



Positive effects of thermosonication in Jamun fruit dairy dessert processing

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ABSTRACT

The effects of thermosonication processing (TS, 90 °C, ultrasound powers of 200, 400, and 600 W) on the quality parameters of Jamun fruit dairy dessert compared to conventional heating processing (high-temperature short time, (HTST), 90 °C/20 s) were evaluated. Microbiological inactivation and stability, rheological parameters, physical properties, volatile and fatty acid profiles, and bioactive compounds were assessed. TS provided more significant microbial inactivation (1 log CFU mL⁻¹) and higher microbial stability during storage (21 days) than HTST, with 3, 2, and 2.8 log CFU mL⁻¹ lower counts for yeasts and molds, aerobic mesophilic bacteria, and lactic acid bacteria, respectively. In addition, TS-treated samples showed higher anti-hypertensive (>39%), antioxidant (>33%), and anti-diabetic (>27%) activities, a higher concentration of phenolic compounds (>22%), preservation of anthocyanins, and better digestibility due to the smaller fat droplet size (observed by confocal laser scanning microscopy). Furthermore, lower TS powers (200 W) improved the fatty acid (higher monounsaturated and polyunsaturated fatty acid contents, 52.78 and 132.24%) and volatile (higher number of terpenes, n = 5) profiles and decreased the atherogenic index. On the other hand, higher TS powers (600 W) maintained the rheological parameters of the control product and contributed more significantly to the functional properties of the products (antioxidant, anti-hypertensive, and anti-diabetic). In conclusion, TS proved to be efficient in treating Jamun fruit dairy dessert, opening space for new studies to define process parameters and expand TS application in other food matrices.

1. Introduction

Dairy desserts can be found worldwide, with many flavors and presentations. According to Brazilian legislation, dairy desserts must contain at least 50% (w/w) of milk or dairy products in their formulation. Therefore, these are excellent options for adding ingredients with functional and sensory appeal [1,2]. *Syzygium cumini* L., known Jamun fruit or Indian blackberry, is a purplish-colored fruit of Indian origin and native to the tropical climate. It is known for several functional

properties, such as anti-diabetes, diuretic, antioxidant, and antioxygenic [3]. Therefore, this fruit can be used in the formulation of dairy desserts.

Emerging food processing technologies have gained increasing prominence in the literature, proportionating products with better sensory and nutritional properties, especially in dairy [4,5] and fruit products [6,7,8]. Thermosonication (TS) is one of the studied emerging technologies, and it consists of treating the products using high-intensity ultrasound (frequencies of 20–500 KHz and intensities >1 W cm⁻²) combined with heating, aiming to preserve bioactive compounds and

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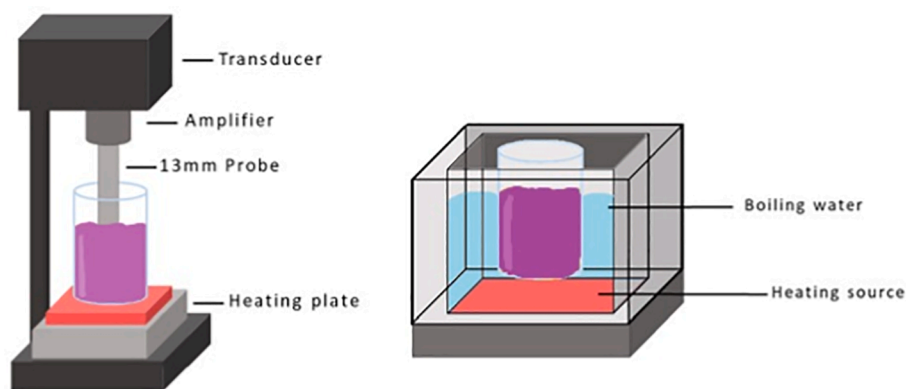


Fig. 1. Scheme of TS (left) and HTST (right) systems.

provide microbiological safety to the food. In addition, TS can also reduce processing time compared to conventional heating treatments [9,10]. TS may disrupt the cells by cavitation and release bioactive or volatile compounds from the matrix. In other cases, TS application may result in lower degradation of bioactive and volatile compounds compared to HTST, such as ascorbic acid, carotenoids, and anthocyanins. Furthermore, TS may generate bioactive peptides [11]. Recent studies have used TS in dairy products, such as cheese [12], yogurt [13], cream cheese [14], and whey drinks [11]. In orange juice whey drink formulation, TS improved the functional properties (antioxidant, anti-hypertensive, and anti-diabetic activities) and maintained important volatile compounds in the products found in the non-treated products [11]. However, additional research is needed to prove the possible effects of thermosonication in other dairy matrices, such as dairy desserts.

The impact of TS on the microbiological, physicochemical, and functional parameters of dairy products depends on the process parameters, mainly on the temperature and power [10,13]. However, the impact of different power of TS on the quality parameters of dairy foods has been scarcely studied [14]. In this way, this study aimed to assess the effect of TS (90 °C and powers of 200, 400, and 600 W) on the functional, physicochemical, and microbiological parameters of Jamun fruit dairy dessert.

2. Materials and methods

2.1. Dairy dessert formulation

All ingredients used were acquired in the local market of Rio de Janeiro, Brazil. The frozen Jamun fruits were thawed, crushed, and sieved. The dairy dessert formulation (% w/w) consisted of pasteurized fluid milk (52.8%), milk cream (10%), Jamun fruit (25%), guar gum (0.1%), xanthan gum (0.1%), powdered milk (5%) and sucrose (7%) [15]. All ingredients were mixed and processed according to section 2.2.

2.2. TS and HTST processing

A scheme of the HTST and TS treatments assembly is presented in Fig. 1. Four treatments were applied: HTST, TS200 (90 °C/200 W), TS400 (90 °C/400 W), and TS600 (90 °C/600 W). The HTST-treated dairy dessert was heated using a bath with boiling water under shaking. The temperature was increased to 90 ± 1 °C (approximately 20 min), held for 20 s, and the product was cooled to 35 °C using an ice bath. Finally, the product was refrigerated and stored (4 ± 2 °C).

The TS-treated dairy desserts were processed using a high-intensity probe-type ultrasound (Unique, Disruptor, Indaiatuba, Brazil) at 20 kHz frequency and with a transducer of 13 mm. The equipment was combined with a heating plate (SL-91, Biolab). For that, 100 mL of the samples were placed in 200-mL glass flasks and subjected to ultrasonic

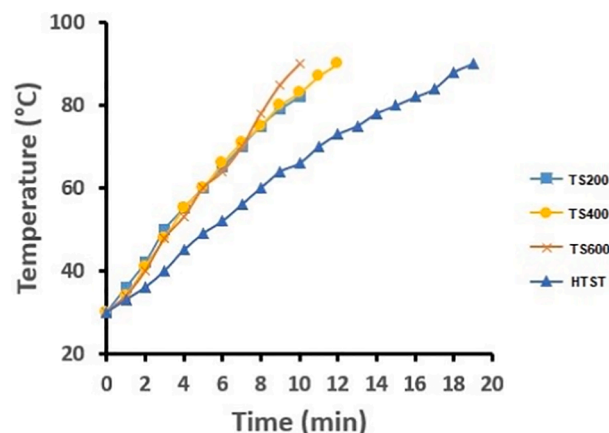


Fig. 2. Temperature profiles for the dairy dessert samples. TS200, TS400, TS600, HTST: TS at 90 °C + ultrasound power of 200, 400 and 600 W and conventional pasteurization (90 °C/20 s).

