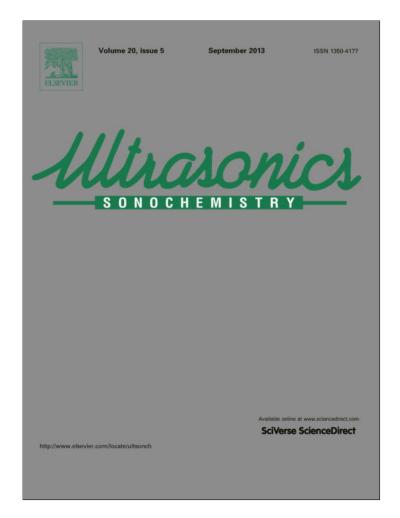
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Effects of ultrasound treatment in purple cactus pear (Opuntia ficus-indica) juice

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ABSTRACT

Cactus pear (*Opuntia ficus-indica*) fruit is a berry with a tasty pulp full of seeds that constitutes about 10–15% of the edible pulp. In Mexico, cactus pear is mainly consumed fresh, but also has the potential to be processed in other products such as juice. The objective of this study was to evaluate the effect of different ultrasound conditions at amplitude levels ranging (40% and 60% for 10, 15, 25 min; 80% for 3, 5, 8, 10, 15 and 25 min) on the characteristics of purple cactus pear juice. The evaluated parameters were related with the quality (stability, °Brix, pH), microbial growth, total phenolic compounds, ascorbic acid and antioxidant activity (ABTS, DPPH and % chelating activity) of purple cactus pear juices. The ultrasound treatment for time period of 15 and 25 min significantly reduced the microbial count in 15 and 25 min, without affecting the juice quality and its antioxidant properties. Juice treated at 80% of amplitude level showed an increased of antioxidant compounds. Our results demonstrated that sonication is a suitable technique for cactus pear processing. This technology allows the achievement of juice safety and quality standards without compromising the retention of antioxidant compounds.

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1. Introduction

Cactus pear (*Opuntia ficus-indica*) can be considered a food of nutraceutical and functional importance [1] due to its high content of chemical constituents as vitamin C, flavonols, phenolic acids and betalains [2,3]. These compounds can help to prevent degenerative diseases such as cancer, diabetes or cardiovascular diseases [4].

In Mexico, cactus pear is mainly consumed fresh, due to its short harvest season. This crop exhibit post-harvest losses that can exceed the 60% primarily because of inadequate handling. The fruit can be processed into other products such as juice [5], and is characterized by a sweet taste derived from a high concentration of sugar and low acidity. However, these characteristics make this fruit susceptible to microbial deterioration and limited shelf life [6,7].

In order to minimize the bacterial load of a product, the manufacturer must reduce the initial contamination, inactivate spoilage microorganisms present in the food and implement procedures to prevent or retard the subsequent growth of microbial populations [8].

Various recent innovative technologies, such as radiation processing, hydrothermal treatments, osmotic dehydration, pulsed electric field applications, and others have been explored to improve shelf life and preserve the nutritional and sensory qualities of fresh fruits or their derived products. Among these technologies the sonication (ultrasound) treatment is an emerging technology that can be cheap, simple, reliable, environmentally friendly, and effective in achieving microbial decontamination [9,10]. When high-power ultrasound propagates in a liquid, cavitation bubbles are generated due to pressure changes. These microbubbles collapse violently in the succeeding compression cycles of a propagated sonic wave. There are also several mechanisms that act when ultrasound is applied in fluids, i.e., thermal effects produced by bubble implosion, mechanical stresses produced by microstreaming, implosion shock waves and, free radical production [11].

Ultrasound has already been evaluated as alternative to heat treatments to process fruit juices without comprising their health benefits and nutritional quality [12,13]. Studies evaluating the characteristics of cactus pear juice subjected to ultrasound treatment are scarce. Therefore, the objective of this study was to evaluate the effect of ultrasound treatments at amplitude levels of 40% and 60% for 10, 15 and 25 min for each amplitude and, 80% with a wide time-range (3, 5, 8, 10, 15 and 25 min) on the characteristics of purple cactus pear juice. The evaluated parameters were juice

Abbreviations: TPC, total plate count; VRBG, violet red bile glucose; ABTS, 2,2'azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt; VCEAC, Vitamin C equivalent antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl. * Corresponding author. Tel.: +52 771 7172000x4213.

