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Effect of ultrasound and storage time on quality attributes of strawberry juice

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Abstract

The aim of this study was to evaluate changes in quality attributes of non-clarified strawberry juice during storage at 5 °C after ultrasonication at 40 kHz for 10 and 30 min in comparison with thermally treated juice at 90 °C for 60 s. Ultrasound treatments maintained color parameters with no significant differences ($p < .05$) from untreated sample, while thermally treated juice showed lower L^* values and higher hue angles than the control. No significant differences in °Brix and total acidity were found between treated and untreated samples, and both parameters remained unchanged throughout storage. Compared to the control, ultrasound treatments showed no significant reductions on day 0 in mesophilic and psychrophilic bacteria, and yeast and molds counts. However, the treatments showed significant decrease in the microbial growth rate through storage time, and significant increase in both polyphenol content and antioxidant activity, compared to control.

Practical applications

With today's tendency towards the consumption of nutritious and fresh-like foods, non-thermal treatments are gaining popularity. Among them, ultrasound has become a feasible alternative against traditional thermal treatments. Strawberry products are considered of great nutritional value, because of their high content in antioxidant compounds and vitamin C. In this study, ultrasound was evaluated in strawberry juice through storage time, where significant reductions on microbial growth rate were achieved; meaning that shelf-life of the product was increased compared to control. Furthermore, physicochemical parameters were maintained through storage, and antioxidant capacity and polyphenol content showed a significant increment, which implies that nutritional value of the product was increased by ultrasound treatments. Therefore, sonication is a potential technology that can easily be applied at the fruit juice industry, replacing the traditional thermal treatment which compromises the organoleptical and nutritional quality of the final product.

1 | INTRODUCTION

The growing interest for fresh-like nutritious foods, has promoted the development of products such as unpasteurized juices, with similar or equivalent quality properties to the natural fresh products (Lin & Zhao, 2007). Among fruit juices, strawberry juice is one of the most popular, due to its attractive color, good aroma and sweet–sour mouthfeel (Wang & Jiao, 2000).

However, fresh strawberry juice is highly perishable, susceptible to spoilage and deterioration, due to the activity of enzymes, and the exposure to oxygen and microorganisms. Therefore, if juices do not receive the proper treatment, enzymatic, microbial, chemical, and physical deterioration can take place, reducing considerably its nutritional and sensory quality, shortening the shelf-life of the product (Bates, Morris, & Crandall, 2001).

Thermal processing technologies use for inactivating the microorganism and enzymes in fruit juices or purees are generally at 70–121 °C for 30–120 s (Cao et al., 2011), and can seriously affect the quality of strawberry juice, losing nutritional components and changing color, flavor, and texture (Patras, Brunton, Da Pieve, & Butler, 2009).

Therefore, new technologies are continuously being investigated to avoid the application of thermal treatments required for the stabilization of food products. Together with UV-C irradiation (Donahue, Canitez, & Bushway, 2004; Wright, Sumnler, Hackney, Pierson, & Zoecklein, 2000), pulsed electric field technology (Vallverdú-Queralt et al., 2013), and high hydrostatic pressure (Briñez, Roig-Sagués, Hernández Herrero, & Guamis López, 2006), the efficacy of ultrasound processing (Patil, Bourke, Kelly, Frías, & Cullen, 2009) as fruit juice processing technique is continuously being explored.

The ultrasound processing (US), also called sonication, is a nonthermal technology that has been effective in the inactivation of microorganisms and enzymes related to degradation of fruit juices, allowing thermo-sensitive food processing (Rawson et al., 2011). Ultrasound refers to pressure waves propagated within a medium with frequency of 20 kHz or more (Butz & Tauscher, 2002). Ultrasound treatments result in the formation of microbubbles in the food system that eventually implode in a process called cavitation. These phenomena eventually can cause microbial inactivation (Patil et al., 2009).

Despite the fact that some authors reported some negative impacts on juice quality due to ultrasound processing, such as a slight deterioration of sensory quality in orange juice with calcium added (Gómez-López, Orsolani, Martínez-Yépez, & Tapia, 2010) and some off-flavor formation in orange juice (Wong, Vaillant, & Pérez, 2010), according to a recent review published by O'Donnell, Tiwari, Bourke, & Cullen (2010) fruit juices treated with ultrasound suffered minimal effects on the quality of the final product and, because of that, this technology has been studied for several applications in food processing.