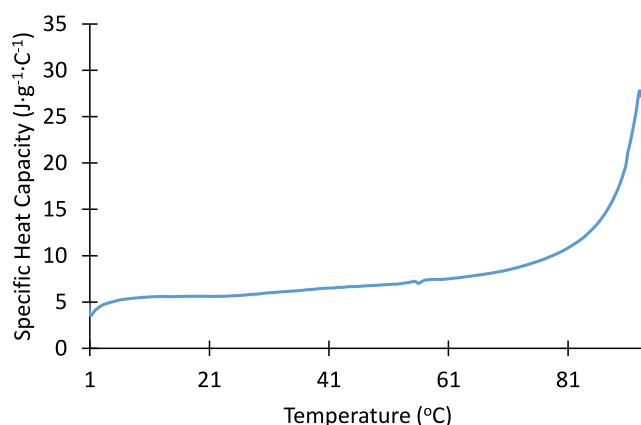


Fig. 3. Measured specific heat capacity of the dairy dessert versus temperature.

treatment (200, 400, and 600 W) for approximately 10–12 min, which was the needed time to reach the temperature of 90 °C. A heating plate at 300 °C was used, and the probe was inserted 10 mm into the samples. The TS was turned off when the samples reached 90 °C. Then, the product was cooled to 35 °C using an ice bath and refrigerated stored (4 ± 2 °C). The temperature profiles are presented in Fig. 2.

The specific heat was determined using a Diamond Differential Scanning Calorimetry (DSC, Perkin Elmer). A scanning rate of 10 °C min^{-1} and a dry nitrogen atmosphere (200 mL min^{-1}) was used. The

experimental procedure consisted of three different runs: the first was performed with an empty aluminum pan (blank test); in the second, a reference sapphire ($\alpha\text{-Al}_2\text{O}_3$) sample was used (10.18 mg); and the third was filled with the experimental sample (11.13 mg), using the same pan (sample test). The blank signal was automatically subtracted from the other runs. The temperature program followed an isotherm at 0 °C for 5 min, heating from 0 to 95 °C using a scanning rate of 10 °C min⁻¹ and another isotherm at 95 °C for 5 min. First, the specific heat curve (Fig. 3) was calculated considering the blank test (baseline) and the sapphire (reference), according to Eq. (1) [16]. Triplicates of the samples were performed, and the specific heat was determined, considering 1–95 °C as the temperature range.

$$C_{p_{\text{sample}}} = C_{p_{\text{reference}}} \times \frac{H_{\text{ax}}M_{\text{r}}}{H_{\text{rx}}M_{\text{a}}} \quad (1)$$

Where H_{a} is the DSC signal of the sample, H_{r} is the DSC signal of the reference, M_{r} is the mass of the reference, and M_{a} is the mass of the sample.

The specific heat of the samples was 8.5 J g⁻¹ °C⁻¹. Then the acoustic power of the process (Eq. (2)), the acoustic intensity (Eq. (3)), the power density (Eq. (4)), and the specific energy (Eq. (5)) were calculated, as suggested by Strieder et al. [17].

$$\text{acousticpower}(W) = mC_p \left(\frac{dT}{dt} \right) \quad (2)$$

Where C_p is the specific heat (J g⁻¹ °C) measured at constant pressure, m is the mass (g) of the sample, and dT/dt is the temperature rise rate in function of process time (°C s⁻¹).

$$\text{acousticintensity} \left(\frac{W}{\text{cm}^2} \right) = \frac{4 \times \text{acousticpower}}{\pi D^2} \quad (3)$$

Where D is the probe diameter (centimeters).

$$\text{powerdensity} \left(\frac{W}{\text{mL}} \right) = \frac{\text{acousticpower}}{\text{samplevolume}} \quad (4)$$

$$\text{specificenergy} \left(\frac{J}{g} \right) = \frac{\text{acousticpower} \times \text{processingtime}}{\text{samplemass}} \quad (5)$$

The temperature rise rate for TS200, TS400, and TS600 was measured without the heating plate used in the TS method. The observed rates were 0.017, 0.046, and 0.076 °C s⁻¹ for TS200, TS400, and TS600. According to the calculations, the ultrasonic parameters of the treatments TS200, TS400, and TS600 applied to the dairy dessert samples were acoustic power of 14.2, 39.4, and 64.76 W; the acoustic intensity of 30.4, 84.7, and 139.4 W cm⁻²; power density of 0.14, 0.39 and 0.64 W cm⁻³; and specific energy of 102, 212.5 and 272 J g⁻¹, respectively.

The energy density (ED) was determined using Equation (6). The ED values were 1.4, 2.6, and 3.6 kJ cm⁻³ for TS200, TS400, and TS600, respectively.

$$ED [J/\text{cm}^3] = \frac{\text{ultrasonicpower}(j/s) \times \text{processtime}(s)}{\text{samplevolume}(\text{cm}^3)} \quad (6)$$

2.3. Microbiological analysis

The dairy dessert formulations were serially diluted in buffered peptone water (0.1 g 100 mL⁻¹), and the lactic acid bacteria (LAB), total aerobic mesophilic bacteria (AMB), and molds and yeasts counts were enumerated. LAB were determined using de Man, Rogosa, and Sharpe agar (MRS, Himedia®, India) added with cycloheximide (100 mg L⁻¹) and anaerobic incubation at 37 °C for 48 h (Anaerobac®, Probac, Brazil). Molds and yeasts were determined using potato dextrose agar (PDA, Himedia®, India) and aerobic incubation at 27 °C for 5 days. AMB were determined using plate count agar (PCA, Himedia®, India) and

aerobic incubation at 37 °C for 48 h [18]. LAB, molds and yeasts, and AMB were enumerated in control (untreated sample), after processing (for microbial inactivation evaluation), and along the storage period (days 7, 14, and 21, for microbial viability evaluation). The log reductions (γ) were calculated considering the counts on control (N0) and using Eq. (7) [19]:

$$\gamma = \log_{10}(N0) - \log_{10}(Nf) \quad (7)$$

where N0 is the counts in the control sample and Nf is the counts after processing.

2.4. Rheology

The samples were analyzed using a controlled stress rheometer (MCR 501 model, Anton Paar Instruments, Canada) with a plate-plate geometry and stainless steel (50 mm diameter), a 0.103 mm gap, and 10 °C. The flow curve assays were performed using shear rates in 0.1 to 200 s⁻¹ [20,21]. The data were fitted to the Herschel-Bulkley model (Eq. (8)).

$$\sigma = \sigma_0 + k\gamma^n$$

where σ is the shear stress (Pa), k is the consistency index (Pa.sⁿ), n is the flow behavior index, and γ is the shear rate (s⁻¹).

2.5. Time-domain nuclear magnetic resonance (TD-NMR)

The samples were analyzed using a low-resolution NMR spectrometer (MARAN Ultra, Oxford Instruments®) at 0.54 T (proton Larmor frequency, 23 MHz) using a probe (18 mm diameter, 3.6 cm height) and at 30 ± 1 °C. The Carr-Purcell-Meiboom-Gill (CPMG) and Small-Angle Flip-Flop (SAFF) sequences were applied [22].