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quality (stability, °Brix, pH), microbial growth, total phenolics, ascorbic acid, and antioxidant activity (ABTS, DPPH, chelating activity).

Our results are expected to contribute to the development of sonication treatments of cactus pear juice, that preserve the quality and assure safety to consumers.

2. Materials and methods

2.1. Chemicals

Anhydrous sodium carbonate (Meyer), gallic acid (Meyer), Folin–Ciocalteu reagent (Sigma–Aldrich, 2N), 2,6-dichloroindophenol sodium (DCPI) (Sigma–Aldrich), oxalic acid (Meyer), anhydrous sodium acetate (Meyer), glacial acetic acid (Meyer), ascorbic acid (Meyer), potassium persulfate crystals (Meyer), 2,2'azino-bis(3ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) \geq 98% (TLC) (Sigma–Aldrich), Trolox 97% (Sigma–Aldrich), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma–Aldrich), absolute ethanol (Meyer), ferric chloride tetrahydrate II \geq 99.9% (RT) (Sigma–Aldrich), ferrozine (Sigma–Aldrich), EDTA 99.4–100.06% powder, methanol (Meyer), peptone water (DIBICO), Plate count agar (DIB-ICO), VRGB (DIBICO). All chemicals and reagents used in the study were of analytical grade(AG).

2.2. Sample and treatments

Purple cactus pears (*Opuntia ficus indica*) were obtained from a local market in Pachuca, Hidalgo, Mexico in spring 2009. Fruits free of external injuries were selected, washed and manually peeled. To elaborate juice, the pulp was homogenate using an industrial blender (model 38BL52 LBC10, Waring Comercial[®], USA) and then passed through a strainer to remove seeds.

Juice samples were treated by ultrasound (ultrasonic processor VCX-1500, Sonics & Materials, Inc. Newtown, CT, USA) at 1500 W and a constant frequency of 20 kHz with 40%, 60% and 80% amplitude levels and time (10, 15 and 25 min) varied with pulse durations of 2 s on and 4 s off. At the maximum amplitude (80%), treatment times of 3, 5 and 8 min were also included to evaluate their behavior within a short time. Temperatures reached after treatment were also monitored (Table 1).

2.3. Stability analysis

Determination of cactus pear juice stability was performed according to Cruz et al. [14]. In brief, 10 mL of juice was centrifuged (Hamilton Bell Clinical Centrifuge, model 6500) at 3400 rpm for

Table 1

Conditions of ultrasonic treatment of purple cactus pear juices.

Treatment		Temperature (°C)	
Amplitude (%)	Time (min)	T^1	T^2
40	10	8	38.4 ± 0.07
40	15	8	50.4 ± 1.27
40	25	8	66.1 ± 2.05
60	10	8	43.3 ± 0.85
60	15	8	53.8 ± 0.21
60	25	8	69.2 ± 0.85
80	3	8	29.3 ± 1.91
80	5	8	37.3 ± 1.06
80	8	8	43.6 ± 0.14
80	10	8	49.9 ± 0.49
80	15	8	63.4 ± 1.98
80	25	8	78.2 ± 1.21

T¹ – Initial temperature.

 T^2 – Final temperature.

20 min and stability was expressed as the percentage (w/w) of settled solids obtained after centrifugation.

2.4. Total soluble solids (°Brix) and pH

Soluble solids were measured using a refractometer (model Brix/ATC FG-113, Hangzhou Chincan Trading Co., Ltd., China), and pH was measured using a potenciometer (model pH 210, Hanna instruments, Microprocessor pH-meter, USA).

2.5. Microbiological analysis

Serial dilutions of juice were performed in peptone water solution for microbial count. Total plate count (TPC) was determined in plate count agar incubated at 30 °C for 48 h. Enterobacteria were determined in violet red bile glucose (VRBG) incubated at 37 °C for 24 h. Results were expressed as log colony forming units (CFU) per milliliter of juice according to Cruz et al. [14].

2.6. Determination of total phenolic content

Samples were centrifuged at 3400 rpm for 20 min and the supernatant was filtered through a pore size of 0.22 μ m (Millipore MillexTM – GV PVDF). The filtrate was used to determinate ascorbic acid and antioxidant activity. Total phenolic content of the juice was determined according to Stintzing et al. [15], diluting the sample in deionized water (1:50). Briefly, 100 μ L sample was mixed with 500 μ L of 1:10 diluted Folin–Ciocalteu reagent. After, 400 μ L (7.5%) sodium carbonate was added, then incubated for 30 min at room temperature. The absorbance of the mixture was measured at 765 nm using a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). Gallic acid was used as a reference standard and the results were expressed as mg gallic acid equivalents per liter of juice (mg GAE/L).

