Thermosonication effect on bioactive compounds, enzymes activity, particle size, microbial load, and sensory properties of almond (Prunus dulcis) milk

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ABSTRACT
The object of this research was to appraise the physicochemical characteristics of almond milk and consumer acceptability after the thermosonication (TS) processing. The almond milk was subjected to TS processing (frequency: 40 kHz; power: 600 W; Temperature: 30, 45, and 60 °C; Time: 10, 20, 30, and 40 min) and pasteurization (for 60 s at 90 °C). After treatments, all samples were analyzed for bioactive compounds, antioxidant activities, microbial, enzymatic, and sensory attributes. The results showed a non-significant difference in total soluble solids and pH while TS processing at 45 and 60 °C significantly increased the cloudiness, viscosity, browning index, and color properties. TS processing increased the bioavailability of total phenolic, flavonols, flavonoids, condensed tannin contents, and antioxidant activity as compared to untreated and pasteurized samples. TS processing also significantly reduced the particle size distribution through acoustic cavitation. Microbial inactivation with TS at 60 °C resulted in ≥ 5 log reduction of total plate count and ≥ 4 log reduction of yeast & mold was achieved. The highest inhibition of lipoxygenase (LOX) and peroxidase (POD) were observed at 60 °C for 30 min. Moreover, the best sensorial properties were observed after TS processing at 60 °C. Thus; TS processing can increase the almond milk quality and safety as a viable substitute for thermal processing.

1. Introduction
Nowadays, consumer demands drive food processing industries to shift the priority to manufacture nutritionally enriched food items from plant sources [1]. Plant-based food products are an essential and sustainable substitute for human nutrition. They are invaluable sources of several bioactive phytochemicals, minerals, and vitamins that ensure the health and nutritional advantages [2,3]. However, plant-based beverages usually tend to deteriorate due to unsuitable processing and storage conditions, resulting in food safety and quality issues [4]. Conventional thermal processing has been studied for the quality preservation of different products, but earlier investigations reported some adverse changes resulting in poor quality. As per these results, dependence on alternative novel processing techniques to satisfy consumer’s interests has become imminent. Recently, Ultrasound (US) is a novel non-thermal processing technique having the advantage of preserving plant-based juices without adverse effects on the quality, sensory properties, and nutritional content than conventional heat treatments. The US processing has attained great attention to fulfill the demands of the Food and Drug Administration (FDA) for juice processing [5].

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Moreover, the combination of US and controlled temperature known as thermosensation (TS), has been stated to enhance the safety and quality attributes of different juices [6–8]. The US processing results in pore formation, membrane disruption, cellular cavitation, and final fragmentation at a frequency of 20–100 kHz [9]. The US treatment is stated to be the most useful technique for microorganism’s inactivation through cavitation, thermal effects, mechanical, and formation of free radicals [10]. In addition, US treatment also inactivates the enzyme activities including polygalacturonase and pectin methylsterase which are capable of breaking down some of the pectins and decreasing the viscosity of juices [11]. Also, TS treatment has gained notable attention in improving bioactive phytochemicals and antioxidant properties of different fruit juices of hog plum [4], star fruit [12], hazelnut milk [13], and pitaya [14].

Hence, the health-promoting compounds needed for the proper function of the human body are preserved in food products. So, the search for novel food processing and preservation of fresh plant-based beverages is becoming an important interest. Almond (Prunus dulcis) milk deserves particular attention as a plant-based product because of an excellent source of dietary fibers, monounsaturated fatty acids, vitamin E, protein, essential minerals, riboflavin, and antioxidants [15]. It has been found that almond milk consumption is also beneficial for coronary heart disease because reducing the plasma LDL cholesterol level [16]. It has been found that almond milk consumption is also beneficial for coronary heart disease because reducing the plasma LDL cholesterol level [16]. Nowadays, almond milk has been used as a substitute for milk in Australia, Europe, and the United States market. This non-dairy drink is a good alternative to cow milk for the hypersensitive and lactose intolerant population. Furthermore, the consumer’s demand has been increasing for dairy milk alternatives and preferable from plant-based sources [17]. To retaining the almond milk quality in processing, TS application will be a novel approach to improve its nutritional significance to its consumers. According to our knowledge, there has been a scarcity of data on TS-treated almond milk. Hence, the goal of this research was to examine the quality characteristics of almonds using the TS processing technique.

2. Material and methods

2.1. Raw material and almond milk extraction

Raw (unroasted) almond seeds were procured and store for 2 days at ambient temperature. Four hundred grams of raw almond seeds were soaked overnight at 4 °C in 1000 mL of distilled water. Then drained and rinsed the soaked almond with cold water and skin was manually removed. Before skin removal, almond seeds were weighed again to estimate the amount of absorbed water. For milk extraction, almonds were mixed with distilled water (1:9 ratio w/v) and ground in a blender. The obtained almond milk was stored for further analysis at 4 °C.

2.2. Thermal processing (TP)

In the thermal processing (TP), almond milk was treated at 90 °C for 1 min in a lab-scale pasteurizer (JBN 26, Cambridge, UK). This modified method was adopted to obtain a 5 log microbial reduction according to the method described by Santhirasagaram, Razali and Somasundram [18]. After that, the almond milk sample was cooled in an ice-water bath and stored (4 °C) for further analysis.

