Ultrasound Processing on Green Cactus Pear (Opuntia ficus Indica) Juice: Physical, Microbiological and Antioxidant Properties

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Abstract

In Mexico, the consumption of green cactus pear fruit it is usually eaten fresh. This fruit could be a natural product with a rapid presence in the market, especially if the production technologies are optimized. The aim of the present study was to evaluate the effect of ultrasound (amplitudes 40, 60 and 80% at different times 10, 15 and 25 min) in the quality (pH, °Brix, stability), microbial growth, phenolic content, ascorbic acid and the antioxidant activity of green cactus pear juice. We found that application of a greater amplitude and time of ultrasound (higher 60% 15 min) significantly decreased the microbial load. Treatment >15 min presented higher total solid soluble (*Brix) with values of 13.00 - 13.40 °Brix. Treatment 60% 15 min, 80% 15 and 25 min showed better physical stability (lower % settled solids) around 16.93%-19.41%. Appreciable amount of bioactive compounds was found mainly in treatment at 80% 25 min in total phenolic contents, while ascorbic acid was the treatments at 60% 25 min and 80% 10 min, preserving the antioxidant activity present in the juice mainly treatments >15 min in ABTS. The amplitude and time of ultrasound applied in the juice might allow the suitable conditions of conservation for industrial use.

Keywords: Ultrasound; Juice; Opuntia; Microorganisms; Physical stability; Ascorbic acid; Total phenolic; Antioxidants

Introduction

Thermal treatments are the most common processing methods for microbial and enzyme inactivation in food and beverages that lead to an extended product shelf-life. High temperatures achieved during processing may be disadvantageous because they can cause undesirable changes in sensory attributes (i.e., texture, flavor, color, smell) and nutritional quality [1]. Emerging processing technologies such as ultrasound are used to improve the shelf life of fresh fruits and derived products, without compromising their nutritional and sensory attributes.

Ultrasound technology is characterized by longitudinal waves that occur when a sonic wave meets a liquid medium creating regions of alternating compression and explosion [2]. These regions of pressure change cause cavitation that generates gas bubbles which are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increase the diffusion of gas, causing the bubble to expand. A point is reached where the ultrasonic energy provided is not sufficient to retain the vapor phase in the bubbles, so that a rapid condensation occurs. The condensed molecules collide violently, creating shock waves. These create regions up to 5500°C and 50,000 kPa that are responsible of the bactericidal effect of ultrasound [3]. The advantages of ultrasound include: reduction of flavor loss (especially in sweet juices), greater homogeneity, better stability, microbial inactivation, physical stability, retention and release of antioxidant compounds [4-6].

The cactus pear (Opuntia ficus-indica) is a fruit originated in America and Mexico. This country has a great diversity of species and cultivars of this fruit and accounts for more than 45% of the worldwide pear cactus production [7-9]. Cactus pear is a good source of nutrients, vitamins and bioactive constituents such as polyphenols and pigments [10], being considered as a food of nutraceutical and functional importance [11]. Due to the short harvest season fruits loss exceeds 60%, due to inadequate handling during post-harvest. These fruits can be processed into other products such as juice [12]. However the processing of this product has been limited because this fruit is classified within the low-acid group pH (pH >4.5) and high soluble-solids content requiring a thermal treatment of 115.5°C or greater to obtain good control of microorganisms [13]. Cactus pear cultivars include green, yellow, purple and red fruits, although the green fruits are the most consumed in fresh. Previous studies with ultrasound technology in purple cultivar showed that this technology can be an interesting alternative to cactus pear processing [6]. Therefore, the objective of this study was to evaluate the effect of ultrasound treatments with different amplitudes and time on the quality, microbial growth, total phenolic and ascorbic acid content, and the antioxidant activity of green cactus pear juice.

Materials and Methods

Sample and treatments

Green cactus pear (Opuntia ficus indica) fruit was obtained from a local market in Pachuca, Hidalgo, Mexico in the spring of 2009. Only fruits without external injuries were selected, washed and manually peeled. To extract juice, the pulp was stirred using an industrial blender

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(model 38BL52 LBC10, Waring Comercial®, USA) and was then passed through a strainer to remove seeds.