2.6. Bioactive compounds

The antioxidant capacity determination followed the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. First, extracts of the samples were obtained using the ethanolic solution, centrifugation, and vacuum filtration. Then, 150 µL of the extracts were mixed with 2.85 mL of DPPH solution (0.006 mol L⁻¹), homogenized in the dark for 60 min, and the absorbance was read at 517 nm [23].

The inhibition of angiotensin-converting enzyme I (ACE) was evaluated by mixing the samples with 20 µL of the ACE enzyme (0.1-unit mL⁻¹) and incubation for 30 min at 37 °C. Then, 250 µL of 1 mol L⁻¹ of hydrochloric acid was added, and the vials dried, resuspended in deionized water, and the absorbance was read at 228 nm [24].

The inhibition of α -amylase and α -glucosidase was determined following Lavelli et al. [25]. The ascorbic acid (AA) content was determined by titration using 2,6-dichlorophenol-indophenol [26]. Finally, the total phenolic compounds (TPC) content was determined following Cappato et al. [4].

Monomeric anthocyanins were measured using the differential pH method, according to Giusti & Wrolstad [27]. The sample was weighed (1 g) and added with acidified ethanol (10 mL, 0.01% HCl), and the content was calculated using Equation (9).

$$\text{Anthocyanins (mg/L)} = \frac{A \times FD \times MM \times 1000}{\epsilon \times b} \quad (9)$$

where A corresponds to absorbance, MM is the molar mass of the standard in g mol⁻¹ (449.2 g mol⁻¹); ϵ is the predominant anthocyanin molar extraction coefficient (26.9 L mol⁻¹ cm⁻¹ of cyanidin-3-glucoside), FD is the sample dilution factor, and b is the path length of the cuvette (cm).

2.7. Fatty acid profile

The fatty acid profile was determined using a gas chromatography-

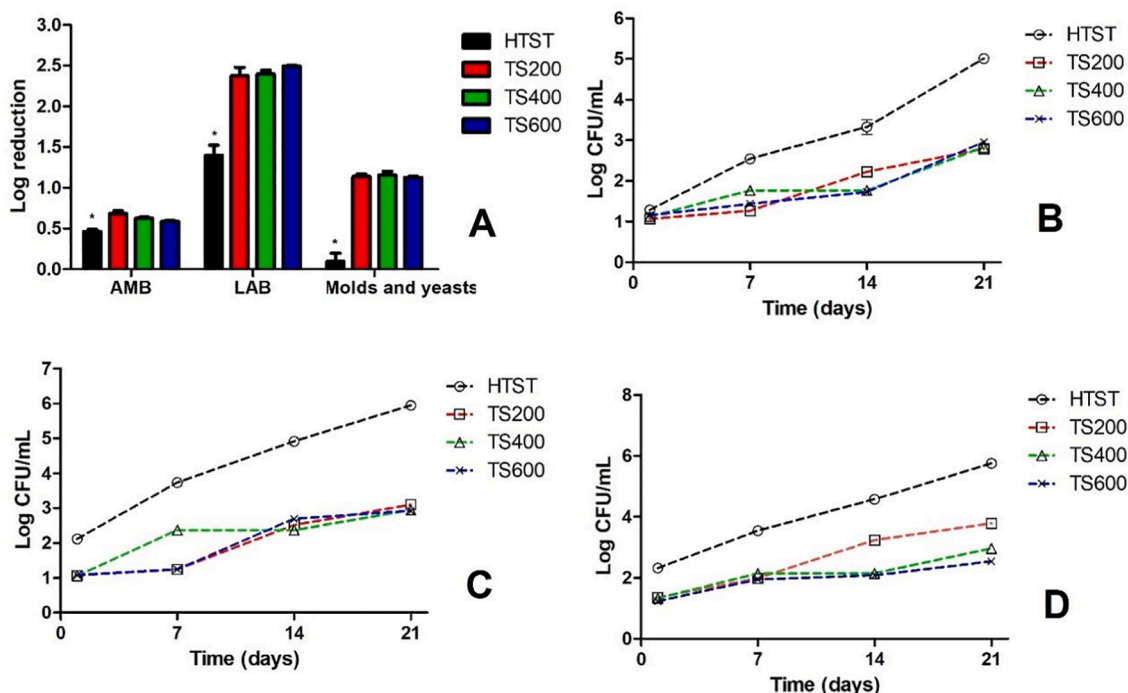


Fig. 4. (A) Microbial inactivation (γ) in TS-treated samples compared to HTST. Marks (*) above the bars denote significant differences between samples. (B) AMB (Aerobic Mesophilic Bacteria) counts during storage in dairy dessert samples. (C) Molds and yeasts count during storage in dairy dessert samples. (D) Lactic Acid Bacteria (LAB) counts during storage in dairy dessert samples. HTST: High-temperature short-time treatment (90 °C/20 s). TS200: Therosonication at 90 °C/200 W. TS400: Therosonication at 90 °C/400 W. TS600: Therosonication at 90 °C/600 W.

mass spectrometer (GC–MS, Agilent Technologies, 7890A-5975C, Santa Clara, California, USA) coupled with a CTC PAL sampler (SPME 120, Agilent Technologies). The column was a DB-FFAP CG (15 m \times 0.10 mm, 0.10 μ m), and the temperature was set at 70 °C for 1 min. Then, ramps of 45 °C min⁻¹ to 115 °C, 40 °C min⁻¹ to 175 °C, and 30 °C min⁻¹ to 240 °C were performed. Finally, the temperature was kept constant at 240 °C for 4 min. The MS detector covered a mass range from 40 and 400 m z⁻¹. Identifying the fatty acids consisted of comparing the retention times of the sample chromatographic peaks with those of reference standards (Sigma FAME 37 18919-1AMP). Furthermore, the mass spectra were compared with the NIST 11 spectra library. The quantification of the fatty acids was assessed following ISO 5508: 1990 and using the Agilent Mass-Hunter Quantitative Analysis software. The health indices (thrombogenic index (TI), atherogenic index (AI), hypercholesterolemic saturated fatty acids (HSFA), and desirable fatty acids (DFA) indices were also calculated [28,29].

2.8. Volatile compounds

The volatile compounds were extracted using solid-phase micro-extraction following Condruso et al. [30] and Cappato et al. [4]. The identification of the compounds was performed using gas chromatography (Agilent Technologies® 7890A GC) coupled with a mass spectrometer (Agilent Technologies® 5975C). Divinylbenzene/carboxy/polydimethylsiloxane (DVB/CAR/PDMS) fibers (50/30 μ m thick Supelco®) and vials (20 mL headspace) were used for extraction in an automated CTC PAL Sampler (Agilent Technologies SPME 120). The equation proposed by Van Den Dool & Dec. Kratz [31] was used to calculate the linear retention index (LRI), and they were compared to those of C8–C40 alkane standards (Supelco, 40127-U). The Agilent Mass Hunter Qualitative Analysis software (Agilent Technologies) was used to identify the compounds. Deconvolution mode and a signal-to-noise ratio above 10, right $\Delta m/z = 0.7$ AMU, and left $\Delta m/z = 0.3$ AMU were used. The National Institute of Standards and Technology Library (NIST/EPA/NIH Mass Spectra Library, version 11, USA) was used to obtain the hints.