2.3. Thermosonication (TS)

TS treatment to almond milk was applied in a sonication bath (Skyen, JP-100S, China; power 600 W; frequency 40 kHz) at an acoustic energy density of 0.348 W/cm². The sonication bath (tank) had a rectangular dimension of 500 × 300 × 200 mm with a maximum tank capacity of 30 L. Almond milk sample (400 mL) was carefully placed in a sonication bath. The TS treatment was carried out at different temperatures (30, 45, and 60 °C) each for different times (10, 20, 30, and, 40 min). To avoid any possible light interference, all sonication treatments were performed in the dark. After treatment, all samples were cooled in an ice water bath and stored at (4 °C) until further analysis.

2.4. Physicochemical analysis

The total soluble solids (TSS) of all samples were measured at ambient temperature (25 °C) with a refractometer (PAL-1, Japan) and results are expressed as °Brix. The pH was measured with a digital pH-meter (PHS-3S, China) and viscosity with Brookfiel viscometer (DV2TLVTJ0, USA) using spindle 3 at 100 rpm.

2.5. Browning indices, cloudiness, and color properties

Browning index (BI) and cloudiness were measured by the method reported by Oladunjoye, Adeboyejo, Okekunbi and Aderibigbe [4]. For BI, the almond milk sample was centrifuged at 12,500 g with centrifuge for 10 min and then the supernatant was collected and clarified by using a 0.45 µm filter. For BI spectrophotometric (TU-1810, UV-visible, China) absorption was taken at 420 nm. For cloud value, 4 mL of almond milk sample was centrifuged at 760 g for 10 min at 4 °C and the supernatant was collected. The spectrophotometric absorbance was measured at 660 nm. The color analysis of almond milk samples was carried out with a digital colorimeter (CR-400, Japan) based on Hunter color values at ambient temperature (25 °C) and the total color difference (DE) was calculated using the following Equation (1).

\[
DE = \sqrt{(\Delta L^2) + (\Delta a^2) + (\Delta b^2)}
\]

(1)

2.6. Bioactive compounds

2.6.1. Total phenolic (TP)

For TPC analysis, the Folin-Ciocalteu protocol proposed by Aadil, Zeng, Han and Sun [19] was followed. Briefly, a reaction mixture was prepared with 0.5 mL of sample, 1 mL Folin-Ciocalteu reagent (10%), and 2 mL Na₂CO₃ (20%). Further, the solution was allowed in a dark room for 60 min at 30 °C. Finally, the spectrophotometric absorbance was calculated at 760 nm. A Standard solution of Gallic acid having a concentration ranging from 10 to 160 mg/mL (r² = 0.9988) was used to acquire calibration curve, the obtained finding was expressed as Gallic acid equivalents (GAE) µg/g.

2.6.2. Total flavonols

The total flavonols in almond milk samples were estimated through the method of Kumanar and Karunakaran [20]. A 2 mL standard solution was first added with 2.0 mL AlCl₃ (2%) mixture. Afterward, 3.0 mL C₂H₅NaO₂ solution with (50 g/L concentration was mixed and the solution was kept for 150 min at 20 °C. Finally, the spectrophotometric absorption was calculated at 440 nm. The acquired results were estimated as catechin equivalents (CE) µg/g based on the calibration curve.

2.6.3. Total flavonoid (TF)

The TF values from all the samples were detected by following the method described by Kim, Jeong and Lee [21] with minor changes. In detail, a known sample (0.25 mL) was first added with 1.25 mL of deionized water in a plastic tube followed by the addition of 75 µL of a 5% sodium nitrite solution. After resting for 6 min, 150 µL of a 10% aluminum chloride mixture was added. Again, 0.5 mL sodium hydroxide (1 M) was added to the solution after 5 min, finally, the volume makes up to 2.5 mL was set by using distilled water and agitated gently. In the end, the absorbance of all the samples was measured at 415 nm. The obtained outcomes were calculated as catechin equivalents (CE) µg/g based on the calibration curve.

2.6.4. Condensed tannin contents

The condensed tannins were estimated using the method of Reátegui,
sults condensed tannin contents were presented as catechin equivalents (CE) μg/g based on the calibration curve.

2.7. Antioxidant activites

2.7.1. Total antioxidant capacity (TAC)

TAC activity of almond milk after pasteurization and TS treatments were estimated through the method of Aadil, Zeng, Rahaman, Siddique, Aadil, Ahmed, Li and Niu [23] with modifications. Firstly, the DPPH solution was made in methanol by adding 50.7 μM DPPH and stored in the dark for 4 h. After that, almond milk samples were diluted with deionized water at a 1:10 ratio. The 300 μL of dilute almond milk solution was added in 3 mL of DPPH solution for reaction. Absorbance was measured at 517 nm at room temperature after 60 min of reaction in the dark. The obtained results for DPPH activity were expressed as % inhibition of the radical.

2.7.2. DPPH radical scavenging activity

The DPPH activity of the almond milk samples was measured through the method of Manzoor, Zeng, Rahaman, Siddique, Aadil, Ahmed, Li and Niu [24] with modifications. Firstly, the DPPH solution was made in methanol by adding 50.7 μM DPPH and stored in the dark for 4 h. After that, almond milk samples were diluted with deionized water at a 1:10 ratio. The 300 μL of dilute almond milk sample was added in 3 mL of DPPH solution for reaction. Absorbance was measured at 517 nm at room temperature after 60 min of reaction in the dark. The obtained results for DPPH activity were expressed as % inhibition of the radical.

