



Influence of thermosonication (TS) process on the quality parameters of high pressure homogenized hazelnut milk from hazelnut oil by-products

Ilyas Atalar¹ · Osman Gul² · Furkan Turker Saricaoglu³ · Aysegul Besir⁴ · Latife Betul Gul⁴ · Fehmi Yazici⁴

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Abstract Hazelnut, an important source of nutrition, is reasonably expensive for hazelnut milk production. Hazelnut cake, a by-product from hazelnut oil production by cold press extraction technique, does not contain any chemical residue and can be used for hazelnut beverage production. This study investigates the effects of thermosonication process on the quality parameters of hazelnut milks and also compares the observed results with the conventional thermal process. Different thermosonication conditions at different amplitude levels (40 and 60% amplitudes for 5, 10, 15, 20 and 25 min and 80% amplitude for 3, 5, 10 and 15 min) were studied for physicochemical and rheological properties, as well as microbial inactivation and bioactive compounds of hazelnut milk produced from the cold pressed hazelnut cake as byproduct of oil production. In general, sonication process significantly improved the total phenolic compounds, antioxidant activity, appearance and structural properties like syneresis, sedimentation, viscosity and consistency of samples. The application of thermosonication at 60% amplitude for 25 min and 80% amplitude for 15 min achieved complete

inactivation of microorganisms (total aerobic mesophilic bacteria and yeast-mould). Complete inactivation of microorganisms was also achieved by conventional pasteurization at 85 °C, but this treatment caused some undesirable changes such as loses of bioactive compounds and deterioration of structural properties. The findings of the present study indicate that thermosonication can be successfully utilized for commercial processing of hazelnut milk with improved quality. This technique allows the production of hazelnut milks in safety and quality standards with highly nutritious then the conventional product.

Keywords Thermosonication · Hazelnut milk · Microbial safety · Bioactive compounds · Structural properties

Introduction

People who suffer from lactose intolerance and milk protein allergy problems tend to consume vegetable based milks. With the growing demand, food industries expand their production volume and numbers of product varieties with different products such as soy, almond, peanut and hazelnut milks (Bernat et al. 2015; Gul et al. 2017). Hazelnut milk is a vegetable-based beverage that can be an alternative to animal based milks. Hazelnut is a nutritional nut type that has a wide area of usage like chocolate and oil industry. After oil production by solvent extraction technique, the remaining part of hazelnut (hazelnut cake) can only be evaluated as animal feed. As an alternative to solvent extraction technique, cold pressing is technically less expensive and labor-intensive. Not only this technique provides better native properties, but also by-products are free of chemicals (Thanonkaew et al. 2012). Because hazelnut cake is rich in hazelnut proteins, phenolic

✉ Ilyas Atalar
atararilyas@gmail.com

¹ Department of Food Engineering, Faculty of Engineering and Architecture, Bolu Abant Izzet Baysal University, 14030 Bolu, Turkey

² Program of Food Technology, Yeşilyurt Demir–Celik Vocational School, Ondokuz Mayıs University, 55300 Samsun, Turkey

³ Department of Food Engineering, Faculty of Engineering and Natural Science, Bursa Technical University, 16310 Bursa, Turkey

⁴ Department of Food Engineering, Faculty of Engineering, Ondokuz Mayıs University, 55139 Samsun, Turkey

compounds, minerals and vitamins, it can be utilized as raw material in hazelnut milk production. Commercial vegetable milks are recommended to be consumed after being sterilized by conventional heating processes. The heat treatment inactivates enzyme and microorganisms ensuring food safety and expanding the shelf life of the products. However high temperatures may cause undesirable modifications and destruction of nutritive compounds (Saeeduddin et al. 2015; Deng et al. 2018). As an innovative technology, ultrasonication (US) has been investigated to preserve the nutritional and sensory properties of fresh products and also to improve their shelf life. Improved cloudiness, minimal loss of flavor and some bioactive compounds are some of the main advantages of ultrasound processing over conventional thermal process (Abid et al. 2014). As for processed vegetable based juices, consumers demand for additive-free and minimally processed products (Anaya-Esparza et al. 2017). By reducing the sedimentation, the ultrasound technology saves the need for adding hydrocolloids to the juice (Zafra-Rojas et al. 2013). Zafra-Rojas et al. (2013) investigated the effect of different ultrasound process conditions on the characteristics of purple cactus pear juice. Authors found that the process period of 15 and 25 min decreased the microbial count without affecting the juice quality and antioxidant content. As an alternative to conventional heating process, US can be a complete or partial solution as a novel food technology for pasteurization process (Knorr et al. 2011). The ultrasound application leads to the formation of microbubbles in the fluid resulting from the cavitation effect. These microbubbles release a high amount of energy and generates high pressure. The release of energy and pressure tends to provide microbial inactivation. US has been acknowledged as a prospective technology to meet the Food and Drug Administration requirement of at least 5 log cycle reduction of related microorganisms in foods (Anaya-Esparza et al. 2017; Salleh-Mack and Roberts 2007). Some researchers revealed that US at a controlled temperature (25 °C) did not show a substantial reduction in some types of microorganisms (Bhat and Goh 2017; Gabriel 2012). In order to produce a sterilized product, US should be applied at moderate heat. This technique, a combination of heat and cavitation effect is called thermosonication (TS) (Anaya-Esparza et al. 2017). The current work deals with the effect of TS treatment on the microbiological quality and physicochemical properties of hazelnut milk. It also aims to enhance quality parameters in view of bioactive compounds, rheological properties, color differences, soluble protein content, syneresis and sedimentation index.