Ultrasound treatment applied to different fruit juices has been proved to inactivate microorganisms and increase antioxidant compounds (Santhirasegaram, Razali, & Somasundram, 2013; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008), however it is important to determine if these effects remain during storage. Therefore, the aim of the present research was to determine the effect of ultrasound treatment in comparison with thermal treatment on the physicochemical properties (color, total soluble solids, and total acidity), microbial growth, total phenolic content, and antioxidant activity (DPPH) of strawberry juice during shelf life.

2 | MATERIALS AND METHODS

2.1 | Juice preparation

Strawberries (*Fragaria* x *ananassa*) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. The strawberries were destemmed and the juices were prepared with a commercial juice extractor. Once the treatments were applied, the strawberry juices were stored in sterile polypropylene flasks at 5 °C for 10 days to assess the evolution of microbial populations, antioxidant capacity, total phenolic content, total soluble solids, total acidity, and color parameters.

2.2 | Ultrasound and thermal treatments

The ultrasound treatments of fresh strawberry juice were performed at 40 kHz frequency, using an ultrasonic cleaning bath (TestLab, Argentine). The ultrasonic bath is a rectangular container (290 × 150 × 150 mm) with the maximal tank capacity of 6.5 L. The 40 kHz transducers at the bottom transmit ultrasound waves of 180 W from bottom to above. Temperature in the ultrasonic bath was monitored at 20 ± 1 °C. The juice level in the flasks was 2 cm below the water surface in the ultrasonic bath. The height of the bottom surface of the flasks from the bottom surface of the tank (face of transducers) is 4 cm. The processing time was 10 and 30 min.

In this study, we used a traditional thermal treatment with the final purpose of comparing its effect on strawberry juice quality against ultrasound. Fruit juice industry usually conducts thermal treatments at 90 °C for 60 s to ensure safety of juices (Nagy, Chen & Shaw, 1993). Therefore, two control samples were used in the present study: a control juice with no treatment (untreated), and a second control thermally treated at 90 °C for 60 s. For the thermal treatment, strawberry juices were immersed in a thermostatic bath (Lauda E300, Germany). After the thermal treatment was applied, the juice samples were immediately cooled by immersing in an ice-water bath.

The following terms were used to describe the different treatments in this study: untreated or control (strawberry juice with no treatment); US10min (ultrasound treatment for 10 min); US30min (ultrasound treatment for 30 min); TT90 °C60s (thermal treatment at 90 °C for 60 s).

2.3 | Sensory evaluation

Sensory evaluation was performed by a quantitative descriptive analysis (QDA). After the treatments were applied, the samples were subjected to QDA by ten trained panelists. Samples labeled with 3 digit code numbers were randomly provided. Water was provided to panelists for eliminating the residual taste between samples.

The attributes evaluated were: color, off-odor, sweetness and acidity. Unstructured line scales (5 cm) anchored at the ends with terms related with minimum and maximum intensities were used to evaluate each attribute. Color was rated using *0 = characteristic red* and *5 = dark red*. Off-odor, sweetness and acidity were rated using *0 = low intensity* and *5 = high intensity*.

2.4 | Physicochemical analysis (total soluble solids and titratable acidity)

Total soluble solids (°Brix) are a measure of soluble solids present in fruit pulp and juice expressed as percentage of sucrose at 20 °C. Soluble solids were measured using an Atago refractometer (Abbe 1T74T, Tokio, Japón). °Brix was measured by duplicate.

For determination of titratable acidity (TA), a known volume of each sample was placed in a 250 mL beaker, and 50 mL of distilled water were added. Further, this solution was titrated against standardized 0.1 N NaOH to the phenolphthalein end point (pH 8.2 ± 0.1). The volume of NaOH was converted to grams of citric acid per 100 mL of juice (% TA) based on the method of Sadler & Murphy (2010), and the total acidity was calculated using equation 1:

$$\%TA = \frac{V\,0.1\,N_{NaOH}\,0.064}{V_S}100\% \quad (1)$$

where V is the titer volume of NaOH and V_s is the volume of the strawberry juice sample.

