integrity.[47]

These observations may explain the results obtained for samples treated at amplitudes <90%, which reached bacteria counts similar to those of the original load after 4 days of storage at 4 °C (Figs. 1A–C and 2A–C). Other authors have observed that refrigeration enhances survival of *E. coli* in an acidic environment,[48–50] which is likely attributed to the reduced permeability of the cell membrane to protons and/or a reduced metabolic activity.[51] Although treatment at 90% for 5 min exhibited bacteria counts <2 log CFU/mL until the last day of storage (Figs. 1D and 2D), ultrasound at amplitudes of 70% and 90% for 1 and 3 min only showed a bacteriostatic effect (Figs. 1B,D and 2B,D). The results demonstrated that treatment at higher amplitudes (90%) and longer times (5 min) were effective in achieving a 5 log reduction. This value complies with the FDA requirement (<5 log CFU) for fruit juices.

Conclusions

The results from this study revealed that ultrasound treatment at 90% amplitude for 5 min resulted in non-detectable levels of *E. coli* in cactus pear juice for 2 days with no effect on pH, TA and soluble solids. In addition, these results complied with the 5 log reduction of *E. coli* recommended by the FDA guidelines for fruit juices. Under the evaluated conditions, ultrasound treatment can be considered an alternative technology for fruit juice preservation. However, further research is required to achieve conditions that prevent re-growth of bacteria, reach total inactivation during storage and confirm if re-growth results from injured cells.

Conflicts of interest

The authors declare that no conflicts of interest exist.

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