After, the samples (250 mL) in a flask (600 mL of capacity) previously presterilized, were treated by ultrasound (ultrasonic processor VCX-1500, Sonics & Materials, Inc. Newtown, CT, USA) at 1500 W and were processed at a constant frequency of 20 kHz at amplitudes of 40%, 60% and 80% for time periods of 10, 15 and 25 min for each amplitude using a probe of 13 mm. At the maximum amplitude (80%) juice was treated also for 3, 5 and 8 min in order to evaluate the effect of short time high amplitude treatment. Temperatures reached after treatment were also monitored (Table I).

**pH and total soluble solids (°Brix)**

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

**Physical stability**

10 mL of juice was centrifuged (Hamilton Bell Clinical Centrifuge, model 6500) at 3400 rpm for 20 min and stability was expressed as the % (w/w) of settled solids obtained after centrifugation [14].

**Microbiological analysis**

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

**Determination of total phenolic content**

The samples were centrifuged at 3400 rpm for 20 min and the supernatant was filtered with a pore size of 0.22 µm (Millipore Millex™ - GV PVDF) (The filtrate was also used to determinate ascorbic acid and antioxidant activity). The sample was diluted in deionized water (1:20). Briefly, 100 µL of the mixture was mixed with 500 µL of 1:10 diluted glucose (VRBG) (The filtrate was also used to determinate ascorbic acid and antioxidant activity). The sample was diluted in deionized water (1:20). A solution ethanolic (7.4 mg/100 mL) of the stable DPPH• radical cation 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS•+) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulfate in the dark at room temperature for 16 h before being used. 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 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 in a microplate reader. The antioxidant capacity was expressed as mg Vitamin C equivalent antioxidant capacity (VCEAC) per 100 mL of juice (mg VCEAC/100 mL) [17].

**Antiradical capacity by ABTS**

The sample was diluted in deionized water (1:5). Briefly, the radical cation 2,2’azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS•+) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulfate in the dark at room temperature for 16 h before being used. 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 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 in a microplate reader. The antioxidant capacity was expressed as mg ascorbic acid equivalents per liter of juice (mg GAE/L) [15].

**Antiradical capacity by DPPH**

Antiradical activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical. The sample was diluted in deionized water (1:20). A solution ethanolic (7.4 mg/100 mL) of the stable DPPH• radical was prepared. Then 100 µL of the sample was taken into vials and 500 µL of DPPH• solution was added. Then it was left to sit at room temperature for 1 h. The solution was stirred and then centrifuged at 3000 rpm for 10 min. Finally, absorbance was measured at 520 nm in a microplate reader and µmol equivalents of Trolox per liter of juice (ET µmol/L) were obtained [18].

**Chelating activity of ferrous ions**

The sample was not diluted. Briefly, 100 µL of the sample was placed in vials and 50 µL of ferric (II) chloride solution (2 mM) and 450 µL of methanol was added. The mixture was mixed by vortexing and then left for 5 min at room temperature. Then 400 µL of ferrozine (5 mM) was added and the mixture was incubated for 10 min. Finally, absorbance was measured at 520 nm in a microplate reader and µmol equivalents of Trolox per liter of juice (ET µmol/L) were obtained [18].

| Treatment | Temperature (°C) |
|-----------|-----------------|
| Amplitude (%) | Time (min) | T<sup>●</sup> | T<sup>●●</sup> |
| 40 | 10 | 8 | 36.1 ± 2.55 |
| 40 | 15 | 8 | 48.2 ± 1.33 |
| 40 | 25 | 8 | 63.0 ± 0.87 |
| 60 | 10 | 8 | 41.2 ± 2.34 |
| 60 | 15 | 8 | 58.2 ± 1.42 |
| 60 | 25 | 8 | 66.4 ± 1.90 |
| 80 | 3 | 8 | 25.5 ± 0.10 |
| 80 | 5 | 8 | 29.4 ± 4.30 |
| 80 | 8 | 8 | 42.0 ± 0.10 |
| 80 | 10 | 8 | 46.0 ± 0.10 |
| 80 | 15 | 8 | 71.6 ± 0.79 |
| 80 | 25 | 8 | 76.3 ± 1.16 |

Table 1: Effect of ultrasonic treatment over the values of temperatures at 76.3 ± 1.16°C.