2.7.3. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging property of all almond milk samples was calculated by using the method of Klein, Cohen and Cederbaum [24]. In short, a 1.0 mL of almond milk sample was mixed with 1.0 mL of iron-EDTA solution having 0.13% ferrous ammonium sulphate 0.26% EDTA, 0.5 mL EDTA solution having 0.018% concentration, 1.0 mL DMSO (0.85%) in 0.1 mol/L phosphate buffer pH 7.4) and 0.5 mL ascorbic acid (0.22%) in tubes and capped tightly. Then the tubes were placed in a water bath for 15 min at 80–90 °C. Further, the reaction was stopped by mixing 1.0 mL of 17.5% ice-cold TCA. Again, 3.0 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2.0 mL of acetylacetone were a mixture and final volume of 1 L was set with distilled water) was mixed and incubated at ambient temperature for 15 min for color development. The color intensity (yellow) was calculated at 412 nm with a spectrophotometer against a reagent blank. Ascorbic acid and gallic acid were employed as standards. The percent inhibition was detected by comparing test and standard.

2.8. Sedimentation index (IS) and particle size distribution (PSD)

After treatment, the PSD of almond milk was measured using a laser scattering PSD analyzer (LA960WET, HORIBA, UK). The mean diameter was measured based on the particle surface area \(D_{4,3}\) and volume-weighted mean diameter \(D_{4,2}\) [25]. Following equations (2) and (3) were used for calculation:

\[
D_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)
\]

\[
D_{4,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (3)
\]

For the calculation of span value, the following equation (4) was used:

\[
\text{Span value} = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}} \quad (4)
\]

The sedimentation index (IS) was determined according to the method of Rojas, Leite, Cristiani, Alvim and Augusto [26]. 50 mL almond milk samples filled in centrifuge tubes (50 mL) and stored for 48 h. Sodium azide (0.04%) was mixed in samples to inhibit microbial growth. The obtained results were described as IS g/100 (w/w).

2.9. Enzymatic activity: Lipooxygenase (LOX) and peroxidase (POD)

The LOX and POD activity of pasteurized and TS treated almond milk was measured using a UV–vis spectrophotometer at 234 nm [27] and 470 nm [28], respectively. The obtained results were displayed as a percent residual activity after treatment, while, the activity of enzymes found in the untreated almond milk was considered 100%. The percent (%) residual enzymatic activity was calculated using the following equation (5):

\[
\text{Residual activity(%) = } \frac{A_t}{A_0} \times 100 \quad (5)
\]

where \(A_t\) and \(A_0\) are the enzyme activity of the treated and untreated samples, respectively.

2.10. Microbial analysis

Microbiological load of yeasts & mold (Y&M) and total plate counts (TPC) were measured through the method reported by Nayak, Rayaguru and Radha Krishnan [29]. Y&M (25 °C for 120 h) and TPC (37 °C for 48 h) were assessed using potato dextrose agar and plate count agar, respectively, via the spread plate method. The obtained results are presented as log CFU/mL.

2.11. Sensory evaluation

All treated samples were analyzed for general acceptability and sensory properties. Sensory evaluation was performed to ascertain the impact of processing on the sensory qualities of almond milk. Seventy-five members were asked to score the samples. The almond milk samples were evaluated at a 9-point hedonic scale method (1 = extremely dislike to 9 = extremely like) [30].

2.12. Statistical analysis

All analyses were conducted in triplicates (n = 3). The obtained data from each treatment were subjected to SPSS software (IBM 24, USA) and Analysis of Variance (ANOVA). Duncan Multiple Range tests were used for comparison of means using significance level at p-value < 0.05.

3. Results and discussion

3.1. TSS (°Brix) and pH

The impact of pasteurization and TS treatments on TSS and pH of the almond milk was non-significant (p > 0.05) as compared to the UAM sample (Table 1). Hence, the TSS (4.27–4.48 °Brix) and pH (6.07–6.14) of the treated almond milk are within acceptable limits like control fresh almond milk. Earlier, Atalar, Gul, Saricagolu, Besir, Gul and Yazici [13] reported that TS processing does not significantly affect the physicochemical properties of hazelnut milk.

3.2. Viscosity and cloudiness

The level of cloudiness significantly reduced in the PAM sample, while the level of cloudiness significantly (p < 0.05) increased in all TS
Table 1

Effect of thermosonicated, and pasteurization on physicochemical and color properties of almond milk.

| Treatments  | pH   | TSS (°Brix) | Browning index | L* | ΔE  |
|-------------|------|-------------|----------------|-----|-----|
| UAM         | 6.12 | 4.48 ± 0.173 | 64.38 ± 0 —    |
| TSAM1       | 6.08 | 4.43 ± 0.111 | 0.03² 0.05³ 0.21³ |
| TSAM2       | 6.08 | 4.43 ± 0.175 | 65.01 ± 0.45² |
| TSAM3       | 6.08 | 4.41 ± 0.173 | 68.11 ± 2.27² |
| TSAM4       | 6.08 | 4.39 ± 0.175 | 66.62 ± 2.39² |
| TSAM5       | 6.07 | 4.37 ± 0.177 | 65.58 ± 3.63³ |
| TSAM6       | 6.07 | 4.37 ± 0.177 | 66.97 ± 3.09³ |
| TSAM7       | 6.09 | 4.37 ± 0.177 | 66.31 ± 3.57² |
| TSAM8       | 6.09 | 4.32 ± 0.139 | 0.08² 0.03³ |
| TSAM9       | 6.10 | 4.36 ± 0.178 | 67.28 ± 3.07² |
| TSAM10      | 6.07 | 4.32 ± 0.175 | 67.12 ± 1.98² |
| TSAM11      | 6.10 | 4.28 ± 0.178 | 64.78 ± 2.53³ |
| TSAM12      | 6.11 | 4.27 ± 0.180 | 63.26 ± 4.22³ |
| PAM         | 6.14 | 4.44 ± 0.188 | 67.75 ± 5.12³ |
|             | 0.02 | 0.19 ± 0.12³ | 0.07² 0.17³ |