2.9. Confocal laser scanning microscopy

The samples were stained with Nile red (NR, Sigma, Brazil, 200 mg mL⁻¹, 15 min, room temperature) and analyzed in an epifluorescence microscope (Leica DMI 6000B, Wetzlar, Germany) using an HCX PLFLUO TAR 100 \times 1.30 objective. A sample drop was included in a glass slide and covered with a coverslip. The samples were excited using a Leica EL6000 mercury short-arc reflector lamp, a Leica I3 fluorescence filter cube (with a BP 450–490 nm excitation filter, a 510 nm dichromatic mirror), and an LP 515 nm suppression filter [32]. The ImageJ software was used to estimate the diameter of Nile red-stained lipid droplets (LDs) from images (n = 300 LDs per sample) [33].

2.10. Statistical analysis

The experiment was repeated three times and followed a complete randomized design. The microbiological analyses were performed along with the storage (days 1, 7, 14, and 21) in triplicates. The other analyzes were performed in triplicates on day 1 after the treatments. The data were submitted to the Analysis of variance (ANOVA) and Tukey test (p < 0.05) or Fisher test (p < 0.05, DSC data) using the XLSTAT (Adinsoft, Paris, France, version 2020) or Statistica (7.0, Statsoft Inc.) software.

3. Results and discussion

3.1. Microbial inactivation and viability

The microbial inactivation is shown in Fig. 4. In all the microbial groups evaluated, there was a lower log reduction (γ) in the sample treated with HTST than in the TS treatments (Fig. 4A). However, there was no significant difference between the different applied ultrasound powers (200, 400, or 600 W, p > 0.05). In this way, considering LAB and molds and yeasts, higher microbial reductions (1 log CFU mL⁻¹) were observed in TS treatments than in HTST. LAB was the microbial group that was most influenced by the TS treatment, with γ values from 2.37 to

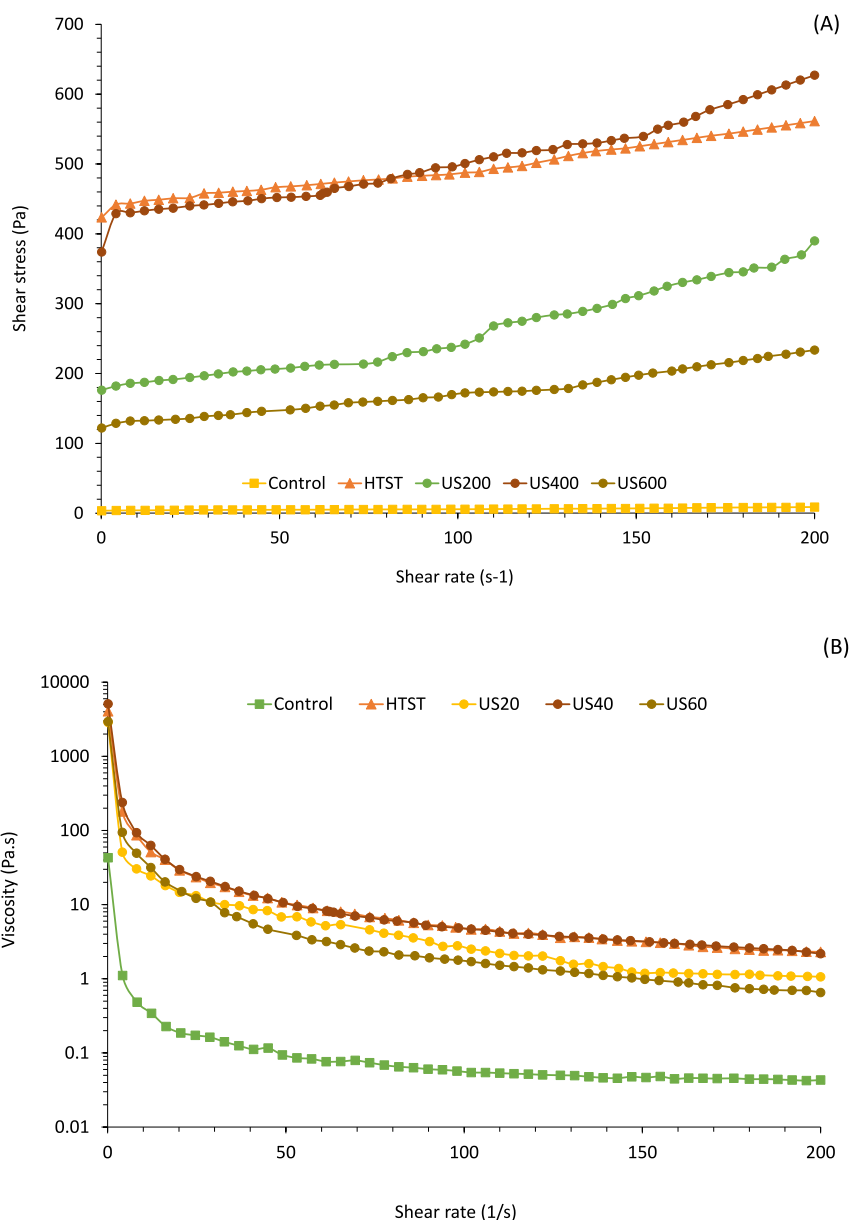


Fig. 5. (A) Steady-state flow curves of the dairy dessert samples. (B) Viscosity obtained from the shear stress sweep tests of the dairy dessert samples. Control, TS200, TS400, TS600, HTST = untreated, thermosonication at 90 °C + ultrasound power of 200, 400 and 600 W and conventional pasteurization (90 °C/20 s).

2.49. At the same time, AMB presented γ values from 0.59 to 0.68, and the reduction in molds and yeasts was in the order of 1.12 to 1.14.

TS treatments maintained lower microbial counts at the end of the

Table 1

Rheological parameters of the dairy dessert samples.

Sample	σ_0 (Pa)	K (Pa.s ⁿ)	N	R^2
Control	3.59 ± 0.07	0.023 ± 0.01 ^d	0.99 ± 0.002 ^a	0.97
HTST	433.82 ± 1.34 ^a	0.606 ± 0.01 ^b	0.99 ± 0.002 ^a	0.98
TS200	159.36 ± 3.41 ^c	1.00 ± 0.02 ^a	0.99 ± 0.003 ^a	0.96
TS400	405.45 ± 2.86 ^b	0.96 ± 0.02 ^a	0.99 ± 0.003 ^a	0.96
TS600	121.28 ± 1.30 ^d	0.51 ± 0.01 ^c	0.99 ± 0.002 ^a	0.99

Different letters in the same column denote significant differences between samples ($p < 0.05$) for the same rheological parameter. HTST: High-temperature short-time treatment (90 °C/20 s). TS200: Thermosonication at 90 °C/200 W. TS400: Thermosonication at 90 °C/400 W. TS600: Thermosonication at 90 °C/600 W. σ_0 : shear stress. n : behavior index. k : consistency index.

storage period, regardless of the microbial group (Fig. 4B–D). For example, at the end of the storage period, the AMB count in the HTST-treated sample was 5.00 log CFU mL⁻¹, while in the TS treatments, the counts ranged between 2.79 and 2.95 log CFU mL⁻¹ (Fig. 4B). Additionally, the mold and yeasts count was 5.95 log CFU mL⁻¹ in the HTST-treated sample, about 3 log CFU mL⁻¹ higher than the counts observed in the TS-treated samples, which ranged from 2.92 (TS600) to 3.10 (TS200) log CFU mL⁻¹ (Fig. 4C). For LAB, HTST treatment generated a final count of 5.76 log CFU mL⁻¹, while samples TS200, TS400, and TS600 exhibited 3.78, 2.96, and 2.55 log CFU mL⁻¹, respectively (Fig. 4D).