TA was measured by duplicate.

2.5 | Color evaluation

Color determination was carried out using a LoviBond colorimeter RT500 (Neu-Isenburg, Germany) with an 8 mm diameter measuring area, calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$, $y = 0.3192$).

Color of strawberry juice was determined by measuring L^*, a^*, and b^* chromaticity coordinates of the CIE-Lab scale (CIE, 1977). Hue angle ($h°$) was calculated according to equation 2:

$$h° = \tan^{-1}\left({}^{b^*}/_{a^*}\right) \quad (2)$$

Three measures were made for each treatment at each storage time.

2.6 | Extraction of antioxidants and phenolic compounds

2 mL of strawberry juice from each treatment was homogenized with 10 mL solution of ethanol (80% v/v) in a vortex. The homogenate was then centrifuged at 8,000 rpm for 15 min at 4 °C. The supernatant was collected and filtered using Whatman filter paper #1. The ethanolic extract was stored at −20 °C to be used in the determinations of total phenolic content (TPC) and antioxidant activity by DPPH method.

2.6.1 | Determination of DPPH radical scavenging activity

The radical scavenging activity was measured in terms of hydrogen-donating or free radical-scavenging using the DPPH methodology proposed by Brand-Williams, Cuvelier, & Berset (1995).

An ethanolic DPPH solution (100 μM) was used for determinations. Ethanol (0.1 mL) was mixed with 3.9 mL of DPPH (100 μM) to determine the initial absorbance of the DPPH solution. Next, 0.1 mL of sample extract was added to 3.9 mL of 100 μM DPPH solution. The mixture was shaken immediately and allowed to stand at ambient temperature in the dark. The decrease in absorbance at 517 nm was measured after 60 min.

The radical scavenging activity was expressed as the inhibition percentage of the DPPH radical and was measured by duplicate.

2.6.2 | Total phenolic content

TPC was determined spectrophotometrically using the Folin–Ciocalteu reagent (FCR) according to the methodology proposed by Singleton, Orthofer, & Lamuela-Raventos (1999) with modifications. Extract samples properly diluted were added to 1000 μL of FCR (diluted 1:10). After 3 min of incubation at ambient temperature, 800 μL of 7.5% Na_2CO_3 solution was added and the reaction mixture was incubated for 2 h at the same temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (1601 PC UV-visible, Shimadzu Corporation, Kyoto, Japan) and TPC was calculated using gallic acid as standard.

Results of TPC were expressed as mg gallic acid equivalents (GAE)/100 mL of juice and were measured by duplicate.

2.7 | Microbiological analysis

The microbial stability of strawberry juices was evaluated through the determination of total aerobic mesophilic and psychrophilic bacteria, and yeast and molds populations. A 10 mL aliquot of juice from each treatment was sampled at different times of refrigerated storage (0, 3, 7, 10 days). Serial dilutions (1:10) of each sample were made in sterile peptonated water (0.1% w/v) and surface spread by duplicate. The enumeration of the microbial populations was performed according to Ponce, Agüero, Roura, Del Valle, & Moreira (2008) by using the following culture media and culture conditions: mesophilic aerobic bacteria on Plate Count Agar (PCA) incubated at 35 °C for 48 h; psychrophilic bacteria on PCA incubated at 7 °C for 7 days; yeast and molds on Yeast-Glucose-Chloramphenicol (YGC) medium incubated at 25 °C for 5 days. All culture mediums were purchased from Britania, Buenos Aires, Argentina. Microbial counts were performed by duplicate and expressed as log CFU/mL.

2.8 | Statistical analysis

A completely randomized design was used. Three independent runs were performed. Data obtained was analyzed using R v. 2.12.2. (R Development Core Team, 2011). Results reported in this article are mean values accompanied by their standard errors (Kuehl, 2001). Analysis of variance ANOVA was performed and Tukey-Kramer comparison test was used to estimate significant differences between treatments ($p < .05$) and between storage days ($p < .05$).