UAM: Untreated almond milk, PAM: Pasteurized almond milk, TSAM: Thermosonicated almond milk. Sample code with detail: TSAM1: 30 °C for 10 min, TSAM2: 30 °C for 20 min, TSAM3: 30 °C for 30 min, TSAM4: 30 °C for 40 min, TSAM5: 45 °C for 10 min, TSAM6: 45 °C for 20 min, TSAM7: 45 °C for 30 min, TSAM8: 45 °C for 40 min, TSAM9: 60 °C for 10 min, TSAM10: 60 °C for 20 min, TSAM11: 60 °C for 30 min, TSAM12: 60 °C for 40 min

Table 2

The bioactive compounds in untreated, pasteurized, and thermosonicated almond milk are presented in Table 2. During TS, the TP contents increased from 712.1 to 746.4 GAE µg/g at 30 °C (TSAM4), and from 718.2 to 7.66.3 GAE µg/g (TSAM8) at 45 °C. The increase in TP contents was observed in samples treated at 60 °C from 732.2 to 692.9 GAE µg/g (TSAM12). A similar trend was also observed in flavonals and TF contents during TS processing at 30, 45, and 60 °C. The TP, flavonals, and TF contents of UAM (702.1 GAE µg/g, 8.90 CE µg/g, and 412.3 CE µg/g) and PAM (678.3 GAE µg/g, 8.12 CE µg/g, and 375.4 CE µg/g), respectively were observed under above conditions. The condensed tannin contents in TS treated almond milk varied between 205.8 and 159.2 CE µg/g. A significant decrease (p < 0.05) in bioactive compounds was observed in PAM compared to untreated almond milk, while TS processing significantly increased the level of the bioactive compounds. An increase in bioactive compounds by TS processing at 30, 45, and 60 °C can be due to the release of bound secondary metabolites during cavitation because the cavitation process enhances mechanical disruption of cell walls. Also, Ghasemzadeh, Jaafar, Juraimi and Tayebi-Meigooni [36] reported that the acoustic wave generates disruption in the biological cell and improves the release of the cell contents. Martínez-Flores, García-Romo, Bermúdez-Aguirre, Pokhrel and Barbosa-Cánovas [37] stated that US provides adequate cavitation to generate shear forces to disrupt the cell walls and improves the diffusion of cell contents. While, a decrease in bioactive compounds at higher temperatures could be associated with processing parameters including treatment time, temperature, and power rating [38]. Earlier similar results of bioactive compounds were reported in TS treated hog plum juice [4].

3.5. Antioxidant activities: TAC, DPPH activity and hydroxyl radicals scavenging activity

The TAC and DPPH values were significantly (p < 0.05) different in UAM, PAM, and TSAM12 as shown in Table 3. The TAC and DPPH activity of UMA was 17.65 mM Trolox equivalent/g and 44.2%, respectively, while 16.12 mM Trolox equivalent/g and 40.54%, respectively were observed in PAM. However, it was observed that TS processing significantly (p < 0.05) increased the TAC and DPPH activity.

During TS, the DPPH activity % inhibition increased from 54.13 to 68.13% at 30 °C (TSAM4), and from 52.65 to 74.71% (TSAM8) at 45 °C. However, the decrease in TP contents from 63.12 to 51.43% (TSAM12) was observed in samples treated at 60 °C. It was also noted that hydroxyl radicals scavenging activities % inhibition were possibly due to the cavitation process, which produced color compounds by partial precipitation of unstable suspended particles. A significant increase in L* TS treated almond milk sample can be associated with the inactivation of polyphenols oxidase [32]. Furthermore, reduction at a long time and elevated temperature has been correlated with the evolution of the Maillard reaction. Earlier, a study reported that a similar impact in TS treated hazelnut milk [13]. Color is an important variable for sensory property and microbiological safety of juices for consumption, processing, and storage [33].

Browning index (BI) significantly increased (p < 0.05) in the PAM sample (0.188) as compared to the UAM sample (0.173) (Table 1). During TS processing, all samples exhibited a raise in the BI with the treatment time, and this can be associated with the formation of the Maillard reaction. Previously, some researchers stated that the TS processing triggers the Maillard reaction above 50 °C and inadequacy of TS to induce the Maillard effect at ≤ 50 °C. Previous studies also reported that the TS processing activates the Maillard reaction above 50 °C [34]. Therefore this statement agrees with the reduction in b* and L* of values in the TSAM12 sample. TS cannot induce a Maillard reaction at ≤ 50 °C and possibly responsible for the trilling impact at TSAM12 [35].

3.3. Color properties and browning index

The color properties (L*) of UAM (64.38) and PAM (67.75) samples are presented in Table 1. Distinctly, TS processing increased b* (yellowness) and L* (lightness) values from TSAM1 to TSAM12, while treatment at 60 °C (TSAM12) decreased these values. During TS processing at 40 and 50 °C, an improvement in L* and b* was observed
significantly increased \((p < 0.05)\) from 43.45 to 57.98\% at 30 \(^\circ\)C (TSAM\(_{1-4}\)) and from 42.35 to 64.57\% (TSAM\(_{5-8}\)) at 45 \(^\circ\)C. While during TSAM\(_{9-12}\) hydroxyl radicals scavenging activity was decreased from 53.12 to 40.67\% at 60 \(^\circ\)C.