The inactivation mechanism of TS may justify the better results observed compared to HTST. The joint application of temperature with ultrasound accentuates the cavitation effect, generating a more significant impact on the collapse of the generated microbubbles and consequently affecting the membranes and cell walls of the microorganisms [11].

Thermosonication has been applied in various food matrices to verify

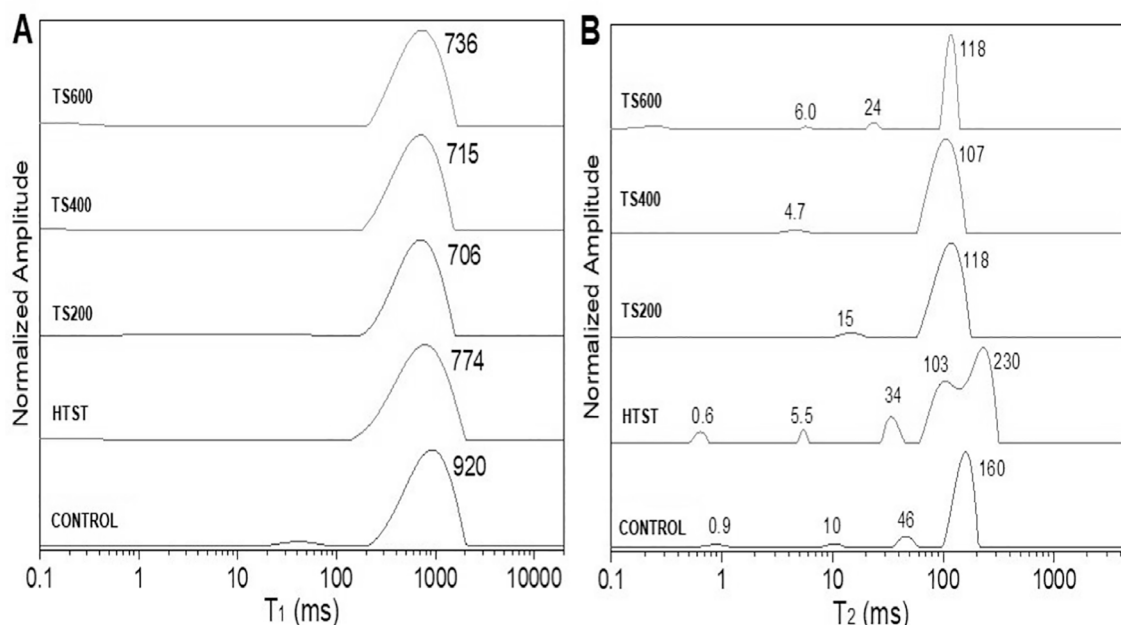


Fig. 6. TD-NMR curves of dairy dessert samples. (A) Distributions of longitudinal relaxation domains. (B) Distributions of transversal relaxation domains. Control, TS200, TS400, TS600, HTST = untreated, thermosonication at 90 °C + ultrasound power of 200, 400 and 600 W and HTST (90 °C/20 s).

the behavior of different microorganisms and bacterial groups, define the best processing parameters, and demonstrate the impacts of the technology on microbial inactivation and nutritional quality [11,34,35,36]. In the present study, adequate efficiency of TS in the microbiology of dairy desserts was observed, similar to previous research addressing the technology in whey beverages [11].

3.2. Rheology

Fig. 5 shows the flow curves and viscosity data. The σ - γ data were adjusted by non-linear regression to the Herschel-Bulkley model ($0.96 < R^2 < 0.99$), and the estimated rheological parameters shear stress (σ_0), consistency index (k), and flow behavior index (n) are shown in Table 1. The application of HTST increased the shear stress values compared to control ($p < 0.05$, Table 1). This is because the thermal treatment

significantly impacts the protein network structure, increasing shear stress. At the same time, the application of TS increased the shear stress, with higher values observed when the system operated at 400 W ($p < 0.05$). This phenomenon might be related to the transient and steady cavitation mechanisms, producing different results according to the sonication intensity. In this way, TS at 200 W and 600 W kept the shear stress more similar to the values found in the control sample.

All treatments (HTST and TS) resulted in increases in the consistency index compared to control ($p < 0.05$, Table 1). However, the increase was more prominent for TS-treated samples at 200 W and 400 W, followed by HTST and 600 W ($p < 0.05$). In this way, the increase in the TS power resulted in decreases in the consistency index of the products ($p < 0.05$). This behavior suggests the ultrasound treatment has an enhancement effect on consistency at low energy. Still, the result was the opposite at high energy values, causing a decrease in the intermolecular

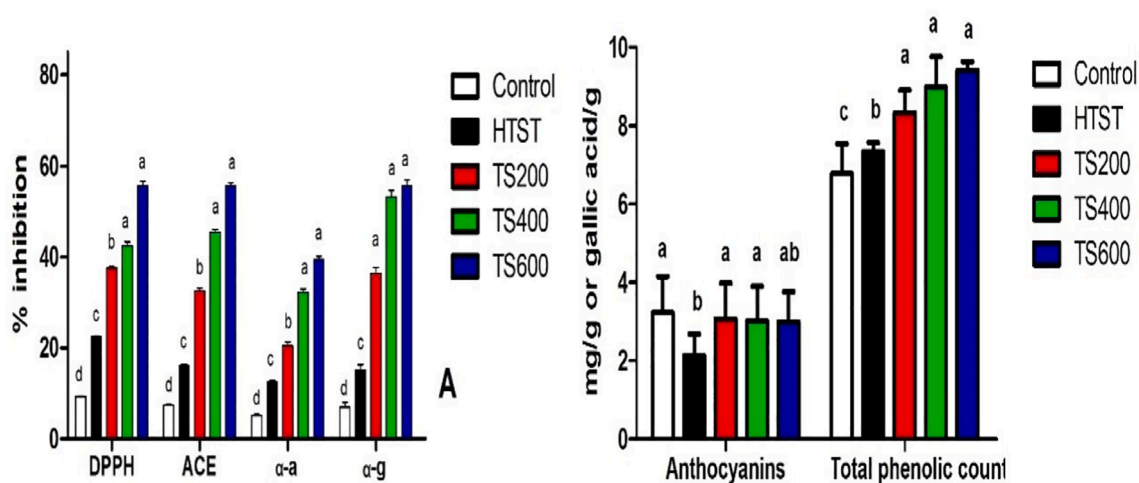


Fig. 7. Functional activities of dairy dessert samples. Different letters denote significant differences between samples ($p < 0.05$). HTST: High-temperature short-time treatment (90 °C/20 s). TS200: Thermosonication at 90 °C/200 W. TS400: Thermosonication at 90 °C/400 W. TS600: Thermosonication at 90 °C/600 W. (A) DPPH: 2,2-diphenyl-1-picrylhydrazyl. ACE: Angiotensin-converting enzyme. α -a: α -amylase. α -g: α -glucosidase. ACE, DPPH, α -g, and α -a are expressed in % inhibition. (B) Anthocyanins are denoted in mg g^{-1} and the total phenolic compound is represented in gallic acid g^{-1} .

forces of attraction between protein molecules and, consequently, promoting more mobility and lower consistency. In this way, TS at 600 W kept the consistency index more similar to the values found in the control sample.