* T<sup>●</sup> Inlet temperature, * T<sup>●●</sup> Outlet temperature in ultrasound-treated cactus pear green juice.
Results and Discussion

pH, total soluble solids and physical stability

Some characteristics of quality that play an important role in cactus pear processing are pH, °Brix and stability (Table 2). Based on its high pH, this fruit is classified in the low-acid group (pH > 4.5) [13]. The ultrasound induced slight changes of pH (range between 4.77 ± 0.05 to 5.12 ± 0.06), which were related to the amplitude increase.

Cactus pear fruit is a source of simple sugars and polysaccharides complex, the total solids content includes most of the sugars (glucose and fructose) that would vary with the degree of maturity [8,20]. Also, this fruit is a source of pectin and mucilaginous components [21-24]. The samples showed °Brix values within ranges established by Mexican standard PC-046-2005 [25] (less than 12 %). Results revealed that applying longer treatment time, the total solids content increased in the juice (°Brix). The increase of total solids was significant in comparison to the control (p<0.05) when treatment time was >15 min. According to Mason et al. [26], the use of power ultrasound could enhance the disruption of cell walls to facilitate the release of their contents. It is also possible that the combined effect of amplitude-time of ultrasound caused cell rupture and hydrolysis of polysaccharides due to cavitation releasing, or fragmenting them in less size [27,28].

Physical stability of juices was assessed by measuring the % (w/w) of total sedimentable solids after centrifugation of samples. The higher the value of this parameter, the lower the stability of juices, as a consequence of particle sedimentation. °Brix results could coincide with the best stability observed in samples treated under amplitude-time of 80% 15 min (16.93 ± 0.47 % settled solids), compared with the control sample (22.04 ± 0.69 %). The settled solids decreased with the ultrasound treatment because of the high shearing effect occurring during cavitation that fragmented colloidal pectin molecules into a smaller particle, which may have helped to stabilize the colloid system. This particle size reduction could explain why more fine particles were retained in the supernatant after centrifugation [27].

Microbiological analysis

Figure 1 shows the effect of ultrasound treatment on the microbial load in the green cactus pear juice. The control sample showed values of 4.6 and 4.2 log CFU/mL for TPC and Enterobacteria respectively. Total and enterobacteria counts were below the detection level when samples were treated at amplitudes of 60 and 80% and times of 15 and 25 min. Among other combined mechanisms, temperature reached during treatment of these samples (58 and 76°C, Table 1) may explain the reduction of microorganisms. A complete microbial inactivation was obtained for sample treated at 60% amplitude level (15 and 25 min) with outlet temperature at 58 - 66°C as well as 80% treatment with higher temperatures (71-76°C). Therefore there are greater effectiveness of inactivation applying high amplitudes levels in long times. Cell destruction occurs when ultrasound treatment is applied for a longer period and is caused by the physicochemical effect of cavitation which thins the microbial cell membrane [29]. In addition, studies suggest that microorganisms subjected at temperatures >50°C show greater sensitivity to the effect of cavitation that cause the weakening of the bacteria membrane [2,30,31].

Total phenolic content

Figure 2 illustrates the content of phenolic compounds in the green cactus pear juice and after the ultrasound treatment. This juice had a phenolic content of 982.2 ± 20.69 mg GAE/L. This value was lower than purple cultivar reported by Zafra-Rojas et al. [6]. However this juice had higher polyphenols content in comparison with other cultivars [9]. Juices subjected to ultrasound exhibited higher phenolics content, mainly the 80% 25 min treatment, about 40% more phenolics content (1376.2 ± 30.70 mg GAE/L) than the control. This result agreed with a similar study on sonicated kasturi lime juice [29] and with purple cactus pear juice [6]. Phenolic compounds are present in the vacuole and cell walls of cactus pear. This interaction is not only attributed to the walls of the cell, but also to the matrix of pectin and cellulose that contributes to stabilize the colloid system. The particle size reduction could explain why more fine particles were retained in the supernatant after centrifugation [27].