3 | RESULTS AND DISCUSSION

3.1 | Effect of ultrasound treatment on sensory quality of strawberry juice

Sensory characteristics of food are of utmost importance as it may contribute significantly to the consumer acceptance or rejection of the product. Therefore, a sensory evaluation was carried out immediately after the application of the treatments in order to assess the effect of ultrasound on the organoleptic characteristics of the strawberry juice compared to both untreated (control) and thermally treated (TT90 °C60s) samples. The results of this sensory analysis are shown on Figure 1.

The application of ultrasound treatments did not affect color and off-odor scores compared to untreated or thermally treated samples. However, both flavor attributes evaluated (acidity and sweetness) were significantly affected by the treatments applied. In this sense, higher acidity scores were observed for both TT90 °C60s and US30min compared to untreated sample. On the other hand, higher sweetness

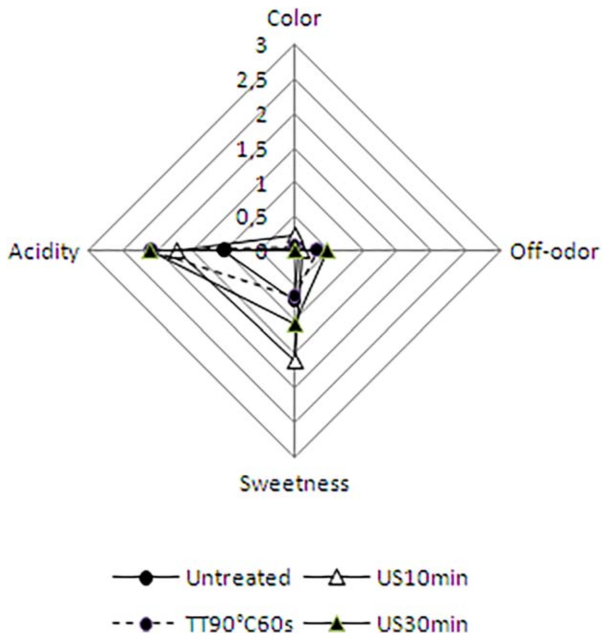

FIGURE 1 Effect of ultrasound treatments on sensory quality of strawberry juice. **TT90 °C60s**: thermal treatment; **US10min**: ultrasound treatment for 10 min; **US30min**: ultrasound treatment for 30 min

scores were found on both sonicated samples compared to untreated and TT90 °C60s. The increase in sweetness flavor might be attributed to the breakage of cell due to ultrasound treatment which extracts sugars from intracellular spaces to the liquid (Abid et al., 2014).

The results presented here confirmed that it is possible to produce strawberry juice with acceptable sensory properties using ultrasound as compared to thermal treatment and hence can be a promising technological alternative to thermal method.

3.2 | Effect of ultrasound treatments on total soluble solids and acidity of strawberry juice

The measurement of total acids (TA) in beverages and juices is required by all producers as a key quality control indicator. Another important quality parameter in fruit juices is total soluble solids (°Brix), which are primarily sugars: sucrose, fructose, and glucose.

Results regarding the effects of sonication and thermal treatment on both °Brix and % TA are shown in Table 1. Sonication and thermal treatment did not induce any changes in the titratable acidity and total soluble solids of strawberry juice compared to untreated samples. Santhirasegaram et al. (2013) found similar results when applying 15, 30 and 60 min ultrasound treatments at 40 kHz frequency, and thermal treatments of 30 and 60 s at 90 °C on mango juice, where no statistically differences were observed at soluble solids content and titratable acidity compared to control. The same correlations were observed by Adekunte, Tiwari, Cullen, Scannell, and O'Donnell (2010), who have shown that 10 min of ultrasound application did not affect the °Brix, pH and acidity of tomato juices.

These results show that sonication as well as thermal treatment have no effect on total soluble solid content and total acidity of strawberry juice, which also remained unchanged during storage.

3.3 | Effect of ultrasound treatments on strawberry juice color parameters

Color is an important attribute of juice quality because it can condition its acceptability by the consumer. Cavitation may induce changes in color due to the acceleration of chemical reactions, raise of diffusion rate, dispersion, formation of aggregates, and particles breakdown (Cruz-Cansino et al., 2015). Color parameters (L^* and $h°$) are shown in Table 2.

At day 0, ultrasonicated samples showed no significant differences from the untreated juice on both color parameters. On the other hand, thermal treatment reduced significantly ($p < .05$) strawberry juice L^* value, while increasing $h°$ compared to the untreated control, which implies a lower sensory quality of the product.