2.7. Determination of ascorbic acid content

Ascorbic acid content of the juice was determined according Dürüst et al. [16]. The sample was diluted 1:20 in 0.4% acid oxalic. Briefly, 100 μ L of the juice was mixed with 100 μ L of acetate buffer and 800 μ L of DCPI. The absorbance of the mixture was measured at 520 nm using a microplate reader. Ascorbic acid was used as a reference standard and the results were expressed as mg ascorbic acid per liter of juice (mg AA/L).

2.8. Antioxidant capacity

2.8.1. Antiradical capacity (ABTS)

Antiradical capacity was measured according to Kuskoski et al. [17]. The sample was diluted in deionized water (1:10). Briefly, the radical cation (ABTS⁺) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulfate under dark conditions and room temperature for 16 h before use. The ABTS⁺ solution was diluted with deionized water to an absorbance of 0.70 ± 0.10 at 754 nm. After the addition of 20 µL of sample to 980 µL of diluted ABTS⁺ solution, absorbance readings were taken after incubation for 7 min at room temperature. The absorbance of the mixture was measured at 754 nm using a microplate reader. The antioxidant capacity (VCEAC) per 100 mL of juice (mg VCEAC/100 mL).

2.8.2. Free radical scavenging activity

Antiradical activity was measured with DPPH⁻ radical as described by Morales et al. [18]. The sample was diluted in deionized water (1:50). An ethanolic solution (7.4 mg/100 mL) of the stable

DPPH' radical was prepared. Then 100 μ L of sample was taken into vials and 500 μ L of DPPH' solution was added, and the mixture was left to stand 1 h at room temperature. The solution was stirred and centrifuged at 3,000 rpm during 10 min. Finally, absorbance was measured at 520 nm using a microplate reader and μ mol Trolox equivalents per liter of juice (μ mol TE/L) were obtained.

2.8.3. Chelating activity of ferrous ions

Chelating activity was determined as described by Gulcin et al. [19]. The sample was diluted in deionized water (1:20). Briefly, 100 μ L of sample was placed in vials and 50 μ L of ferric (II) chloride solution (2 mM) and 450 μ L of methanol was added. The mixture was vortexed and left for 5 min at room temperature. Then 400 μ L of ferrozine (5 mM) was added and the mixture was vortexed again and allowed to settle for 10 min at room temperature. The absorbance was read at 562 nm using a microplate reader. As reference chelating agent was used (0.1 M EDTA), and deionized water was used as control. The chelating activity was calculated using the following equation:

% chelating activity = $[(A_0-A_1)/A_0]^*100$

where:

 A_0 = absorbance of the control sample.

A₁ = absorbance of the sample.

2.9. Statistical analysis

All values were obtained by triplicate and expressed as mean ± standard deviation (SD). Data were analyzed performing a one-way analysis of variance (ANOVA) and differences among means were determined using a Tukey test with a level of significance of p < 0.05. The statistical package SPSS[®] System for WINTM version 12.0.1 was used.

3. Results and discussion

3.1. Stability, total soluble solids and pH

The ripening degree of cactus pear fruit is determined by the total solids content (°Brix) and pH, parameters influenced by physical and chemical factors such as place of origin, plant species, state of maturation, and the cultivar [2]. According to the Mexican standard PC-046-2005 [20], °Brix value of a commercial-quality cactus pear should be at least 12%. Due to the high sugar content and high pH juice of these fruits, they are characterized by a sweet taste. The sonication treatment and time had no effect on these juice