The flow curves seem to behave as a Bingham plastic; however, it is possible to notice a slight shear-thinning behavior at a logarithmic scale. According to the non-linear regression, all samples presented a flow behavior slightly lower than 1 (approximately 0.99), indicating a tenuous shear-thinning behavior, where a viscosity decrease is observed with increasing shear rate (Fig. 5B). No significant differences were observed between the evaluated samples.

3.3. Time-domain nuclear magnetic resonance (TD-NMR)

Nuclear magnetic relaxation analyses using TD-NMR were performed from the determination of longitudinal and transversal relaxation times. The longitudinal relaxation time for liquid systems is primarily sensitive to the diffusion processes of water molecules, presenting higher time values, close to 1 s. The transversal relaxation time, in turn, can identify physical and chemical interactions between water molecules, proteins, carbohydrates, and fats. The distribution of the transversal relaxation domains (Fig. 6) observed that the TS-treated samples presented a more similar profile to the control sample. On the other hand, the HTST sample had a more heterogeneous behavior, with five distinct relaxation domains.

The distribution of the longitudinal relaxation domains shows the relaxation of the aqueous phase of the samples being inversely proportional to the viscosity of the systems [37]. Therefore, the TS samples have slightly lower viscosities than the HTST sample.

3.4. Bioactive compounds

The bioactive compounds of dairy dessert formulations are presented in Fig. 7.

The TS-treated samples showed higher values for DPPH, ACE, α -glucosidase, and α -amylase inhibitory activities (Fig. 7A) and TPC content compared with the HTST and with even more satisfactory results when compared with the control sample ($p < 0.05$). Among the TS samples, the use of the highest ultrasound powers (400 and 600 W) generated significantly better results in DPPH, ACE, α -glucosidase, and α -amylase inhibitory activities compared with the lowest power (200 W) ($p < 0.05$), but without differences in the TPC content ($p > 0.05$, Fig. 7B). The higher antioxidant activity in the samples treated by TS can be explained by the more significant release of compounds with antioxidant activity after thermosonication application, such as peptides, associated with the phenomenon of cavitation [38]. The more significant ACE inhibitory activity is related to bioactive peptide fragments resulting from protein hydrolysis, which seems to occur more intensely in thermosonication and other emerging technologies [4]. Furthermore, the α -glucosidase and α -amylase inhibitory activities are associated with kinetic processes related to dairy dessert proteins. Bioactive peptides can demonstrate a certain level of binding affinity with these enzymes, generating greater maintenance of blood glucose levels [1]. It is important to highlight that TS resulted in lower processing times (10–12 min) compared to HTST (20 min) (Fig. 2), which may have contributed to the higher functional properties of TS-treated products.

Considering the monomeric anthocyanin concentration, the application of HTST decreased its content ($p < 0.05$), while TS showed no effect ($p > 0.05$) compared to control. Anthocyanins are water-soluble flavonoids contained in fruits and vegetables. Their consumption may be linked with the prevention of cardiovascular and neurodegenerative diseases and influences the regulation of the intestinal microbiota [39]. Thus, it is interesting to study the different types of treatment in foods to preserve these compounds as much as possible in fruit-based products, such as Jamun fruit dairy dessert, since conventional heat treatment degrades a considerable portion of anthocyanins. In this way, TS

Table 2
Fatty acid profile of dairy desserts treated by thermosonication.

Fatty acid (mg L ⁻¹)	Control	HTST	TS200	TS400	TS600
Butyric Acid (C4:0)	76.25 ± 11.81 ^a	77.12 ± 30.62 ^a	53.08 ± 3.38 ^a	51.21 ± 6.02 ^a	58.72 ± 0.72 ^a
Caproic Acid (C6:0)	78.32 ± 2.31 ^b	85.76 ± 3.48 ^a	88.72 ± 0.71 ^a	83.46 ± 6.74 ^a	75.97 ± 2.70 ^b
Caprylic Acid (C8:0)	57.86 ± 1.01 ^c	64.72 ± 6.75 ^b	73.48 ± 5.64 ^a	65.84 ± 5.17 ^b	56.72 ± 1.42 ^c
Capric Acid (C10:0)	96.49 ± 5.02 ^b	111.95 ± 2.55 ^a	125.56 ± 16.70 ^a	102.94 ± 15.28 ^a	92.56 ± 1.70 ^{ab}
Lauric Acid (C12:0)	113.45 ± 1.00 ^b	133.82 ± 0.39 ^{ab}	151.5 ± 25.41 ^a	118.58 ± 21.16 ^b	110.58 ± 1.10 ^b
Tridecanoic Acid (C13:0)	13.16 ± 1.00	–	–	–	–
Myristic Acid (C14:0)	382.8 ± 2.49 ^c	500.78 ± 28.01 ^a	590.44 ± 98.78 ^a	453.37 ± 95.06 ^a	423 ± 7.28 ^b
Myristoleic Acid (C14:1)	47.46 ± 1.40 ^b	57.17 ± 1.13 ^a	68.55 ± 17.23 ^a	50.09 ± 8.87 ^a	47.1 ± 1.15 ^b
Pentadecanoic Acid (C15:0)	57.5 ± 1.46 ^b	67.62 ± 0.77 ^a	82.54 ± 21.88 ^a	59.87 ± 10.18 ^{ab}	55.53 ± 0.45 ^b
Palmitic Acid (C16:0)	1143.2 ± 189.67 ^b	1441.38 ± 88.27 ^a	1762.87 ± 366.38 ^a	1340.6 ± 230.80 ^{ab}	1326.54 ± 7.71 ^b
Palmitoleic Acid (C16:1)	95.63 ± 2.29 ^c	111.83 ± 1.59 ^{ab}	141.4 ± 43.42 ^a	100.9 ± 13.87 ^b	93.62 ± 1.08 ^c
Heptadecanoic Acid (C17:0)	280.00 ± 0.01 ^a	280.01 ± 0.01 ^a	280.01 ± 0.01 ^a	280.00 ± 0.01 ^a	280.00 ± 0.01 ^a
Stearic Acid (C18:0)	515.54 ± 31.42 ^c	593.39 ± 10.60 ^{ab}	809.91 ± 295.60 ^a	563.28 ± 53.19 ^b	564.14 ± 7.11 ^b
Elaidic Acid (C18:1n9t)	104.08 ± 1.15 ^b	141.22 ± 1.10 ^b	–	255.14 ± 1.22 ^a	124.39 ± 0.71 ^b
Oleic Acid (C18:1n9c)	1088.58 ± 156.35 ^b	1305.94 ± 75.52 ^{ab}	1704.27 ± 487.80 ^a	1238.89 ± 170.34 ^b	1233.83 ± 2.10 ^b
Linoleic Acid (C18:2n6c)	120.51 ± 3.54 ^b	138.12 ± 2.45 ^{ab}	159.41 ± 32.56 ^a	136.97 ± 0.83 ^{ab}	117.82 ± 7.12 ^b
Linolenic Acid (C18:3n3)	28.26 ± 5.95 ^{ab}	28.92 ± 1.96 ^{ab}	33.42 ± 4.40 ^a	31.3 ± 1.41 ^a	24.54 ± 1.59 ^b
Arachidic Acid (C20:0)	11.89 ± 0.92 ^b	14.74 ± 0.52 ^a	14.86 ± 0.70 ^a	15.03 ± 0.93 ^a	12.45 ± 0.71 ^b
cis-11-Eicosenoic Acid (C20:1)	10.38 ± 0.73 ^b	–	23.02 ± 0.82 ^a	16.2 ± 8.50 ^b	–
cis-11,14-Eicosadienoic Acid (C20:2)	5.54 ± 0.07 ^c	–	13.39 ± 0.71 ^a	11.34 ± 1.15 ^b	–
cis-8,11,14-Eicosatrienoic Acid (C20:3n6)	–	–	11.11 ± 0.82	–	–
Arachidonic Acid (C20:4n6)	9.85 ± 0.71	–	–	–	–
Behenic Acid (C22:0)	10.6 ± 0.35 ^a	11.03 ± 0.71 ^a	11.47 ± 0.21 ^a	7.03 ± 0.71 ^b	–
Tricosanoic Acid (C23:0)	7.10 ± 0.01 ^b	–	13.55 ± 0.01 ^a	6.23 ± 0.01 ^b	–
Lignoceric Acid (C24:0)	7.39 ± 0.85	–	–	–	–
∑ MUFA	1639.45 ± 12.36	2075.98 ± 96.40	2504.78 ± 65.40	1912.55 ± 87.50	1859.58 ± 100.56
∑ PUFA	164.26 ± 11.40	167.77 ± 9.66	217.23 ± 10.10	179.5 ± 11.7	142.94 ± 13.90