Materials and methods

Materials

Whitened hazelnuts (*Corylus colurna*) were obtained from Gursoy Hazelnut Production Factory (Ordu, Turkey). The oil was extracted from hazelnut by using a headed cold press machine (Ekotok 1, Izmir, Turkey). The cold pressed hazelnut cake (9.95% moisture, 38.92% protein, 31.27% carbohydrate, 15.58% lipid and 5.28% ash) was used as a main ingredient for the milk production.

Preparation of hazelnut milks

Hazelnut cakes were grounded by using a blender (Waring laboratory blender, Conair Corporation, Stamford, CT, USA) for 2 min. Powder cakes were diluted with distilled water (25 °C) in order to obtain the target total solid concentration (10% w/v). Hazelnut slurry was homogenized with an ultra-turrax homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 10,000 rpm for 10 min. Finally, hazelnut milks were treated with the two stage high pressure homogenizer (GEA Niro Soavi-Panda PLUS 2000 Homogenizer, GEA Niro Soavi S.P.A., Parma, Italy) by applying 100 MPa pressure. After the homogenization step, temperature of hazelnut milk was measured by a thermometer (37 °C), and the homogenized samples were quickly cooled using an ice bath.

Thermosonication and heat treatments

TS treatment was carried out using an ultrasonic processor (VCX 750, Sonics & Materials, Inc., USA). Different amplitudes and sonication times (40 and 60% amplitudes for 5, 10, 15, 20, 25 min; 80% amplitude for 3, 5, 10 and 15 min) were applied with titanium probe at 13 mm diameter. Ultrasonic intensities applied to hazelnut milks were given in Table 1 and calculated by dividing the total energy to ultrasound time and probe area. Total energy was displayed as joule in the screen of ultrasonic processor. Probe was immersed to a depth 2 cm below the liquid surface. Samples were covered with stretch film to prevent the loss caused by evaporation. Control sample was prepared without ultrasound treatment. Experimental design was shown in Table 1. Conventional heat treatment was conducted at 85 °C for 2 min in a water bath (Nuve BM-30 model, Turkey).

pH measurement and protein solubility

pH values of samples were measured by using a calibrated pH meter (Eutech Cyberscan pH 2700, Ayer Rajah

Table 1 Process conditions and pH, soluble index and color values of untreated (C), heat treated (HT) and therosonicated hazelnut milks

Amplitude (%)	Time (min)	Temperature (°C)	Intensity (W/cm ²)	pH	Soluble protein (%)	L*	a*	b*	ΔE
C				6.51 ± 0.03 ^a	4.09 ± 0.16 ^a	75.16 ± 0.08 ^{ab}	- 1.25 ± 0.01 ^{def}	9.39 ± 0.03 ^a	-
HT	2	85		6.44 ± 0.01 ^a	3.62 ± 0.67 ^a	73.40 ± 0.11 ^f	- 1.26 ± 0.01 ^{def}	9.18 ± 0.06 ^b	1.76 ± 0.11 ^a
40	5	45	28.16 ± 1.26	6.48 ± 0.02 ^a	3.88 ± 0.01 ^a	75.04 ± 0.02 ^{ab}	- 1.22 ± 0.02 ^{cde}	9.41 ± 0.01 ^a	0.12 ± 0.02 ^g
	10	54	26.96 ± 0.32	6.50 ± 0.04 ^a	3.63 ± 0.16 ^{ab}	75.26 ± 0.05 ^a	- 1.16 ± 0.01 ^{ab}	9.30 ± 0.02 ^a	0.15 ± 0.03 ^g
	15	57	26.18 ± 0.05	6.51 ± 0.01 ^a	2.92 ± 0.01 ^{cd}	74.50 ± 0.04 ^{cd}	- 1.23 ± 0.01 ^{cde}	9.06 ± 0.01 ^c	0.73 ± 0.04 ^{def}
	20	62	26.33 ± 0.06	6.45 ± 0.04 ^a	2.85 ± 0.06 ^{cd}	74.19 ± 0.2 ^{de}	- 1.26 ± 0.02 ^{def}	9.01 ± 0.01 ^{cd}	1.04 ± 0.19 ^{cd}
	25	65	26.07 ± 0.08	6.44 ± 0.06 ^a	3 ± 0.06 ^{cd}	74.01 ± 0.07 ^e	- 1.30 ± 0.02 ^f	8.86 ± 0.01 ^f	1.26 ± 0.07 ^{bc}
60	5	46	43.62 ± 0.88	6.50 ± 0.01 ^a	3.28 ± 0.01 ^{bc}	75.22 ± 0.07 ^{ab}	- 1.15 ± 0.02 ^a	9.30 ± 0.01 ^a	0.13 ± 0.04 ^g
	10	58	43.11 ± 0.01	6.44 ± 0.01 ^a	3.21 ± 0.16 ^{bcd}	74.50 ± 0.21 ^{cd}	- 1.28 ± 0.01 ^{ef}	9.04 ± 0.04 ^{cd}	0.74 ± 0.21 ^{de}
	15	63	43.32 ± 0.17	6.44 ± 0.03 ^a	3.03 ± 0.11 ^{cd}	74.10 ± 0.01 ^{de}	- 1.23 ± 0.01 ^{cde}	8.93 ± 0.02 ^{ef}	1.15 ± 0.01 ^{bcd}
	20	70	40.95 ± 0.41	6.43 ± 0.04 ^a	2.78 ± 0.06 ^d	73.81 ± 0.06 ^{ef}	- 1.26 ± 0.04 ^{def}	8.94 ± 0.01 ^{ef}	1.42 ± 0.06 ^{abc}
	25	75	40.55 ± 0.02	6.44 ± 0.01 ^a	2.96 ± 0.06 ^{cd}	73.99 ± 0.07 ^{ef}	- 1.23 ± 0.01 ^{cde}	8.92 ± 0.01 ^{ef}	1.49 ± 0.07 ^{ab}
80	3	40	65.25 ± 0.73	6.45 ± 0.03 ^a	3.31 ± 0.13 ^{bc}	74.86 ± 0.04 ^{abc}	- 1.19 ± 0.01 ^{abc}	9.34 ± 0.02 ^a	0.30 ± 0.03 ^{fg}
	5	45	64.22 ± 1.38	6.42 ± 0.01 ^a	3.07 ± 0.06 ^{cd}	74.77 ± 0.17 ^{bc}	- 1.22 ± 0.02 ^{bcd}	9.18 ± 0.03 ^b	0.44 ± 0.16 ^{ef}
	10	60	63.49 ± 0.27	6.44 ± 0.07 ^a	2.93 ± 0.11 ^{cd}	74.17 ± 0.08 ^{de}	- 1.26 ± 0.01 ^{def}	8.89 ± 0.04 ^f	1.10 ± 0.09 ^{cd}
	15	75	62.37 ± 0.37	6.42 ± 0.01 ^a	3.1 ± 0.12 ^{cd}	73.91 ± 0.21 ^e	- 1.28 ± 0.02 ^{ef}	8.86 ± 0.04 ^f	1.36 ± 0.20 ^{ab}