On day 3 US10min, US30min and untreated samples showed an important decrease in both L* and $h°$, while the thermally treated sample maintained its color parameters unchanged from day 0. During the rest of storage, every sample except for the pasteurized juice exhibited a similar trend (Table 2).

TABLE 1 Effects of ultrasound treatments on total soluble solids (°Brix) and total acidity (%TA) in strawberry juice during refrigerated storage at 5 °C

Parameters	Storage day	Control	Treatments TT90 °C60s	US10min	US30min
°Brix	0	10.06 ± 0.06^{aA}	10.15 ± 0.06^{aA}	10.25 ± 0.05^{aA}	10.25 ± 0.05^{aAB}
	3	10.30 ± 0.12^{aA}	10.29 ± 0.14^{aA}	10.38 ± 0.07^{aA}	10.58 ± 0.08^{aA}
	7	10.38 ± 0.22^{aA}	10.43 ± 0.10^{aA}	10.40 ± 0.06^{aA}	10.53 ± 0.16^{aA}
	10	10.13 ± 0.22^{aA}	10.43 ± 0.19^{aA}	10.38 ± 0.22^{aA}	10.20 ± 0.12^{aB}
% TA	0	1.13 ± 0.01^{aA}	1.13 ± 0.01^{aA}	1.08 ± 0.01^{aA}	1.07 ± 0.02^{aA}
	3	1.08 ± 0.03^{Aa}	1.13 ± 0.08^{aA}	1.05 ± 0.06^{aA}	1.05 ± 0.03^{aA}
	7	1.04 ± 0.02^{aA}	1.06 ± 0.01^{aA}	1.04 ± 0.04^{aA}	1.05 ± 0.03^{aA}
	10	1.12 ± 0.01^{aA}	1.08 ± 0.01^{aA}	1.08 ± 0.04^{aA}	1.15 ± 0.02^{aA}

Data is shown as means ± standard errors. Values with different lower case letters in the same row indicate significant differences ($p < .05$) between treatments. Values with different capital letters in the same column indicate significant differences ($p < .05$) with respect to storage days.**TT90 °C60s**: thermal treatment; **US10min**: ultrasound treatment for 10 min; **US30min**: ultrasound treatment for 30 min.

TABLE 2 Effects of ultrasound treatments on lightness (L^*) and hue angle ($h°$) in strawberry juice during refrigerated storage at 5 °C

			Treatments		
Parameters	Storage day	Control	TT90 °C60s	US10min	US30min
L^*	0	44.69 ± 1.09^{aA}	33.21 ± 1.62^{bA}	46.12 ± 0.67^{aA}	44.56 ± 1.11^{aA}
	3	30.98 ± 0.32^{aB}	33.19 ± 1.10^{aA}	27.87 ± 0.84^{bB}	26.69 ± 0.11^{bB}
	7	29.69 ± 0.20^{aB}	30.17 ± 0.72^{aA}	29.30 ± 0.80^{aB}	28.19 ± 0.35^{aB}
	10	28.00 ± 0.29^{bC}	30.54 ± 0.66^{aA}	27.98 ± 0.29^{bB}	26.44 ± 0.19^{bB}
$h°$	0	24.22 ± 0.30^{aA}	26.46 ± 0.65^{aA}	24.53 ± 0.18^{aA}	24.02 ± 0.60^{aA}
	3	20.64 ± 0.18^{cB}	27.00 ± 0.40^{aA}	21.45 ± 0.22^{bcB}	22.59 ± 0.35^{bB}
	7	21.88 ± 0.15^{bB}	27.95 ± 0.21^{aA}	21.31 ± 0.34^{bB}	22.14 ± 0.23^{bB}
	10	21.63 ± 0.20^{bcB}	27.66 ± 0.12^{aA}	20.95 ± 0.39^{cB}	22.21 ± 0.20^{bB}

Data is shown as means ± standard errors. Values with different lower case letters in the same row indicate significant differences ($p < .05$) between treatments. Values with different capital letters in the same column indicate significant differences ($p < .05$) with respect to storage days. **TT90 °C60s**: thermal treatment; **US10min**: ultrasound treatment for 10 min; **US30min**: ultrasound treatment for 30 min.