* Results are expressed as mean ± standard deviation. Different letters on the same line indicate a difference. HTST: High-temperature short-time treatment (90 °C/20 s). TS200: Thermosonication at 90 °C/200 W. TS400: Thermosonication at 90 °C/400 W. TS600: Thermosonication at 90 °C/600 W. MUFA: monounsaturated fatty acids. PUFA: polyunsaturated fatty acids.

treatment exhibits significant advantages in preserving and generating bioactive compounds, resulting in greater antioxidant, antihypertensive, and anti-diabetes activities [11,40,41].

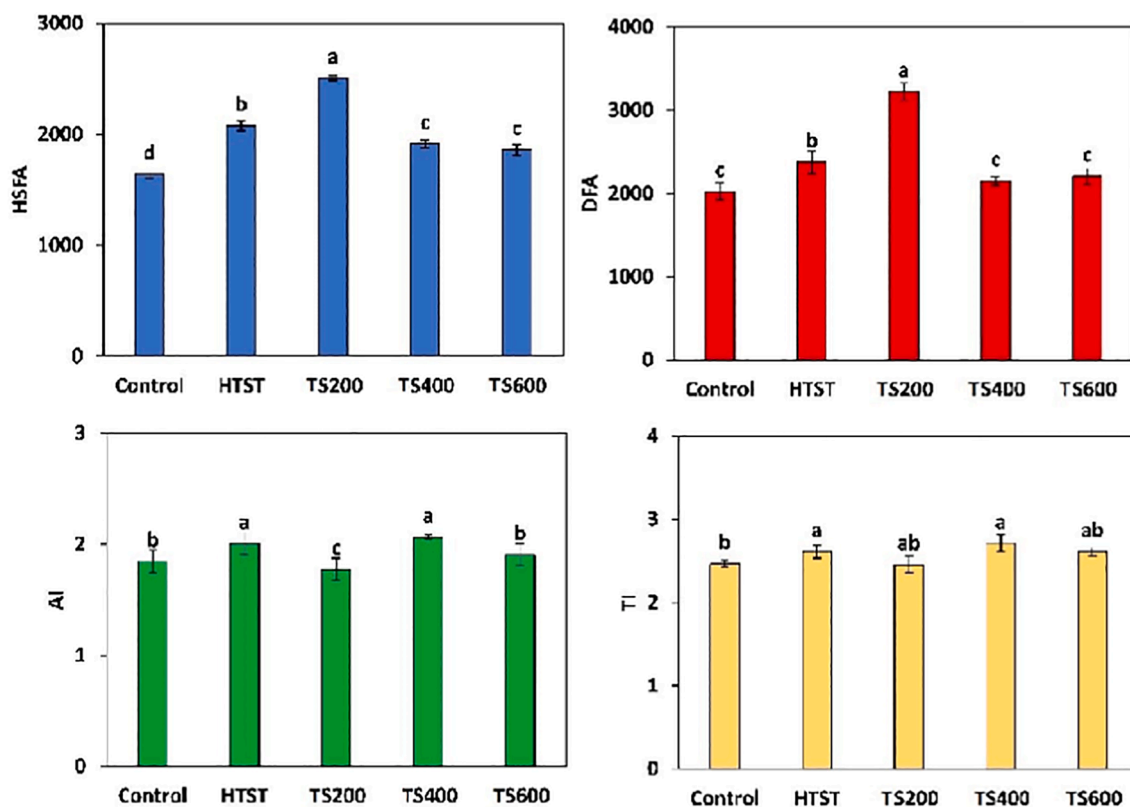


Fig. 8. Nutritional lipid indexes based on fatty acids. HSFA: Hypercholesterolemic saturated fatty acids. DFA: Desired fatty acids. TI: Thrombogenic Index. AI: Atherogenic index. HTST: High-temperature short-time treatment (90 °C/20 s). TS200: Thermosonication at 90 °C/200 W. TS400: Thermosonication at 90 °C/400 W. TS600: Thermosonication at 90 °C/600 W.

3.5. Fatty acid profile

Dairy fat has as main lipid the triacylglycerides, representing >98% of total fat, while the remaining 2% comprise diacylglycerides, monoacylglycerides, free fatty acids, phospholipids, sterols, and hydrocarbons [42]. The fatty acid profile of ultrasound-treated dairy desserts is shown in Table 2. Considering the quantitative point of view, the most important fatty acid in milk is palmitic acid (16:0), representing about 30% of the total weight of the fatty acids [43], confirming the results observed in this study.

HTST and TS processing changed the fatty acid profile of the products compared to control ($p < 0.05$). HTST sample showed a higher concentration of saturated fatty acids than the control, such as capric, myristic, pentadecanoic, palmitic, and stearic acids ($p < 0.05$). At the same time, decreases in some MUFA and PUFA occurred ($p < 0.05$). These results may be associated with the thermal effects, which may have provided changes in the molecular structure, breaking double bonds and increasing saturated fatty acid content [44]. The TS power had a significant impact on the fatty acid profile, with TS600 resulting in minor changes compared to TS200 ($p < 0.05$). In the TS200, it is noted that the concentration of fatty acids tends to increase, especially the concentration of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), compared to the control sample. This increase was associated with increases in the oleic and linoleic fatty acids ($p < 0.05$). The samples subjected to more energetic treatments (TS400 and TS600) had lower fatty acid contents than TS200 but higher concentrations than the control treatment ($p < 0.05$). It was also observed that increasing the dissipated ultrasound energy reduced the concentration of unsaturated fatty acids (MUFA and PUFA). This behavior may be due to the heat of processing and the aggregation of air bubbles with oxygen, accelerating the oxidation process of unsaturated fatty acids [10].

Compared to other technologies such as pasteurization and ultraviolet light [45], ultrasound was the technique that improved the availability of fatty acids in milk. Monteiro et al. [46] and Guimarães et al. [47] observed that ultrasound modified the fatty acid profile of dairy beverages. The fatty acid content increased when açai and buriti pulps were treated with ultrasound [48]. The ultrasound process promotes the breakdown of milk fat globules, improving the concentration and bioavailability of fatty acids.