 Table 2

 Effect of sonication on stability, °Brix and pH in purple cactus pear juice. ^A

Amplitude (%)	Stability	°Brix	рН
Control	37.48 ± 3.37 ^d	12.52 ± 0.49^{abc}	5.09 ± 0.14^{a}
40/10 min	25.12 ± 2.13 ^{ab}	12.05 ± 0.41 ^a	5.00 ± 0.20^{a}
40/15 min	27.47 ± 2.02 ^{bc}	13.13 ± 0.83 ^{bc}	5.06 ± 0.19^{a}
40/25 min	29.01 ± 1.52 ^c	13.15 ± 0.81 ^{bc}	5.09 ± 0.18^{a}
60/10 min	23.61 ± 1.80 ^a	12.22 ± 0.33 ^{ab}	5.03 ± 0.19^{a}
60/15 min	26.59 ± 1.12 ^{ab}	13.22 ± 0.84 ^{bc}	5.11 ± 0.19^{a}
60/25 min	24.66 ± 3.02 ^{ab}	13.00 ± 1.21 ^{abc}	5.08 ± 0.08^{a}
80/3 min	25.59 ± 2.10 ^{ab}	12.13 ± 0.22 ^{ab}	5.03 ± 0.25^{a}
80/5 min	24.76 ± 1.45 ^{ab}	12.16 ± 0.23 ^{ab}	5.00 ± 0.24^{a}
80/8 min	23.78 ± 1.50 ^a	12.20 ± 0.30 ^{ab}	5.01 ± 0.21^{a}
80/10 min	24.76 ± 2.68 ^{ab}	12.20 ± 0.30 ^{ab}	5.00 ± 0.23^{a}
80/15 min	23.65 ± 1.33 ^a	13.04 ± 0.96 ^{abc}	5.05 ± 0.05^{a}
80/25 min	25.47 ± 1.92 ^{ab}	13.33 ± 1.05 ^c	5.04 ± 0.05^{a}

^ADifferent letters in the same column indicate significant differences (p < 0.05). Values are mean ± SD (n = 9).

parameters maintaining its quality characteristics (Table 2). In addition, ultrasound treated juices showed good stability (measured as% settled solids). According to other studies [21,13] ultrasound reduces the particle size due to the collapse of cavitation bubbles formed on the surface, this allows fine particles to remain in the supernatant after centrifugation and adds stability to the juice.

3.2. Microbiological analysis

Conventional thermal pasteurization and sterilization are widely used to reduce the microbial load in food products. However, these thermal methods adversely affect the sensory quality and nutritional value of liquid foods [22]. For this reason, emerging technologies are suitable alternatives to treat food products with minimal effects on their characteristics. Table 3 shows the effect of sonication treatment on the microorganisms content of cactus pear juice. The control sample showed values of 4.07 and 4.40 log CFU/mL for TPC and Enterobacteria respectively. TPC and Enterobacteria was reduced when juice was sonicated for \geq 15 min inclusive this microbiological load was not detected with the increase of amplitude and time. The results obtained with the ultrasound treatment are considered satisfactory compared with sanitary regulations for pasteurized juice (2 log CFU/mL to TPC and Enterobacteria) [23,24]. Similar results has been reported suggesting that microbial cells destruction occur only if the treatment is applied for longer periods time [12]. Cell disruption may be caused by several factors such as the combined physical and chemical mechanisms that occur during cavitation, the formation of free radicals and hydrogen peroxide [25], leading to a thinning of microbial cell membranes, and the restricted mild heating that occurs during sonication treatments [12]. Sonication treatments at 80% amplitude level for 15 and 25 min reached similar pasteurization temperature (Table 1) and decreased microbial loads (Table 3). In contrast, TPC increased in juices treated at 40% and 60% amplitude levels for 10 min, and at 80% for 3 and 5 min. Other authors found that during sonication there is a released of specific compounds that may be used by microorganisms [26]. In this study these released compounds were not specific by Enterobacteria.

3.3. Total phenolics

The content of phenolic compounds in the control sample was 1654.6 ± 23.4 mg GAE/L. After sonication, all juice samples showed contents above this value (Fig. 1). Sonication treatments at 40% and

Table 3

Effect of sonication treatment on the survival of microorganisms in purple cactus pear juices. $^{\rm A}$

Amplit (%)	ude Total plate	e count (log CFU/mL)	Enterobacteria (log CF	U/mL)
Control	4.07 ± 0.12	2 ^d	4.40 ± 0.18^{h}	
40/10 r	nin 4.72 ± 0.17	7 ^g	3.85 ± 0.05^{f}	
40/15 r	nin 4.12 ± 0.12	7 ^{de}	1.52 ± 0.05^{b}	
40/25 r	nin ND ^B		ND	
60/10 r	nin 4.37 ± 0.15	5 ^{ef}	4.10 ± 0.00^{g}	
60/15 r	nin 2.42 ± 0.38	3 ^b	2.37 ± 0.25 ^c	
60/25 r	nin ND		ND	
80/3 m	in 4.40 ± 0.00) ^{ef}	3.77 ± 0.15^{f}	
80/5 m	in 4.60 ± 0.20) ^{fg}	4.22 ± 0.05^{gh}	
80/8 m	in 4.37 ± 0.15	5 ^{ef}	3.52 ± 0.28^{e}	
80/10 r	nin 3.70 ± 0.00) ^c	3.27 ± 0.05^{d}	
80/15 r	nin ND		ND	
80/25 r	nin ND		ND	

^ADifferent letters in the same column indicate significant differences (p < 0.05). Values are mean ± SD (n = 9).