3.4 | Effect of ultrasound treatments on antioxidant capacity of strawberry juice

The antioxidant activity of certain foods depends on the content of a mixture of antioxidants with different mechanisms of action. In this study, antioxidant capacity was measured through the DPPH radical scavenging assay, which measures the hydrogen donating capacity of antioxidants to stable free radical DPPH, which forms diphenylpicrylhydrazine (Shon, Kim, & Sung, 2003). The results are shown on Figure 2a.

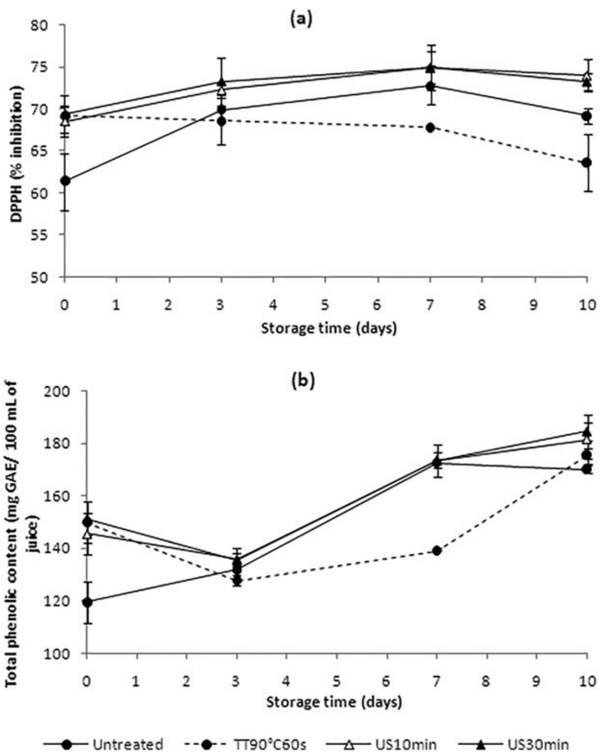

FIGURE 2 Effect of ultrasound treatments on antioxidant capacity of strawberry juice through refrigerated storage at 5°c: (a) DPPH radical scavenging activity, (b) total phenolic content. bars indicate standard errors. **TT90 °C60s**: thermal treatment; **US10min**: ultrasound treatment for 10 min; **US30min**: ultrasound treatment for 30 min

On day 0, both ultrasonicated and thermally treated juices exhibited higher DPPH radical scavenging activity compared to the untreated sample. From day 3 until the end of storage antioxidant capacity of the control sample remained unchanged (70.66% inhibition). On the other hand, samples treated with ultrasound (both US10min and US30min) showed significantly higher values of DPPH radical scavenging activity respect to both untreated and TT90 °C60s, reaching values of approximately 75% inhibition at day 7.

The increase in the antioxidant capacity may be explained by the effect of ultrasound on the extraction of bioactive compounds and antioxidant molecules (Vilkhu, Mawson, Simons, & Bates, 2008). The application of ultrasound improves the extraction yield of bioactive compounds (Vilkhu et al., 2008). This positive effect of ultrasound is assumed to be due to the effective removal of occluded oxygen from the juice (Knorr, Zenker, Heinz, & Lee, 2004).

These results indicate the non-destructive effects of ultrasound on the antioxidant compounds of strawberry juice.

3.5 | Effect of ultrasound treatments on total phenolic content of strawberry juice

Results regarding the effects of ultrasound and thermal treatments on total phenolic content (TPC) of strawberry juice are shown on Figure 2b. Initial TPC measured in this research for the untreated sample (119.5 mg of GAE/100 mL of juice) was significantly lower respect to results found in other studies, (Heinonen, Meyer, & Frankel, 1998; Proteggente et al., 2002). It is known that differences in the concentration of total phenolics in fruits and vegetables can fluctuate according to the variety, development stage, environmental conditions, sample treatment, extraction process, and quantification method.