An increase in antioxidant activity at 30 and 45 \(^\circ\)C might be linked with the release of phenolic contents during cavitation \([39]\). Earlier similar trends were reported in carrot juice with the same treatment conditions \([37]\). Some researchers also reported that during TS processing, a decrease in the formation of free hydroxyl radicals is associated with an increase in antioxidant activities \([40]\). However, the high level of free hydroxyl radicals for a long time can inhibit antioxidant activities \([41]\). So we can say that higher temperature (60 \(^\circ\)C) produces free hydroxyl radical’s species resulting in a reduction of antioxidant activities. Muñiz-Márquez, Martínez-Avila, Wong-Paz, Belmares-Cerda, Rodríguez-Herrera and Aguilar \([42]\) stated that the decrease in antioxidant activities might be associated with the degradation of phenolic compounds when employing the high temperature and high power, through generating cavitation bubble collapse. Also, Qu, Yu, Luo, Zhao and Huang \([43]\) also reported that the hydroxyl radical scavenging activity was lower at the higher ultrasonic time and temperature, while higher at a low ultrasonic time and temperature.

### 3.6. Colloidal stability: Sedimentation index (IS) and particle size distribution (PSD)

TS treatment at 30, 45, and 60 \(^\circ\)C for 10, 20, 30, and 40 min caused significant \((p < 0.05)\) reduction in \(D_{(3,2)}\) and \(D_{(4,3)}\) values of samples (Table 3). The highest \(D_{(3,2)}\) (6.81 \(\mu\)m) and \(D_{(4,3)}\) (7.95 \(\mu\)m) values were recorded in the PAM sample. TS treatment reduces the \(D_{(3,2)}\) and \(D_{(4,3)}\) values from 5.22 to 3.96 \(\mu\)m and 7.02 to 6.46 \(\mu\)m, respectively. TS treatment disintegrates the almond milk particles through acoustic cavitation. Earlier, a notable reduction in particle size of coconut milk was observed after the ultrasound treatment at 20 kHz for 13 min \([44]\). Tiwari, Muthukumarappan, O’donnell and Cullen \([45]\) reported a significant variation in PSD due to cavitation in sonicated orange juice. The increase in the treatment time led to reducing average PSD. During the sonication process of guava juice, the impact of high shearing led to the change of the colloidal pectin molecules into a smaller size \([46]\). The span values significantly \((p < 0.05)\) decreased after the TS treatment (TSAM\(_{1-12}\)), while significantly \((p < 0.05)\) increased in pasteurization.
The span value of almond milk describes the PSD width, so this decline presents better-homogenized almond milk. The US cavitation caused by the low-frequency further promotes the damage to droplets. Furthermore, microbubbles generated and subsided regularly near the interface, ending in localized turbulence. High local shear force and acoustic waves produced by high-pressure shock and microflow guide to larger droplets exploding [44]. Consequently, the variations in smaller cell fragments and various cell components, as aggregates) look to be more valuable than those on the bigger particles. It can describe a potential deviation in the mechanical resistance between the whole cells and its particles, which can be explained by several potential mechanisms: the region exposed, the diverse compositions, the internal cell pressure, consequent mechanical resistance of the intercellular liquid, and susceptibility to cavitation. This outcome highlights that the suspended particle disruption by the US processing is a complicated phenomenon, which needs to be fully understood.

Almond milk is emulsified product with different process steps allowing modifications in components arrangement [47]. In the liquid phase, particles are scattered in different aspects including oil droplets, natural protein aggregates, and proteins and oil droplets aggregates. In almond milk, the stability issues including oil droplets creaming, sedimentation of solid particles, and both dependent upon PSD. By measuring the solid sedimentation of almond milk after TS processing, and PSD, the colloidal stability was observed as shown in Table 3. In the case of macrostructure, phase separation was observed in all samples after 48 h, except the TSAM2 sample (Supplementary Fig. 1). After TS processing it was observed that the sedimentation rate significantly decreased (p < 0.05) in TSAM2, possibly associated with centrifugation than the UMA sample. While sedimentation rate significantly increased (p < 0.05) in the PAM sample. Therefore, TS processing seems to support a weak gel formation associated with subsequent protein solubilization and denaturation, and both of the stabilization of the PSD to evading phase separation.