L* represent the lightness with values from 0 (black) to 100 (white); a* positive values are red and negative values are yellow and negative ones are blue; ΔE color difference

^{a–g} Means within the same column with different letters are significantly different at *p* < 0.05

Crescent, Singapore). Protein solubility of sonicated, heated and untreated hazelnut milk samples were determined by using the Biuret method (Robinson and Hogden 1940). One mL sample was mixed with 1 mL of Biuret reagent and homogenized by vortex for 1 min. After 20 min, the absorbance of the samples was measured at 550 nm by UV spectrophotometer and protein solubility was calculated from a standard curve of Bovine Serum Albumin (BSA).

Color properties

Color values (L^* , a^* and b^*) of the samples were measured by using a colorimeter (Minolta Chroma Meter, CR-400, Osaka, Japan). Color values as L^* (Lightness), a^* (red-green) and b^* (yellow-blue) were used for evaluation of color difference (ΔE) (Eq. 1) with the following equation;

$$\text{Colour difference} = \Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Particle size

The particle size measurements of hazelnut milks were conducted using a laser diffractometer (Mastersizer 3000, Malvern Instruments Ltd., Worcestershire, UK). The mean diameter was evaluated based on volume weighted mean diameter $d_{4,3}$; Eq. (2) and the particle surface area $d_{3,2}$; Eq. (3). This approach is useful when the particles are not ideal spheres, and the $d_{3,2}$ is more influenced by the smaller particles, whereas the $d_{4,3}$ is more influenced by the larger ones (Leite et al. 2014). Cumulative percentiles $d_{0,1}$, $d_{0,5}$ and $d_{0,9}$ indicate that 10, 50 and 90% of the particles fell under the specified diameter, respectively.

$$d_{4,3} = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad (2)$$

$$d_{3,2} = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (3)$$

Sedimentation index and syneresis

Sedimentation was evaluated using 50 mL centrifuge tubes filled with the samples and stored at 4 °C for 15 days. In order to prevent microbial growth, 0.04% of sodium azide (NaN_3) was added. Sedimentation index (IS) was evaluated according to Eq. (4) (Rojas et al. 2016).

$$IS\% = \frac{S(t)}{V} * 100 \quad (4)$$

where $S(t)$ is sediment volume and V is total volume of samples. The IS of the all samples modelled as a function of storage time (4 °C) using an exponential decay function according to Eq. (5).

$$IS = I_{\text{equilibrium}} + (I_{\text{initial}} - I_{\text{equilibrium}}) \cdot e^{-k \cdot t} \quad (5)$$

where $I_{\text{equilibrium}}$ final read after sedimentation (%), I_{initial} is initial read before sedimentation (%), t is days, k is reaction kinetic.

Syneresis test was performed as centrifugation of 10 g hazelnut milk at $2400 \times g$ for 10 min and expressed as the percentage (w/w) of the supernatant part after centrifuge.

Microbiological analysis

Serial dilutions were prepared with 0.1% (w/v) peptone water. One milliliter of decimal dilutions of samples was pipetted into Petri dishes. Total aerobic mesophilic bacteria (TAMB) was enumerated by using Plate Count Agar (Merck, GmbH, Darmstadt, Germany) incubated at 30 °C for 72 h. Yeast and mold counts were enumerated by using Yeast Extract Glucose Chloramphenicol Agar (Merck, GmbH, Darmstadt, Germany) at 25 °C for 72 h. Results were expressed as log colony forming units (CFU) per milliliter.

Bioactive properties

Total phenolic content (TPC) was determined according to Bhat et al. (2011). Extraction was carried out as the procedure described by Behrad et al. (2009) with some modification. Two mL of hazelnut milk sample was mixed with 4 mL of 80% methanol, vortexed for 1 min and centrifuged at $5000 \times g$ for 10 min, and then supernatant was collected. The extracts were stored at 4 °C for further analysis. Extract (0.5 mL) was mixed with 2.5 mL 0.2 N Folin Ciocelteau's phenol reagent and 2 mL of 7.5% Na_2CO_3 , and incubated at room temperature for 30 min. After incubation, absorbance was measured at 760 nm using a UV/VIS spectrophotometer. Results were expressed as μg gallic acid equivalents (GAE) per gram of sample (calibration curve linearity range: $R^2 = 0.998$). DPPH free radical scavenging activity of the extracts was measured according to the method described by Behrad et al. (2009). An aliquot of 0.1 mL supernatant, was added to 4.9 mL DPPH solution (0.1 mM in methanol) and mixed vigorously. After 90 min incubation in the dark environment at room temperature, the absorbance was measured at 517 nm by a spectrophotometer (Helios Gamma, Cambridge, UK).