At day 0, both ultrasonicated and thermally treated juices exhibited higher TPC compared to the untreated sample. Polyphenols and anthocyanins are enzymatically degraded in the presence of polyphenol oxidase (Patras, Brunton, O'Donnell, & Tiwari, 2010), and this enzyme can be inactivated by thermal treatment. Although it is known that exposure to high temperatures may degrade phenolic compounds (Escribano-Bailon & Santos-Buelga, 2003), some authors have reported

TABLE 3 Effects of ultrasound treatments on microbial counts (log cfu/ml) of strawberry juices during storage at 5 °C

Microorganisms	Storage day	Control	Treatments		
			TT90 °C60s	US10min	US30min
Total mesophilic bacteria	0	4.72 ± 0.18^{aB}	ND^{bB}	4.76 ± 0.18^{aB}	4.42 ± 0.12^{aB}
	3	4.67 ± 0.29^{aB}	ND^{bB}	4.68 ± 0.21^{aB}	4.46 ± 0.14^{aB}
	7	6.60 ± 0.17^{aA}	ND^{cB}	5.70 ± 0.33^{abB}	5.13 ± 0.25^{bB}
	10	7.12 ± 0.14^{aA}	3.20 ± 0.05^{cA}	7.05 ± 0.14^{aA}	6.34 ± 0.05^{bA}
Total psychrophilic bacteria	0	4.64 ± 0.13^{aB}	ND^{bB}	4.70 ± 0.13^{aC}	4.70 ± 0.13^{aB}
	3	4.77 ± 0.30^{aB}	ND^{bB}	4.54 ± 0.14^{aC}	4.38 ± 0.18^{aB}
	7	5.72 ± 0.15^{aB}	ND^{bB}	5.70 ± 0.30^{aB}	5.12 ± 0.28^{aB}
	10	7.09 ± 0.50^{aA}	3.44 ± 0.14^{bA}	7.11 ± 0.07^{aA}	6.45 ± 0.04^{aA}
Yeast and molds	0	4.73 ± 0.15^{aC}	ND^{bA}	4.48 ± 0.08^{aC}	4.37 ± 0.15^{aB}
	3	4.45 ± 0.45^{aC}	ND^{cA}	4.00 ± 0.08^{aC}	3.70 ± 0.15^{bC}
	7	6.58 ± 0.16^{aB}	ND^{dA}	5.67 ± 0.17^{bB}	4.05 ± 0.05^{cBC}
	10	7.95 ± 0.03^{aA}	ND^{dA}	7.04 ± 0.17^{bA}	5.45 ± 0.10^{cA}

Data is shown as means ± standard errors. Values with different lower case letters in the same row indicate significant differences ($p < .05$) between treatments. Values with different capital letters in the same column indicate significant differences ($p < .05$) with respect to storage days. **TT90 °C60s**: thermal treatment; **US10min**: ultrasound treatment for 10 min; **US30min**: ultrasound treatment for 30 min.
ND. Not detected.

that the inclusion of a blanching step can have a positive effect on phenolic compounds with antioxidant capacity (Patras et al., 2010).

On day 0, similar results has been reported by Lieu and Le (2010) for grape, Cruz-Cansino et al. (2015) for pear, and Sathirasegaram et al. (2013) for mango juice, which presented higher total phenolic content after sonication. Phenolic compounds are found in soluble form in the vacuole or bound to the pectin, cellulose, hemicellulose, and lignin traces of the cell wall (Escarpa & Gonzalez, 2001). The increase of TPC on sonicated samples may be explained by the release of these compounds from the cell wall, through the collapse via cavitation in the surroundings of colloidal particles (Cheng, Soh, Liew, & The, 2007). Furthermore, increasing trends in phenolic content with ultrasound treatments can also be explained by the addition of hydroxyl radicals to the aromatic ring of phenolic compounds during sonication (Bhat, Kamaruddin, Min-Tze, & Karim, 2011). The sonochemically generated hydroxyl radicals lead to the hydroxylation of aromatic ring of the phenolic compounds in the ortho-, meta- and para- positions. This reaction has been suggested as a reason for increasing the properties of antioxidant molecules that can be extracted from food materials (Ashokkumar et al., 2008).

With the advance of storage, every sample including the control had an increase of these compounds. In accordance with our results, Cruz-Cansino et al. (2015) studied ultrasound effects on pear juice and also found that control samples incremented their TPC during storage. The changes that caused the senescence and decomposition of the cell structure and thus the liberation of free phenolic acids and free amino acids, may contribute to the increase in total polyphenols (Puttongsiri & Haruenkit, 2010).