^BNot detected.

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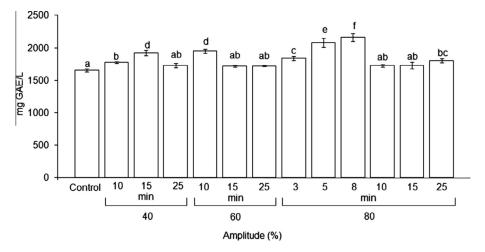


Fig. 1. Content of total phenolic compounds in purple cactus pear juice, mg gallic acid equivalents per liter of juice (mg GAE/L). ^aDifferent letters indicate a significant difference (*p* < 0.05) between treatments.

60% amplitude levels for 15 and 10 min, respectively, had between 1925 to 1950 mg GAE/L, and the treatments at 80% amplitude level for 5 and 8 min had a significant release of total phenolics content (2077.4 ± 68.1 and 2160.7 ± 58.2 mg GAE/L, respectively). These results agreed with other findings [12]. Phenolic compounds are present in the vacuole in soluble form or bound to the cell wall such as pectin, cellulose, hemicellulose, and lignin traces [27]. It is possible that the use of power ultrasound enhanced the disruption of biological cell walls, and facilitates the release of their contents [21] via cavitational collapse in the surroundings of colloidal particles [13].

3.4. Ascorbic acid content

Ascorbic acid is a good indicator of commercial maturity of the fruits [28] and is an important antioxidant nutrient [29]. The content of ascorbic acid found in the cactus pear juice was $352.6 \pm 4.3 \text{ mg} \text{ AA/L}$ (Fig. 2), which indicates a good fruit commercial maturity according to other related studies [30]. The content of ascorbic acid in all the treatments was similar to the control sample except for the juice treated at 80% amplitude level for 25 min (415.6 \pm 7.0 mg AA/L) where the values resulted higher. These results agree with other similar studies such as sonicated guava juice [13]. The increase of these compounds could be attributed to the elimination of dissolved oxygen which is essential for ascorbic acid degradation during cavitation produced by the sonication treatment [13]. However, in juice treated at the same

amplitude (80%) at 8 and 15 min the content of ascorbic acid decreased. Several authors [11,31] had explained the loss of ascorbic acid during the sonication process is caused by oxidative processes in aerobic and anaerobic environments associated with the production and use of hydroxyl radicals.

3.5. Antioxidant activity

The antioxidant capacity of a food is determined by its content of a mixture of antioxidants with different mechanisms of action such as synergistic interactions. Therefore it is necessary to combine more than one method to determine *in vitro* antioxidant capacity of foodstuffs [32,17]. In this study we use three methods (ABTS, DPPH, chelating activity) to determinate the antioxidant activity in purple cactus pear. In general, we found that the treatments with ultrasound did not affect adversely the antioxidant activity of the juice as other conventional treatments do [33].

3.5.1. Antiradical capacity by ABTS

ABTS is frequently used by the food industry and agricultural researches to measure the antioxidant activity of food. It measures the ability of pure substances or crude extracts to trap free radicals by donating hydrogen atoms or electrons [30]. One of the most convenient ways to express this antiradical activity is to refer to their equivalent values of vitamin C (VCEAC). This is one of the major active substances responsible for the antioxidant activity [34]. The antiradical capacity of purple cactus pear juice varied with the ultra-

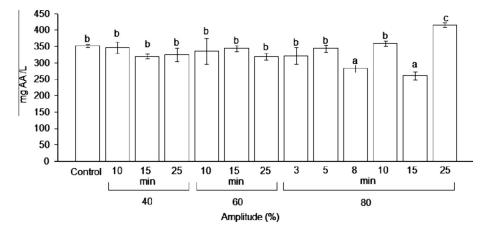


Fig. 2. Ascorbic acid in purple cactus pear juice, mg ascorbic acid per liter of juice (mg AA/L).^aDifferent letters indicate a significant difference (p < 0.05) between treatments.