### Table 3

| Treatments | DPPH (% inhibition) | TEAC (mM Trolox eq. per g) | Hydroxyl radical scavenging activity | D$_{50.2}$ µm | D$_{45.3}$ µm | Span value | Sedimentation Index (g/100 w/w) |
|------------|----------------------|----------------------------|-------------------------------------|---------------|-----------------|------------|-------------------------------|
| UAM        | 44.12 ± 0.12         | 17.65 ± 0.14               | 35.14 ± 0.11                        | 5.97 ± 0.26   | 7.35 ± 0.12     | 1.45 ± 0.12 | 3.97 ± 0.21                   |
| TSAM$_{1}$ | 54.13 ± 0.21         | 21.02 ± 0.10               | 43.45 ± 0.15                        | 5.22 ± 0.27   | 7.02 ± 0.15     | 1.34 ± 0.17 | 3.75 ± 0.18                   |
| TSAM$_{2}$ | 58.17 ± 0.16         | 25.87 ± 0.22               | 48.67 ± 0.22                        | 5.01 ± 0.31   | 6.90 ± 0.15     | 1.28 ± 0.10 | 3.58 ± 0.20                   |
| TSAM$_{3}$ | 60.35 ± 0.12         | 23.28 ± 0.18               | 51.76 ± 0.14                        | 4.82 ± 0.12   | 6.88 ± 0.15     | 1.24 ± 0.07 | 3.43 ± 0.17                   |
| TSAM$_{4}$ | 68.13 ± 0.19         | 25.91 ± 0.12               | 57.98 ± 0.08                        | 4.71 ± 0.28   | 6.65 ± 0.12     | 1.21 ± 0.13 | 3.25 ± 0.13                   |
| TSAM$_{5}$ | 52.65 ± 0.12         | 21.06 ± 0.09               | 42.35 ± 0.11                        | 5.14 ± 0.29   | 6.96 ± 0.13     | 1.31 ± 0.13 | 3.62 ± 0.15                   |
| TSAM$_{6}$ | 61.35 ± 0.11         | 24.17 ± 0.11               | 51.23 ± 0.16                        | 4.97 ± 0.33   | 6.81 ± 0.12     | 1.26 ± 0.16 | 3.41 ± 0.11                   |
| TSAM$_{7}$ | 70.47 ± 0.21         | 25.31 ± 0.15               | 60.87 ± 0.18                        | 4.89 ± 0.45   | 6.68 ± 0.12     | 1.23 ± 0.24 | 3.23 ± 0.24                   |
| TSAM$_{8}$ | 74.71 ± 0.20         | 29.43 ± 0.19               | 64.56 ± 0.21                        | 4.68 ± 0.39   | 6.37 ± 0.15     | 1.18 ± 0.10 | 2.95 ± 0.09                   |
| TSAM$_{9}$ | 63.12 ± 0.15         | 25.12 ± 0.13               | 53.12 ± 0.12                        | 4.87 ± 0.47   | 6.75 ± 0.15     | 1.23 ± 0.24 | 3.25 ± 0.14                   |
| TSAM$_{10}$| 60.12 ± 0.18         | 22.17 ± 0.08               | 50.02 ± 0.09                        | 3.96 ± 0.41   | 6.45 ± 0.08     | 1.14 ± 0.14 | 3.03 ± 0.07                   |
| TSAM$_{11}$| 57.23 ± 0.17         | 23.17 ± 0.16               | 47.42 ± 0.15                        | 4.24 ± 0.22   | 6.51 ± 0.11     | 1.17 ± 0.12 | 2.85 ± 0.12                   |
| TSAM$_{12}$| 51.43 ± 0.14         | 20.58 ± 0.12               | 40.67 ± 0.17                        | 4.46 ± 0.29   | 6.62 ± 0.15     | 1.20 ± 0.11 | 2.55 ± 0.11                   |
| PAM        | 40.54 ± 0.15         | 16.12 ± 0.17               | 30.48 ± 0.13                        | 6.81 ± 0.33   | 7.95 ± 0.16     | 1.48 ± 0.23 | 4.22 ± 0.23                   |

UAM: Untreated almond milk, PAM: Pasteurized almond milk, TSAM: Thermosonicated almond milk, Sample code with detail: TSAM$_{1}$: 30 °C for 10 min, TSAM$_{2}$: 30 °C for 20 min, TSAM$_{3}$: 30 °C for 30 min, TSAM$_{4}$: 30 °C for 40 min, TSAM$_{5}$: 45 °C for 10 min, TSAM$_{6}$: 45 °C for 20 min, TSAM$_{7}$: 45 °C for 30 min, TSAM$_{8}$: 45 °C for 40 min, TSAM$_{9}$: 60 °C for 10 min, TSAM$_{10}$: 60 °C for 20 min, TSAM$_{11}$: 60 °C for 30 min, TSAM$_{12}$: 60 °C for 40 min.

All results are present as ± SD and n = 3. Values with different superscripts are significantly (p < 0.05) different from each other.
3.7. Enzymes activity

The residual LOX and POD enzyme activities for the almond milk after TS treatment are shown in Table 4. TS treatments were more effective against enzyme inactivation as compared to pasteurization. The inhibition of LOX and POD enzymes in the PAM sample was 7.12 and 8.24 %, respectively. During TS, the residual activity of LOX was decreased from 85.5 to 70.3% at 30 °C (TSAM4), and from 65.7 to 34.9 % (TSAM4) at 45 °C. However, the decrease in LOX activity from 42.2 to 5.12 % (TSAM12) was observed in samples treated at 60 °C. The residual activity of POD was reduced from 88.1 to 6.35 % in TSAM12 samples. The LOX and POD activities of almond milk reduced by increasing the treatment time and temperature. Earlier, POD residual activity decreased in the US-treated tomato by increasing treatment temperature was reported by Ercan and Soysal [48]. Cao, Cai, Wang and Zheng [49] reported seventeen times more reduction of POD enzyme activity in thermal and the US treated bayberry juice at 55 to 60 °C. The TS treatment time and the temperature were important in decreasing LOX and POD activity. The formation of free radicals during sonolysis and cavitation bubbles responsible for the denaturation of the enzymes [50]. The production and disappearance of cavities due to the bubble’s evolution is also linked to enzyme inactivation [48,51]. This can produce an obvious improvement in pressure and temperature in a localized US-producing area, which possibly an influential constituent in enzyme inactivation. Hence, the temperature with other mechanical forces during US pasteurization has a joined role to inactivate the enzymes. The TS treated samples presents better results for enzyme inactivation at different time and temperature combinations. TS processing initially assured mechanical and thermal shock into endogenous enzymes, subsequently damaging the structure of a protein. Finally, these critical physical conditions create the inactivation of enzymes [11].