Antiradical activity (ARA, %) was described by the following equation:

$$ARA(\%) = \left(\frac{A_c - A_s}{A_c} \right) \times 100 \quad (6)$$

A_c is absorbance of control (methanol) and A_s is absorbance of sample. Results were expressed as μM Trolox equivalents (TE) per gram of sample (calibration curve linearity range: $R^2 = 0.999$).

Rheological measurement

Rheological characterization of hazelnut milk was carried out by using Haake Mars III rheometer (Thermo Scientific, Germany) with a cone and plate system (35 mm diameter, 0.105 mm gap, 2° angle). The temperature was maintained constant at 25°C by a Peltier plate system. The steady state shear experiments measured by shearing the samples at linearly increasing shear rates from 1 to 100 s^{-1} through 120 s. Product flow behavior was modeled using Ostwald-de-Waele model (Eq. 7).

$$\eta_{app} = K\dot{\gamma}^{n-1} \quad (7)$$

where η_{app} is apparent viscosity (Pa s), $\dot{\gamma}$ the shear rate (s^{-1}), K the consistency index (Pa s^n) and n the flow behavior index (dimensionless). Rheowin 4 Data Manager software (version 4.20, Haake) was used for calculations.

Data and statistical analysis

All values obtained in this study were expressed as mean \pm standard deviation. Data were analyzed by one-way analysis of variance (ANOVA). Differences between means were determined by using Tukey post hoc test with the level of significance of 95% ($p < 0.05$). Statistical analyses were performed using software IBM SPSS Statistics 21 (IBM SPSS, USA).

Results and discussion

pH and protein solubility

Applying TS and heat treatment resulted in a slight decrease (6.42–6.51) in pH but it was not significant (Table 1; $p < 0.05$). The change in pH of TS treated hazelnut milk is in accordance with findings of sonicated apple juice, kasturi lime juice and cranberry juice (Bhat et al. 2011; Abid et al. 2013; Gomes et al. 2017). The application of TS led to significant reduction in the content of soluble protein ($p < 0.05$) and this reduction was only low in the heat treated sample. This behavior may be

attributed to the protein unfolding/denaturation due to disruption in secondary, tertiary and quaternary structures and may cause to protein aggregation leading to decrease in protein solubility (Dhakal et al. 2014; Chen et al. 2015). Floury et al. (2002) were found that cavitation phenomena led to a strong decrease in the globulin solubility for egg/dairy emulsion.

Color properties

Color parameters of all samples (L^* : brightness, a^* : redness, b^* : yellowness, and ΔE : color difference value) are shown in Table 1. Heat treatment caused a reduction in L^* values ($p < 0.05$). After 10 min of application at 40% amplitude and more than 5 min of sonication for 60 and 80% amplitude led to decrease in L^* values ($p < 0.05$). The value of a^* did not change in the TS treated samples, whereas b^* reduced. Changes in ΔE are classified as not noticeable (0–0.5); slightly noticeable (0.5–1.5); noticeable (1.5–3.0) and easily noticeable (3.0–6.0) (Cserhalmi et al. 2006). Visible difference that can be perceived by humans does not occur under 3 value. The largest color difference was observed in heat treated hazelnut milk (1.76) compared to control. In the short time TS treatments, the color difference was below 0.5 and there was no visible change. As the process time increased, slightly noticeable change was observed due to the effect of temperature. So, only applying heat treatment caused noticeable differences for hazelnut milks. The changes in color values of TS treated hazelnut milks might be due to alone or combined effects of extrinsic control treatment variables of temperature and time (Rawson et al. 2011). Tiwari et al. (2009b) studied the effect of sonication in blackberry juice and they found that ΔE values were significantly influenced by process time whereas amplitude level was found as insignificant. Khandpur and Gogate (2015) obtained slight raise for L^* , a^* , b^* values by applying ultra-sonication in spinach juice. For kasturi lime and carrot juice it was found that sonication process decreased L^* , a^* values and increased b^* value (Jabbar et al. 2014; Khandpur and Gogate 2015). Applying TS at higher temperatures (40 and 60°C) caused to increase in L^* , a^* , b^* values of watermelon juice (Rawson et al. 2011).

Particle size

TS applications at 40 and 60% amplitudes for 5 and 10 min and 80% amplitudes for 3 and 5 min caused a decrease in the average particle size values ($d_{0.5}$) of samples (Table 2; $p < 0.05$). The highest average particle size was observed in control sample as $33\ \mu\text{m}$ and the lowest as $26\ \mu\text{m}$ at 80% amplitude for 5 min treatment. Ultrasound process disintegrates particles in the hazelnut milks by acoustic

Table 2 Particle size parameters ($d_{0.1}$, $d_{0.5}$, $d_{0.9}$, $d_{4.3}$, $d_{3.2}$) of untreated (C), heat treated (HT) and thermosonicated hazelnut milks