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in a soluble form or bound to the cell wall with dietary fiber like pectin, cellulose, hemicellulose, and lignin traces [27,32]. According to Escarpa and Gonzalez [33] the high amplitudes-time could contribute to the release of these phenolic compounds into the juice upon ultrasound via cavitation, collapse in the surroundings of colloidal particles.

Ascorbic acid content

Ascorbic acid has potential antioxidant activity; it is known to protect cells against damage caused by free radicals, contributing to the prevention of cardiovascular diseases and cancer [34]. The content of ascorbic acid in green cactus pear juice was of 510.16 mg AA/L. This content was into the range of the values found for other cultivars [9]. Juices ultrasound treated at 80% 10 min and 60% 25 min presented a higher concentration of ascorbic acid (551 and 544.33 mg AA/L respectively) compared to the control (Figure 3). However, these values should be corroborated using methodologies such as HPLC. In previous works with purple cactus pear juice was found an increase of ascorbic acid only in the 80% 25 min treatment [6]. Therefore, is important to consider the response of ultrasound between different cultivars. Studies of ultrasound on kasturi lime and guava juices have reported an increase of ascorbic acid, which is attributed to the elimination of dissolved oxygen. This is essential for ascorbic acid degradation during the cavitation produced by sonication [27,29].

Antioxidant activity

The antioxidant activity is determined by the different mechanisms of action of the antioxidants present in the food that may exhibit synergistic interactions. For this reason it is necessary to combine several methods to determine in vitro antioxidant capacity of foodstuffs [35]. In this study three parameters were used to determine the antioxidant capacity of cactus pear juice (Figure 4).

Antiradical capacity by ABTS and DPPH

The antiradical capacity by ABTS in control sample presented values of 5.16 mg VCEAC/100 mL (Figure 4a). Juices treated with ultrasound above 15 min regardless amplitude presented higher antiradical capacity, especially at 80% 15 min (102.60 mg VCEAC/100 mL). Our result showed that sonication time is important in the release of antioxidants (mainly polyphenol compounds). Therefore, these treatments had a higher antiradical capacity by ABTS, in comparison with sonicate samples with low time that had a decrease of this activity. In the other hand, the results of antiradical capacity by DPPH (Figure 4b) showed that the majority of ultrasound treated juices were similar to the control (3340 µmol ET/L), with exception treated juices at 40% 15 min, 80% 5 min, and 8 min which presented higher values in a range of 4600 to 4800 µmol ET/L.

% Chelating activity of Ferrous Ions

The main compounds responsible of chelating activity of ferrous ions are the phenolic compounds. They can react as metal chelating agents (form σ-bonds) which are effective as secondary antioxidants, because they reduce the redox potential, thereby, stabilizing the oxidized form of the metal ion [9,36].

All treated juices exhibited similar chelating activity to the control sample (54.2%), which indicates that the ultrasound treatment did not affect the compounds responsible of the chelating activity. The total antiradical activity in cactus pear is the result from the sum of the antiradical activities of the substances that make up its composition, such as taurine, vitamins, betalainas, ascorbic acid and phenolic compounds [9]. The majority of phenolic compounds found in cactus pear are flavonoids, mainly quercentin, kaemferol and isorhamnetin [23,37]. However, chelating activity of phenolic compounds can depend on the structure of each specific flavonoid [38]. The unmodified or increased
antioxidant (ABTS, DPPH) and quelling activity in cactus pear juices could be due to the release of ascorbic acid or phenolic compounds which may act as radical acceptors and chain terminators [39]. Other studies by ultrasound have reported the release of phenolic compounds [27,29,6] or the sonochemically generation of hydroxyl radicals (HO•) of the aromatic ring in these compounds [39,40]. Finally, in this study, the release of compounds that increased the antioxidant activity could be attributed to the synergism between ultrasound-time and outlet temperatures reached during processing.

Conclusion

Green cactus pear juice treatment with ultrasound technology reduced total cell counts and enterobacteria to levels of no detection. Juices presented good quality parameters and ultrasound contributed to the release of bioactive compounds. This technology is a promising alternative to heat treatments that allows the processing of fruit products with good quality and better functional characteristics. Further studies are necessary to evaluate the characteristics of cactus pear juices treated by ultrasound during storage.

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