3.8. Microbial load

PAM sample displayed no detectable microbiological load, while the UAM sample showed TPC and Y&M (5.21 and 4.40 CFU/mL, respectively) (Table 4). After TS processing at 30 °C (TSAM4), the Y&M count decreased from 4.18 to 3.54 log CFU/mL, while TPC was decreased from 4.75 to 2.55 log CFU/mL. Moreover, TS processing at 45 °C (TSAM4) and 60 °C exhibited no detectable microbiological growth in TPC and Y&M. During pasteurization, complete inactivation might be due to the rupturing of nuclear components and cell membrane leading to cell destruction [52]. Microbial inactivation through TS is mainly linked with cavitation on the cellular structure. This acoustic cavitation could either be stable or transient [6]. During TS processing, the almond milk acidity produced osmotic pressure synchronically with other reactions and possibly improve the cavitation influence on the microbiological structure leading to the release of lipids, protein, and nuclear compounds [53,54]. Earlier similar results have been reported to increase microbial inactivation in TS-treated carrot juice [37] and hog plum juice [4]. Nevertheless, partial inactivation of TPC and Y&M at 30 °C and 45 °C might be due to spore formation which could endure these processing conditions [18,55]. But, increasing treatment temperatures, complete TPC, and Y&M inactivation were achieved which could fulfill accepted regulatory obligations. The inactivation mechanism of microorganisms through the US the result of many complex physical processes. The impact of the US is also associated with chemical influences linked to the formation of free radicals through the disintegration of water inside oscillating bubbles. The US processing to microorganisms punctures their cell membranes and extrusion of the intracellular matrix and generates free radicals finally eliminates the microorganisms [56].

| Treatments | Residual activity (%) | LOX (%) | Total plate count (log CFU/mL) | Yeast and mold count (log CFU/mL) |
|------------|----------------------|--------|--------------------------------|----------------------------------|
| UAM        | 100.0 ± 0.00         | 100.0 ± 0.00 | 5.21 ± 0.09                    | 4.40 ± 0.11                     |
| TSAM1      | 88.1 ± 0.23          | 85.5 ± 0.35  | 4.75 ± 0.06                    | 4.18 ± 0.13                     |
| TSAM2      | 83.3 ± 0.43          | 82.1 ± 0.23  | 3.98 ± 0.12                    | 4.05 ± 0.16                     |
| TSAM3      | 79.2 ± 0.34          | 76.8 ± 0.45  | 3.73 ± 0.11                    | 3.94 ± 0.12                     |
| TSAM4      | 75.1 ± 0.18          | 70.3 ± 0.65  | 2.55 ± 0.10                    | 3.54 ± 0.09                     |
| TSAM5      | 74.4 ± 0.22          | 65.7 ± 0.53  | 2.02 ± 0.07                    | 2.98 ± 0.07                     |
| TSAM6      | 61.4 ± 0.56          | 60.2 ± 0.42  | 1.04 ± 0.07                    | 2.12 ± 0.08                     |
| TSAM7      | 49.2 ± 0.28          | 46.7 ± 0.33  | ND                             | 1.68 ± 0.11                     |
| TSAM8      | 39.5 ± 0.39          | 34.9 ± 0.49  | ND                             | ND                              |
| TSAM9      | 46.8 ± 0.47          | 42.2 ± 0.23  | ND                             | ND                              |
| TSAM10     | 32.4 ± 0.21          | 28.4 ± 0.19  | ND                             | ND                              |
| TSAM11     | 18.6 ± 0.19          | 15.3 ± 0.38  | ND                             | ND                              |
| TSAM12     | 6.35 ± 0.17          | 5.12 ± 0.27  | ND                             | ND                              |
| PAM        | 8.24 ± 0.11          | 7.12 ± 0.17  | ND                             | ND                              |

Table 4: Effect of thermosonicated, and pasteurization on enzymes activity and microbial load of almond milk.

4. Conclusions

In this research, almond milk was treated with TS as a way forward to enhance the overall quality and consequently its economic worth over traditional processing techniques. The TS retained the quality parameters of the almond milk, mainly with increasing the cloudiness, color properties, and browning index. It also improved phenolic, flavonoids, flavonoids, condensed tannins contents, DPPH activity, and total antioxidant activity. Significant reduction in residual activities of enzymes (LOX and POD) and microbiological inactivation was observed with better sensory scores. The TS processing of almond milk can be useful in enhancing quality without disturbing any quality parameters. The study shows that TS is a viable processing technique for almond milk processing as compared to thermal processing. Moreover, the storage shelf stability of the TS treated almond milk is open to further research for its economic potentials.