Amplitude (%)	Time (min)	$d_{0.1}$ (μm)	$d_{0.5}$ (μm)	$d_{0.9}$ (μm)	$d_{4.3}$ (μm)	$d_{3.2}$ (μm)
C	–	1.13 \pm 0.01 ^d	33.05 \pm 0.07 ^{ab}	88.00 \pm 2.26 ^a	38.95 \pm 0.78 ^a	4.65 \pm 0.01 ^{fgh}
HT	–	1.31 \pm 0.25 ^{abc}	32.55 \pm 0.64 ^{bc}	86.90 \pm 0.28 ^a	38.45 \pm 0.21 ^{ab}	4.96 \pm 0.44 ^{cde}
40	5	1.11 \pm 0.01 ^d	32.40 \pm 0.26 ^c	82.33 \pm 0.86 ^b	36.90 \pm 0.46 ^{de}	4.51 \pm 0.03 ^{hi}
	10	1.15 \pm 0.01 ^d	30.83 \pm 0.06 ^e	69.83 \pm 0.12 ^g	33.57 \pm 0.06 ^{hi}	4.60 \pm 0.01 ^{gh}
	15	1.21 \pm 0.01 ^{bc}	31.35 \pm 0.07 ^d	71.50 \pm 0.57 ^{fg}	33.45 \pm 0.21 ^{gh}	4.86 \pm 0.02 ^{defg}
	20	1.32 \pm 0.01 ^{abc}	31.70 \pm 0.17 ^d	70.33 \pm 0.12 ^g	33.47 \pm 0.12 ^{gh}	5.20 \pm 0.03 ^{bc}
	25	1.37 \pm 0.01 ^{ab}	33.17 \pm 0.06 ^a	79.30 \pm 0.1 ^{de}	36.87 \pm 0.06 ^{de}	5.37 \pm 0.01 ^{ab}
60	5	1.19 \pm 0.01 ^{bc}	32.70 \pm 0.1 ^{abc}	82.70 \pm 0.35 ^b	37.53 \pm 0.15 ^{cd}	4.89 \pm 0.01 ^{def}
	10	1.24 \pm 0.01 ^{bcd}	31.70 \pm 0.1 ^d	73.13 \pm 0.06 ^f	34.10 \pm 0.01 ^{fg}	4.99 \pm 0.01 ^{cde}
	15	1.18 \pm 0.01 ^{bc}	32.83 \pm 0.06 ^{abc}	86.80 \pm 0.44 ^a	38.57 \pm 0.12 ^{ab}	4.73 \pm 0.01 ^{efgh}
	20	1.41 \pm 0.01 ^a	32.77 \pm 0.06 ^{abc}	80.00 \pm 0.1 ^{cd}	37.10 \pm 0.20 ^{de}	5.46 \pm 0.01 ^{ab}
	25	1.19 \pm 0.01 ^{bc}	32.70 \pm 0.1 ^{abc}	82.70 \pm 0.35 ^b	38.00 \pm 0.1 ^{bc}	5.06 \pm 0.01 ^{cd}
80	3	1.12 \pm 0.01 ^d	28.07 \pm 0.06 ^e	81.17 \pm 0.15 ^{bc}	34.43 \pm 0.06 ^f	4.24 \pm 0.01 ^{ij}
	5	1.11 \pm 0.01 ^d	26.03 \pm 0.06 ^f	79.40 \pm 0.17 ^{de}	33.17 \pm 0.06 ⁱ	4.17 \pm 0.01 ^j
	10	1.40 \pm 0.01 ^a	33.03 \pm 0.21 ^{ab}	77.97 \pm 0.4 ^e	36.73 \pm 0.15 ^e	5.51 \pm 0.02 ^a
	15	1.42 \pm 0.01 ^a	32.37 \pm 0.07 ^{abc}	82.7 \pm 0.17 ^b	37.43 \pm 0.15 ^{cde}	5.33 \pm 0.01 ^{ab}

$d_{4.3}$ Volume weighted mean diameter; $d_{3.2}$ Surface weighted mean diameter; $d_{0.1}$ The diameter below which 10% of the volume of particles is found; $d_{0.5}$ The diameter below which 50% of the volume of particles is found; $d_{0.9}$ The diameter below which 90% of the volume of particles is found). HT: Heat treated samples at 85 °C for 2 min

^{a–j} Means within the same column with different letters are significantly different at $p < 0.05$

cavitation. A significant change in particle size distribution was detected in sonicated orange juice due to the cavitation effect (Tiwari et al. 2009a). Cheng et al. (2007) stated that the effect of high shearing during sonication process leads to transform fragment colloidal pectin molecules of guava juices into smaller size. The increase in process time led to increase in average particle size. It has been observed that the treatment after 10 min in all amplitude applications increased particle size. This can be attributed to the change in the protein conformation (denaturation) and the particle aggregation events due to increase in temperature during the process (Bernat et al. 2015). Another theory is that water penetrates inside of the cell and causes swelling due to permeability of the cell wall changes by cavitation effect (Rojas et al. 2016). Heat treatment without TS did not change average particle size significantly compared to control ($p > 0.05$). Bernat et al. (2015) applied low heat treatment (85 °C for 30 min) to hazelnut and almond milks and did not observe important particle size changes as compared to control (not-heated). Volume weighted mean diameter $d_{4.3}$ values showed similar results with average particle size values $d_{0.5}$ (Table 2). The minimum $d_{4.3}$ and $d_{3.2}$ values were obtained at 80% amplitude for 5 min. At this parameter, the reduction (17.02%) for $d_{4.3}$ was higher than the reduction (10.32%) in the $d_{3.2}$. That means that considerable decrease was occurred for large particles. Rojas et al. (2016) observed that the diameter reduction for

the $d_{4.3}$ (18% of reduction in relation to the unprocessed sample) was lower than the reduction in the $d_{3.2}$ (53% of reduction in relation to the unprocessed sample), which was different from our findings. They also stated that suspended particle disruption by TS is a complex phenomenon that can show differences due to process parameters.