3.6 | Effect of ultrasound treatments on strawberry juice native microflora

The nutritional richness of fruit juice makes the product a good medium for microbial growth, vehicle of foodborne pathogens and associated hazards (Al-Jedah & Robinson, 2002). Table 3 shows the microbial growth (total mesophilic bacteria, total psychrophilic bacteria and yeast and molds) in strawberry juices during storage.

At day 0, TT90 °C60s was able to reduce every population levels below the detection limits (< 2 log). On the other hand, the ultrasound treatments applied showed no significant initial effect in native microflora. However, the growth rate of every microbial population was significantly decreased by the ultrasound treatments. Furthermore, the decrease in the growth rate was higher in US30min samples than in US10min, meaning that higher ultrasound times produce significantly higher decrements in the growth rate of native microflora of strawberry juice.

According to the Spanish Regulation (BOE, 2001), 7 log CFU/mL is the maximum limit of microorganisms (at expiry date) in minimally processed foods. Therefore, the untreated sample would not be commercially accepted by the end of storage (10 days), while ultrasound-treated samples for 30 min and TT90 °C60s would still be safe for consumption (Table 3). These results support the use of ultrasound to improve the microbiological shelf-life of strawberry juice as an alternative to thermal treatment.

The significant reductions on microbial growth rate of ultrasound-treated samples could be justified through the physical phenomenon of cavitation that occurs when applying ultrasound treatments (Vercet, Sanchez, Burgos, Montañes, & Lopez, 2002). Cavitation can break molecules or particles through different mechanisms that can occur individually or combined: thermal effects produced by bubble implosion, microstreaming and implosion shock waves produced by mechanical stresses, and free radical production. However, radical production has been considered the most probable mechanism that explains the inactivation of microorganisms. Mañas and Pagan (2005) noticed the inhibitory effect of ultrasound treatments on the microbial growth, attributing this effect to the water cavitation and the generation of radicals. It is known that hydrogen ions (H^+), free radicals ($O_2^{\cdot}$, $OH^{\cdot}$, $HOO^{\cdot}$)

and hydrogen peroxide (H_2O_2) are formed during the sonolysis of water molecules (Petrier, Combet, & Mason, 2007) present in juice samples. Those radicals produce oxidative damage due to their high reactivity, damaging microorganism cell walls and inactivating the enzyme activity of mitochondria, leading to microbial inactivation.

4 | CONCLUSIONS

Ultrasound treatments did not affect the physicochemical parameters of the strawberry juice. Furthermore, sonicated samples showed higher values of DPPH inhibition percentage compared to untreated and thermally treated samples. This result means higher antioxidant capacity of the juice, which is an important nutritional property of strawberry products.

Through refrigerated storage, a significant reduction was shown in the microbial counts of the samples sonicated with respect to untreated sample, which implies a longer shelf-life of the product. Ultrasound treatments extended strawberry juice microbiological shelf-life, from 7 days in untreated samples, to more than 10 days for sonicated samples for 30 min. Although TT90 °C60s showed a more important reduction in every microbial population compared tosonicated samples, hue angles through storage were higher. High values of $h°$ indicate an important deterioration in strawberry juice color, which may lead to consumers' unacceptance.

In conclusion, in comparison with conventional thermal treatment, sonication of strawberry juice showed better results in several quality parameters, such as color and antioxidant capacity. Thermal treatment was more effective in inactivating microbial growth, however, significant quality loss was observed. This indicates that ultrasound could be a viable alternative to traditional thermal treatment of strawberry juice, avoiding the sensory deterioration of the product and extending its shelf-life. Further studies should be made in order to evaluate hurdle technologies, using ultrasound treatment in combination with different non-thermal technologies in order to increase the microbial inactivation, extending the shelf-life of the product without altering its physicochemical, sensory and nutritional properties.

Sonicated fruit juices might be the best option for consumers preferring health benefits from their food along with taste and fresh like attributes. Overall it can be said that non-thermal techniques could be helpful for food industry to produce fruit juices with fresh like attributes and holds good promise for commercial application.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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