CRediT authorship contribution statement

Muhammad Faisal Manzoor: Conceptualization, Methodology, Formal analysis, Writing–original draft. Rabia Siddique: Software. Abid Hussain: Investigation, Nazir Ahmad: Writing–review & editing. Abdur Rehman: Data curation, Software. Azhari Siddique: Data curation, Software. Ammar Alfarga: Data curation, Software. Ghedir M. Alshammari: Data curation, Funding acquisition. Mohammed A. Yahya: Data curation, Funding acquisition.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] E. Medawar, S. Huhn, A. Villringer, A.V. Witte, The effects of plant-based diets on the body and the brain: a systematic review, Transl. Psychiatry 9 (2019) 1–17.
[2] H.M. Shabbar, J.U. Kim, S.H. Kim, J. Park, The Inactivation of Pathogens in Fruit Juice: Escherichia coli O157: H7, Salmonella Typhimurium, and Listeria monocytogenes, in: Fruit Juices, Elsevier, 2018, pp. 341–361.
[3] M.F. Manzoor, X.-A. Zeng, N. Ahmad, Z. Ahmad, A. Rehman, R.M. Aadil, U. Roobab, R. Siddique, A. Rahaman, Effect of pulsed electric field and thermal treatments on the bioactive compounds, enzymes, microbial, and physical stability of almond milk during storage, J. Food Processing Preservation 44 (7) (2020), https://doi.org/10.1111/jfpp.2019.44.7.
[4] A.O. Oladunjoye, F.O. Adeboyejo, T.A. Okekunbi, O.R. Aderibigbe, Effect of thermosonication on quality attributes of hog plum (Spondias mombin L.) juice, Ultras. Sonochem. 70 (2021), 105316.
[5] R.M. Aadil, X.A. Zeng, Z.H. Zhang, M.S. Wang, Z. Han, D.-W. Sun, Effects of ultrasound treatments on pathogenic bacteria in almond milk, J. Food Process Eng 42 (8) (2019) e12099.
[6] M.F. Manzoor, N. Ahmad, R.M. Aadil, A. Rahaman, Z. Ahmed, A. Rehman, A. Siddeeg, X.-A. Zeng, A. Manzoor, Impact of pulsed electric field on rheological, structural, and physicochemical properties of almond milk, J. Food Process Eng 42 (8) (2019) e12099.
[7] V. Sahinissegaram, R. Razali, C. Somasundaram, Effects of thermal treatment and sonication on quality attributes of Chokanan mango (Mangifera indica L.) juice, Ultras. Sonochem. 20 (2013) 1276–1282.
[8] M.F. Manzoor, N. Ahmad, R.M. Aadil, A. Rahaman, Z. Ahmed, A. Rehman, A. Siddeeg, X.-A. Zeng, A. Manzoor, Impact of pulsed electric field on rheological, structural, and physicochemical properties of almond milk, J. Food Process Eng 42 (8) (2019) e12099.
[9] A. Kumaran, R.J. Karunakaran, In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India, LWT-Food Science Technology 40 (2007) 344–352.
[10] M.D. Klein, G. Cohen, A.L. Cederbaum, Production of formaldehyde during thermosonication (TS) process on the quality parameters of high pressure homogenized hazelnut milk from hazelnut oil by-products, Int. J. Food. Sci. Technol. 55 (2019) 1405–1415.
[11] H. Liao, W. Zhu, K. Zhong, Y. Liu, Evaluation of colour stability of clear red pitaya juice treated by thermosonication, J. Food Sci. Technol. 121 (2020), 108997.
[12] S. Yada, G. Huang, K. Lapsley, Natural variability in the nutrient composition of California-grown almonds, J. Food Composition Analysis 30 (2013) 80–85.
[13] A. Kumaran, R.J. Karunakaran, In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India, LWT-Food Science Technology 40 (2007) 344–352.
[14] D. Barreira, S.M. Nabavi, A. Sureda, M. Rasekhian, R. Raciti, A.S. Silva, G. Annunziata, A. Arnese, G.C. Tenore, I. Sünztar, Almonds (Prunus dulcis Mill. DA Webb): a source of nutrients and health-promoting compounds, Nutrients 12 (2020) 672.
[15] M.F. Manzoor, N. Ahmad, R.M. Aadil, A. Rahaman, Z. Ahmed, A. Rehman, A. Siddeeg, X.-A. Zeng, A. Manzoor, Impact of pulsed electric field on rheological, structural, and physicochemical properties of almond milk, J. Food Process Eng 42 (8) (2019) e12099.
[16] V. Sahinissegaram, R. Razali, C. Somasundaram, Effects of thermal treatment and sonication on quality attributes of Chokanan mango (Mangifera indica L.) juice, Ultras. Sonochem. 20 (2013) 1276–1282.
[17] M.A. Islam, M. Zhang, B. Adhikari, The inactivation of enzymes by ultrasound—a review of potential mechanisms, Food Reviews Int. 20 (2014) 1–21.
[18] P.K. Nayak, C.M. Chandrashekar, R.K. Kesavan, Effect of thermosonication on the quality attributes of star fruit juice, J. Food Process Eng 41 (7) (2018) e12857.
[19] I. Atalor, G. Gou, F.T. Sarcaicoagu, A. Besir, L.B. Gul, F. Yazici, Influence of thermosonication (TS) process on the quality parameters of high pressure homogenized hazelnut milk from hazelnut oil by-products, Int. J. Food. Sci. Technol. 55 (2019) 1405–1415.
[20] H. Liao, W. Zhu, K. Zhong, Y. Liu, Evaluation of colour stability of clear red pitaya juice treated by thermosonication, J. Food Sci. Technol. 121 (2020), 108997.
[21] S. Yada, G. Huang, K. Lapsley, Natural variability in the nutrient composition of California-grown almonds, J. Food Composition Analysis 30 (2013) 80–85.
[22] C. Somasundaram, Effects of thermal treatment and sonication on quality attributes of Chokanan mango (Mangifera indica L.) juice, Ultras. Sonochem. 20 (2013) 1276–1282.
[23] R.M. Aadil, X.A. Zeng, Z. Han, D.-W. Sun, Effects of ultrasound treatments on quality of grapefruit juice, J. Food Chem. 141 (2013) 3201–3206.
[24] A. Kumaran, R.J. Karunakaran, In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India, LWT-Food Science Technology 40 (2007) 344–352.
[25] D.-O. Kim, S.W. Jeong, C.Y. Lee, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem. 81 (2003) 321–326.
[26] A. Kumaran, R.J. Karunakaran, In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India, LWT-Food Science Technology 40 (2007) 344–352.
[27] Z. Ahmed, M.F. Manzoor, N. Begum, A. Khan, I. Shah, U. Farooq, R. Siddique, X.-A. Zeng, A. Rahaman, A. Rehman, A. Siddeeg, X.-A. Zeng, A. Manzoor, Impact of pulsed electric field on rheological, structural, and physicochemical properties of almond milk, J. Food Process Eng 42 (8) (2019) e12099.