Sedimentation index and syneresis

Vegetable based beverages are emulsified products with different process steps such as homogenization and heat treatments usually generating changes in the arrangement of their components (Bernat et al. 2015). The changes in the milk microstructure can explain the observed behavior in relation to sedimentation. Sedimentation mechanisms can be mainly explained by the Stokes Law. Particle sedimentation velocity is related to the square diameter and the difference between the densities of particle and dispersant medium (Kubo et al. 2013; Rojas et al. 2016). The mean particle size of samples decreased by the application of ultrasound treatment. The minimum sedimentation index was found as 14.5% at 40% amplitude for 5 min. Due to predominant large particles $d_{4.3}$, the sedimentation index values of control and heated samples were higher than the TS treated samples. Owing to the cavitation effect of TS, cell damage and disruption caused to release of

intracellular compounds resulting in the increase of serum viscosity. The increase in viscosity can explain the reduction in the sedimentation (Rojas et al. 2016). Sedimentation index values of samples during storage period was modelled as a function of storage time by using an exponential decay function (Eq. 4). The coefficient of determination values of all samples were satisfying and ranged from 0.915 to 0.994. The kinetic parameter obtained by modeling the sedimentation index displays the rate of sedimentation during the shelf life. The precipitation rate of ultrasound treated samples was lower than the control sample. The lowest kinetic parameter was found as 0.14 day⁻¹ for 80% and 60% amplitude for 5 and 25 min treatments, while the highest values for control and heat treated samples as 0.38 day⁻¹. In a similar study, the application of ultrasonication for 3 min led to a decrease in the rate of precipitation in peach juice from 1.4 to 0.26 day⁻¹ (Rojas et al. 2016).

Syneresis values of TS treated samples at short times showed a parallel decrease with particle size. The highest syneresis value was observed in the control and heat treated samples as 63.39 and 63.91% while the lowest values were observed in the TS applications of 80% amplitude for 3 and 5 min and 40% amplitude for 5 min as 59.03, 59.93 and 59.84%, respectively (Table 3). The increase in temperature depending on the extension of the ultrasound time increased the syneresis level of hazelnut milks. Results of syneresis are consistent with sedimentation index results in view of structural improvement of hazelnut milks with TS treatment.

Microbiological assessment

Microbiological results of control, heat-treated and TS treated samples were given at Table 4. Control sample had values of 5.98 and 3.65 log CFU/mL for TAMB and yeast-mold, respectively. Microorganism counts started to reduce when samples were sonicated at 40% amplitude for 15 min (57 °C); 60% amplitude for 10 min (58 °C) and 80% amplitude for 5 min (45 °C) (*p* < 0.05). In this study both heat treated and the TS treated samples at 60% amplitude for 25 min (75 °C) and 80% amplitude for 15 min (75 °C) had a complete inactivation of microorganisms in hazelnut milk.

Effect of sonication on microbiological reduction is stem from cell disruption during cavitation and also formation of free radicals and hydrogen peroxide, causing thinning of cell membranes of microorganisms (Bhat et al. 2011; Ertugay and Başlar 2014; Zafra-Rojas et al. 2013). There are some studies suggesting that only sonication treatment was ineffective for complete inactivation of microorganisms (Adekunte et al. 2010; Bhat et al. 2011; Zafra-Rojas et al. 2013). Inactivation of microorganisms by ultrasound treatment was effective when it was used in combination with other techniques such as heating (Abid et al. 2013). Bacterial cells become more sensitive after TS treatment by absorbing energy into membranes and biomaterials. This causes weakening of cell membranes with the combine effect of heat and ultrasound waves. Deng et al. (2018) applied TS for 2 min at 40, 50, and 60 °C to a typical Chinese lager beer. They found that both TS

Table 3 Syneresis values and mathematical modelling parameters of sedimentation during 15 days of storage (4 °C) for untreated (C), heat treated (HT) and thermosonicated hazelnut milks

Amplitude(%)	Time(min)	Syneresis (%)	<i>I.S. Eq</i> (%)	<i>I.S. Dif</i> (%)	<i>K</i> (1/days)	<i>R</i> ²
C	–	63.39 ± 0.66 ^{ab}	80	20	0.38	0.969
HT	–	63.91 ± 0.61 ^a	77.9	22.1	0.38	0.983
40	5	59.84 ± 0.99 ^{cde}	85.5	14.5	0.23	0.994
	10	60.37 ± 1.18 ^{cde}	83.5	16.5	0.24	0.985
	15	61.43 ± 0.49 ^{bcd}	83.65	16.35	0.36	0.915
	20	61.42 ± 0.21 ^{bcd}	83.5	16.5	0.24	0.983
	25	62.90 ± 0.83 ^{abc}	77.5	22.5	0.19	0.992
60	5	61.40 ± 0.97 ^{cde}	82	18	0.14	0.967
	10	60.61 ± 1.27 ^{cde}	83.5	16.5	0.33	0.972
	15	61.65 ± 1.80 ^{cde}	79	21	0.21	0.958
	20	62.13 ± 0.98 ^{abc}	79.5	20.4	0.20	0.963
	25	60.02 ± 1.11 ^{cde}	80.4	19.6	0.14	0.966
80	3	59.03 ± 2.62 ^e	83.1	16.9	0.15	0.971
	5	59.93 ± 1.7 ^{de}	84.1	15.9	0.13	0.98
	10	61.65 ± 1.42 ^{bcd}	84.5	15.5	0.14	0.957
	15	60.92 ± 0.44 ^{bcd}	79	21	0.14	0.982

