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

Nelly del Socorro Cruz-Cansino[a], *Isidro Reyes-Hernández*[a], *Luis Delgado-Olivares*[a], *Diana Pamela Jaramillo-Bustos*[b], *José Alberto Ariza-Ortega*[a], *Esther Ramírez-Moreno*[a,*]

[a] *Centro de Investigación Interdisciplinario, Área Académica de Nutrición, Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, San Agustín Tlaxiaca, Hidalgo, México, Mexico*
[b] *Mikuna Group Erie, PA, USA*

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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.

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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]

* *Corresponding author.*
E-mail: rme1234@yahoo.com (E. Ramírez-Moreno).
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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

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