sound amplitude and time. Antioxidant activity after sonication treatments at 60% and 80% amplitude levels for 15 min were similar to the control sample value $(26.3 \pm 0.1 \text{ mg VCEAC}/100 \text{ mL})$ (Fig. 3a). Our results showed that sonication at higher amplitudes and time released higher levels of antioxidants. Therefore, these had a higher antioxidant activity in comparison with those with low amplitude.

treatments had an antioxidant activity similar to control sample (4368.5 \pm 182.5 μ mol TE/L). A higher antioxidant activity was observed in juice treated at 80% amplitude level for 15 min (4812.9 \pm 166.6 μ mol TE/L). As in other fruits, the total antiradical activity in cactus pear is the sum of the antiradical activities of all antioxidant compounds in the fruit, such as phenolic compounds, taurine, vitamins, betalains and ascorbic acid [29]. Then, the stability or increase of the antioxidant activity in the juice may be attributed to the ascorbic acid and all the phenolic compounds that are

exhibited a lower antioxidant activity, juice subjected to sonication

3.5.2. Free radical scavenging activity

Fig. 3b shows the results of DPPH of cactus pear juice. Despite that low amplitude levels (40% for 25 min and 60% for 10 min)

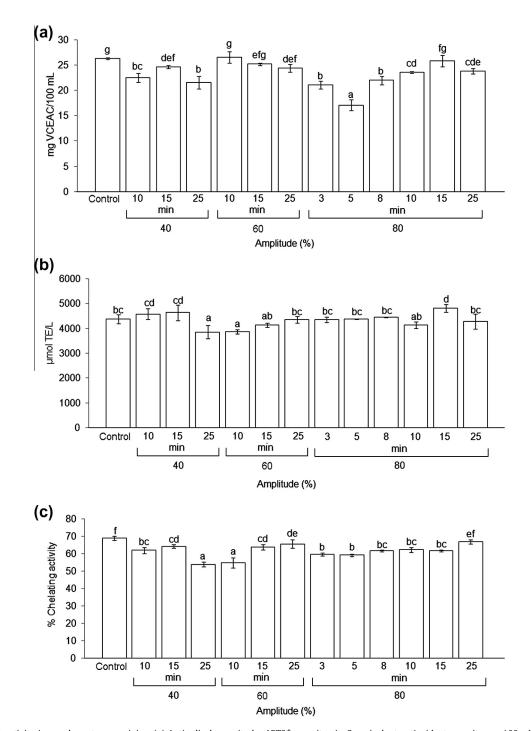


Fig. 3. Antioxidant activity in purple cactus pear juice: (a) Antiradical capacity by ABTS⁺, mg vitamin C equivalent antioxidant capacity per 100 mL of juice (mg VCEAC/ 100 mL). ^aDifferent letters indicate a significant difference (*p* < 0.05) between treatments. (b) Free Radical Scavenging Activity, µmol Trolox equivalents per liter of juice (µmol TE/L). ^aDifferent letters indicate a significant difference (*p* < 0.05) between treatments. (c) Percentage of chelating activity ferrous ions. ^aDifferent letters indicate a significant difference (*p* < 0.05) between treatments. (c) Percentage of chelating activity ferrous ions. ^aDifferent letters indicate a significant difference (*p* < 0.05) between treatments.

free, and involved as radical acceptors and chain terminators, acting as antioxidants [35].

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3.5.3. % Chelating activity of ferrous ions

Fig. 3c shows the percentage of chelating activity of ferrous ions. The chelating activity in sonicated juices decreased in comparison to the control (69.1 \pm 1.0%), except in juices treated at 80% amplitude level for 25 min (67 \pm 1.1%). Phenolic compounds are the main responsible for the chelating activity, as described previously, these compounds were retained or even increased after the ultrasonic treatments. Phenolic compounds can react as metal chelating agents (form σ -bonds) being effective as secondary antioxidants because they reduce the redox potential, stabilizing the oxidized form of the metal ion [29,36]. However, this activity depends on the type of the chelating activity pertaining to phenolic compounds released by ultrasound [37].

4. Conclusions

The sonication treatment of purple cactus pear juice significantly reduced microbial counts without affecting its quality parameters and antioxidant properties. Further research is needed to assess if this emerging technology could be used to increase the shelf life of fruit juices by itself or combined with other conventional technologies. It is also necessary to evaluate the relation between released compounds and certain health benefits obtained from consuming these juices. It is expected that our study will support these future research.

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