(*I_{S_{Eq}}*

^{a-e} Means within the same column with different letters are significantly different at *p* < 0.05

Table 4 Effect of heat treatment (HT) and sonication on the survival of microorganisms of hazelnut milks

Amplitude (%)	Time (min)	Yeast (Log CFU/mL)	TAMB (Log CFU/mL)
C	–	3.65 ± 0.25 ^a	5.98 ± 0.03 ^a
HT	–	ND	ND
40	5	3.63 ± 0.55 ^{ab}	5.41 ± 0.08 ^{abc}
	10	3 ± 0.3 ^{abc}	5.42 ± 0.07 ^{abc}
	15	2.28 ± 0.03 ^{bc}	4.83 ± 0.02 ^{cde}
	20	2.65 ± 0.03 ^{cd}	4.58 ± 0.19 ^{de}
	25	1.65 ± 0.06 ^d	4.68 ± 0.52 ^{de}
60	5	3 ± 0.1 ^{abc}	5.17 ± 0.2 ^{bcd}
	10	2.07 ± 0.11 ^{cd}	4.53 ± 0.21 ^{de}
	15	0.43 ± 0.75 ^e	3.04 ± 0.04 ^f
	20	ND	2.13 ± 0.27 ^g
	25	ND	ND
80	3	2.97 ± 0.06 ^{abc}	5.54 ± 0.07 ^{ab}
	5	2.59 ± 0.52 ^{cd}	4.16 ± 0.16 ^c
	10	ND	2.34 ± 0.44 ^g
	15	ND	ND

ND not detected, HT heat treated samples at 85 °C for 2 min. TAMB total aerobic mesophilic bacteria

^{a–g} Means within the same column with different letters are significantly different at $p < 0.05$

treatments at 50 and 60 °C resulted in inactivation of yeast and aerobic microorganisms in beer. Saeeduddin et al. (2015) studied the quality assessment of pear juice treated with ultrasound and commercial pasteurization processing conditions. Authors found out that a complete inactivation of yeast and aerobic microbes was achieved in sonicated juice at 65 °C. However, pasteurization without sonication at 65 °C for 10 min could not inactivate microorganisms completely. Authors interpreted that inactivation occurs due to the possible synergistic effect existing between ultrasound and heat and a sudden increase in temperature and pressure of the localized area forming explosion of bubbles that cause the breakdown of microbial cells.

Bioactive properties

The total phenolic content (TPC) of control sample was found as 162.78 µg GAE/g. Applying heat treatment caused to a significant decrease in TPC (150.74 µg GAE/g) ($p < 0.05$). Sonication treatments at 40, 60 and 80% amplitudes significantly increase the release of TPC. Expanding of sonication time increased TPC except 80% amplitude for 10 and 15 min (Fig. 1a). The highest TPC content was observed for 60% amplitude level for 25 min as 178.82 µg GAE/g. Similar findings were obtained for cactus pear juice and kasturi lime juice samples (Bhat and Goh 2017; Bhat et al. 2011; Zafra-Rojas et al. 2013). Phenolic compounds are embedded at the vacuole in soluble form or bound to cell wall such as pectin, cellulose, hemicellulose and lignin (Escarpa and González 2001). The enhancement of TPC with sonication treatment

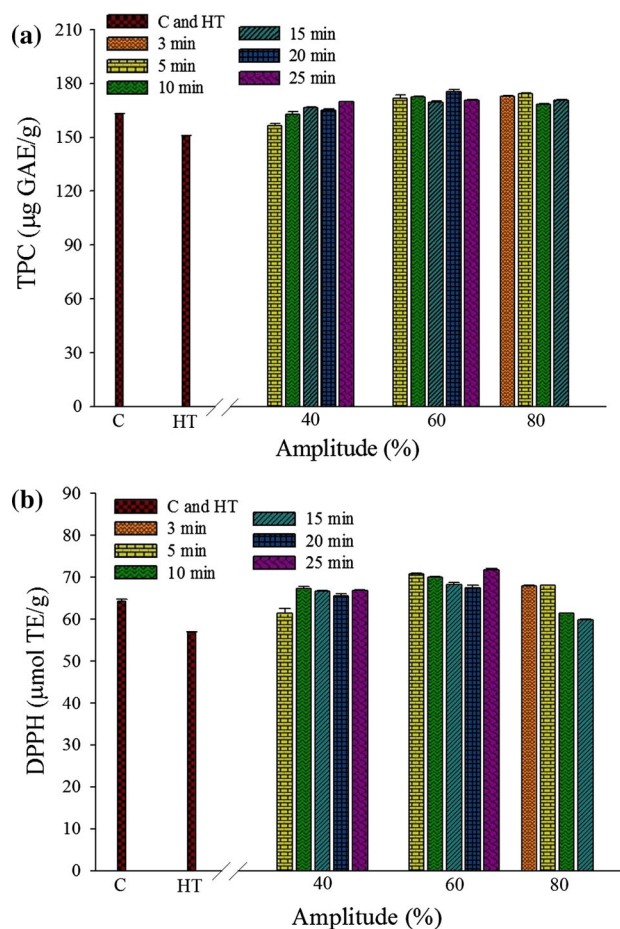


Fig. 1 Total phenolic compounds **a** antioxidant activity, **b** in hazelnut milks treated with thermosonication at different amplitudes and times. C control; HT heat treated hazelnut milks; TPC Total phenolic compounds. DPPH: DPPH free radical scavenging activity (color figure online)

Table 5 Rheological properties of untreated (C), heat treated (HT) and thermosonicated hazelnut milks

Amplitude (%)	Time (min)	K (Pa s ⁿ)	n (–)	R^2	η_{50} (Pa s)
C	–	0.37 ± 0.03 ^{cde}	0.33 ± 0.04	0.991	0.027 ± 0.001 ^{de}
HT	–	0.65 ± 0.10 ^a	0.37 ± 0.03	0.989	0.051 ± 0.007 ^b
40	5	0.33 ± 0.01 ^{cde}	0.33 ± 0.01	0.994	0.025 ± 0.003 ^{efg}
	10	0.30 ± 0.01 ^{de}	0.35 ± 0.01	0.990	0.023 ± 0.001 ^{fg}
	15	0.27 ± 0.05 ^e	0.37 ± 0.06	0.974	0.022 ± 0.002 ^g
	20	0.36 ± 0.06 ^{cde}	0.32 ± 0.02	0.990	0.025 ± 0.004 ^{efg}
	25	0.39 ± 0.04 ^{cde}	0.32 ± 0.04	0.989	0.029 ± 0.001 ^{de}
60	5	0.33 ± 0.01 ^{cde}	0.34 ± 0.06	0.989	0.028 ± 0.001 ^{de}
	10	0.34 ± 0.001 ^{cde}	0.34 ± 0.01	0.991	0.025 ± 0.002 ^{efg}
	15	0.32 ± 0.007 ^{cde}	0.36 ± 0.02	0.984	0.025 ± 0.004 ^{efg}
	20	0.43 ± 0.006 ^{bcd}	0.30 ± 0.05	0.983	0.032 ± 0.005 ^d
80	25	0.44 ± 0.007 ^{bc}	0.37 ± 0.01	0.977	0.032 ± 0.007 ^d
	3	0.35 ± 0.003 ^{cde}	0.27 ± 0.02	0.990	0.041 ± 0.008 ^c
	5	0.54 ± 0.003 ^{ab}	0.38 ± 0.01	0.980	0.029 ± 0.002 ^{de}
	10	0.54 ± 0.016 ^{ab}	0.34 ± 0.01	0.985	0.039 ± 0.006 ^c
	15	0.64 ± 0.001 ^a	0.28 ± 0.02	0.992	0.064 ± 0.004 ^a

HT: Heat treated samples at 85 °C for 2 min. K : Consistency index; n : flow behavior index; η_{app} : apparent viscosity at 50 s⁻¹ shear rate; R^2 : determination coefficient of Ostwald de-Waele equation

^{a–g} Means within the same column with different letters are significantly different at $p < 0.05$

attributed to the disruption of cell walls, that could make easier the release of bound TPC (Bhat and Goh 2017; Bhat et al. 2011). The highest increase in the total antioxidant activity was observed for 60% amplitude-25 min TS treated sample from 64.34 to 71.78 µmol TE/g ($p < 0.05$). This increase may be related to the uprising unbound TPC contents with cavitation effect. Heat treated samples had the lowest value of 56.75 µmol TE/g for hazelnut milks (Fig. 1b). This observation is also supported by the findings of Khandpur and Gogate (2015) who determined that sonication improved TPC and antioxidant activity, but heat treatment affected adversely. Del Socorro et al. (2015) compared TS with conventional pasteurization for purple cactus pear juice in view of antioxidant properties. They stated that pasteurization reduced antioxidant activity, whereas TS application led to enhancement.

Rheological parameters

The rheological parameters (K , n , and η_{50}) of control, heat and TS treated samples are shown in Table 5. The results are modeled according to the Ostwald-de Waele model because of the high correlation coefficient (0.974–0.994). Flow behavior index values of all samples were determined under 1 and all samples showed non-Newtonian flow characteristics. The highest apparent viscosity was determined as 0.064 Pa s for 80% amplitude 15 min at 50 s⁻¹ shear rate (shear rate in the mouth). In low amplitude and time periods, apparent viscosity and consistency index values of samples decreased compared to control. It has

been found that viscosity values increased at 80% amplitude for all time periods and 40 and 60% amplitudes for 20–25 min. Application of ultrasound at low intensities decreased the viscosity, whereas high intensity caused permanent changes on the rheological properties (Chemat and Zill-E-Huma 2011; Santhirasegaram et al. 2013). There are different explanations about the effects of ultrasound on the viscosity in the literature. Rojas et al. (2016) sonicated peach juice with different process times and they indicated that the effect of ultrasound process on apparent viscosity showed complex behavior. Application of ultrasonication significantly decreased viscosity on strawberry juice and the reduction in viscosity has been assigned to cavitation that might have led to pressure, shear and temperature changes. These changes triggered the fragmentation of polymeric structures (Bhat and Goh 2017). The heat treatment application raises the viscosity of the control sample from 0.027 to 0.051 Pa sⁿ accompanied by an increase in the consistency index from 0.37 to 0.65 Pa sⁿ ($p < 0.05$). Bernat et al. (2015) also mentioned that thermal treatments of homogenized almond and hazelnut milks lead to an increase in viscosity that may related to protein aggregation. These findings verify our results.

Conclusion

This study evaluated the effect of TS on the microbial, physical and structural properties of hazelnut milks. It was found that TS treatment improved the structural properties

such as sedimentation index and syneresis during the shelf life compared to conventional heating process. TS treatment also enhanced the bioactive compounds and antioxidant activity of hazelnut milk samples. Microbial inactivation was observed for 60% amplitude for 25 min (75 °C) and 80% amplitude for 15 min (75 °C). Synergistic use of ultrasound in a simultaneous application with heat could offer some advantages over the conventional thermal process in terms of sensory and nutritional quality of the foods. These findings shed light to food industry in view of the potential usage of TS for food safety and quality.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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