Nutritional indices based on fatty acids are important to understand different processes and their effect on foods rich in oils and fats [47]. The HSFA, DFA, AI, and TI indices are shown in Fig. 8.

HTST treatment originated products with higher TI, AI, HSFA, and DFA ($p < 0.05$) than the control. In this way, HTST contributed negatively to the fatty acid profile, as lower TI, AI, and HSFA values are desired. These results may be associated with increased saturated fatty acids in this formulation, mainly myristic, palmitic, and stearic ($p < 0.05$).

The effect of TS was dependent on the used power. TS200 improved fatty acid profile compared to control and HTST, with lower AI and higher DFA ($p < 0.05$). This result may be associated with the increased MUFA and PUFA levels in this formulation. On the other hand, TS400, similar to HTST, showed higher TI, AI, and HSFA ($p < 0.05$), associated with their similar fatty acid profile. Finally, no significant differences in the health indices were observed for TS600, except for an increase in HSFA ($p < 0.05$). Cell disruption caused by the high energy of ultrasonic waves favors the release of intracellular substances that are more available for absorption by the human body [10]. Although the increase in HSFA may be associated with the concentration of saturated fatty acids, the rise in DFA may be important for improving the nutritional quality of sonicated dairy desserts. Even though saturated fatty acid consumption may be associated with increased cholesterol levels [49], regular consumption of HSFA may not be related to hypertension [50].

Table 3
Volatile compounds of orange whey drink submitted to thermosonication and HTST treatment.

Group	Compound	LRI	CONTROL	HTST	TS200	TS400	TS600
Carboxylic acid	Acetic acid	1421	X	X	X	X	X
	Hexanoic acid	1821	X	X	X	X	X
	Octanoic acid	2038	X	X	X	X	X
	n-Decanoic acid	2249	X	X	X	X	X
Alcohol	1-Pentanol	1193	X	–	–	–	X
	1-Butanol, 3-methyl-	1199	X	X	X	X	–
	1-Pentanol, 4-methyl-	1326	–	X	X	X	–
	3-Oxetanol, 2,2,3-trimethyl-	1358	–	X	X	X	–
	Phenylethyl Alcohol	1906	X	X	X	X	X
	3-Furaldehyde	1438	X	–	X	X	X
Aldehyde	3-Furaldehyde	1438	X	–	X	X	X
	Dimethyl ether	1070	X	X	X	X	X
Ether	Butane, 1-(2-propenyloxy)-	1034	–	X	–	–	–
	Oxalic acid, pentyl 2-phenylethyl ester	1845	X	X	X	X	X
	Benzoic acid 2-bromoethyl ester	2395	X	–	–	–	–
Ketone	2-Propanone, 1-methoxy-	1277	–	X	X	X	–
	2-Nonanone	1363	X	X	X	X	X
Terpene	β -Phellandrene	1097	–	X	X	X	X
	β -Pinene	1121	–	–	–	X	–
	D-Limonene	1155	–	X	X	X	–
	α -Cubebene	1457	–	X	X	X	X
	Copaene	1473	X	–	–	–	–
	Caryophyllene	1565	X	X	X	X	X
	β -Ocimene	1209	–	–	X	X	–
	Humulene	1638	–	X	–	–	–
	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	1082	X	X	X	X	X
	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	1096	–	–	–	–	X
	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	1096	X	–	–	–	–
	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	1126	X	–	–	–	–
	Cyclohexene, 4-methylene-1-(1-methylethyl)-	1128	–	–	–	–	X
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	1189	–	–	–	–	X	
1,3-Cyclohexadiene, 1,3,5,5-tetramethyl-	1338	–	X	–	–	–	
Cyclohexene, 3-methyl-6-(1-methylethylidene)-	1343	–	–	X	X	–	
Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	1577	–	X	X	X	–	
1,4,7,7-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	1644	X	–	–	–	X	
Amine	Ethylamine, N,N-dinonyl-2-(2-thiophenyl)-	1076	X	X	X	–	–
	Spiro[2.4]heptane, 1,5-dimethyl-6-methylene-	1185	–	X	–	–	–
Other							
	Total of volatile organic compounds		19	23	22	23	17

*LRI – Linear Retention Index. HTST: High-temperature short-time treatment (90 °C/20 s). TS200: Thermosonication at 90 °C/200 W. TS400: Thermosonication at 90 °C/400 W. TS600: Thermosonication at 90 °C/600 W. X- presence, – absence.

The atherogenic index (AI) considers the ratio between non-HDL and HDL cholesterol [51]. The thrombogenic index (TI) characterizes the thrombogenic potential of fatty acids, indicating potential clot formation in blood vessels, and provides the contribution of different fatty acids, which denotes the ratio between pro-thrombogenic fatty acids (C12:0, C14:0, and C16:0) and anti-thrombogenic (MUFA and n-3 and n-6 families) [48]. For the desserts treated with ultrasound, it is noted that TS200 presented lower AI. At this level of ultrasound, unsaturated fatty acids are released in greater concentration, improving the nutritional profile of desserts. The lower the AI and TI values, the greater the amount of anti-atherogenic and anti-thrombogenic fatty acids present in a given oil/fat and, consequently, the greater the potential for preventing the onset of coronary heart disease [52].

3.6. Volatile compounds

Table 3 shows the volatile compounds of the dairy dessert formulations, where 17 to 23 volatile compounds were identified, depending on the treatment applied. The most identified organic groups were hydrocarbons (10) and terpenes (8). All formulations presented caryophyllene as a terpene. At the same time, the application of HTST and TS contributed to the appearance of terpenes compounds, such as β -phellandrene and α -cubene, regardless of the TS power used. Sonication and increased temperatures may have caused the rupture of Jamun fruit cells, resulting in extraction of terpenes [53]. However, it is important to mention that TS samples, mainly TS200 and TS400, presented more terpene compounds (n = 5–6) than the other formulations (n = 2–4). Terpenes are part of the characteristic flavor volatile profile of Jamun

fruit [54]. In addition, phenyl ethyl alcohol and 2-nonanone, found in all formulations, are compounds associated with fruity odor [55,56], and D-limonene is related to sweet and citrusy notes [57]. Other compounds identified in all formulations were related to fruit (octanoic acid), flowery (hexanoic acid), and buttery-like (n-decanoic acid and nonanoic acid) notes [58,59].

No compounds associated with the lard reaction were identified, such as 5-hydroxymethylfurfural, furfural, and pyranone. This result indicates that HTST and TS did not trigger the lard reaction, not generating advanced glycation end products and off-flavors compounds. In a previous study evaluating the thermosonication treatment in orange juice whey drink, the formation of compounds from the lard reaction was also not observed, reinforcing that the application of TS is an exciting option in this sense [11]. It is essential to evaluate this aspect when applying emerging technologies in food since the formation of lard reaction products varies according to the process parameters used [4].

3.7. Confocal laser scanning microscopy

Fig. 9 displays fluorescence microscopy images for all samples. The mean size of fat droplets differed significantly ($p < 0.05$) between TS samples (values between 2.2 and 2.3 μm) and control and HTST samples (values between 3.2 and 3.3 μm). However, no significant impact of TS power was observed ($p > 0.05$). These results indicate that the samples treated by TS have better digestibility when compared to the control sample and the sample treated by HTST due to the smaller size of the fat droplets observed [60,61].

Ultrasound treatment has already been shown to reduce the size of

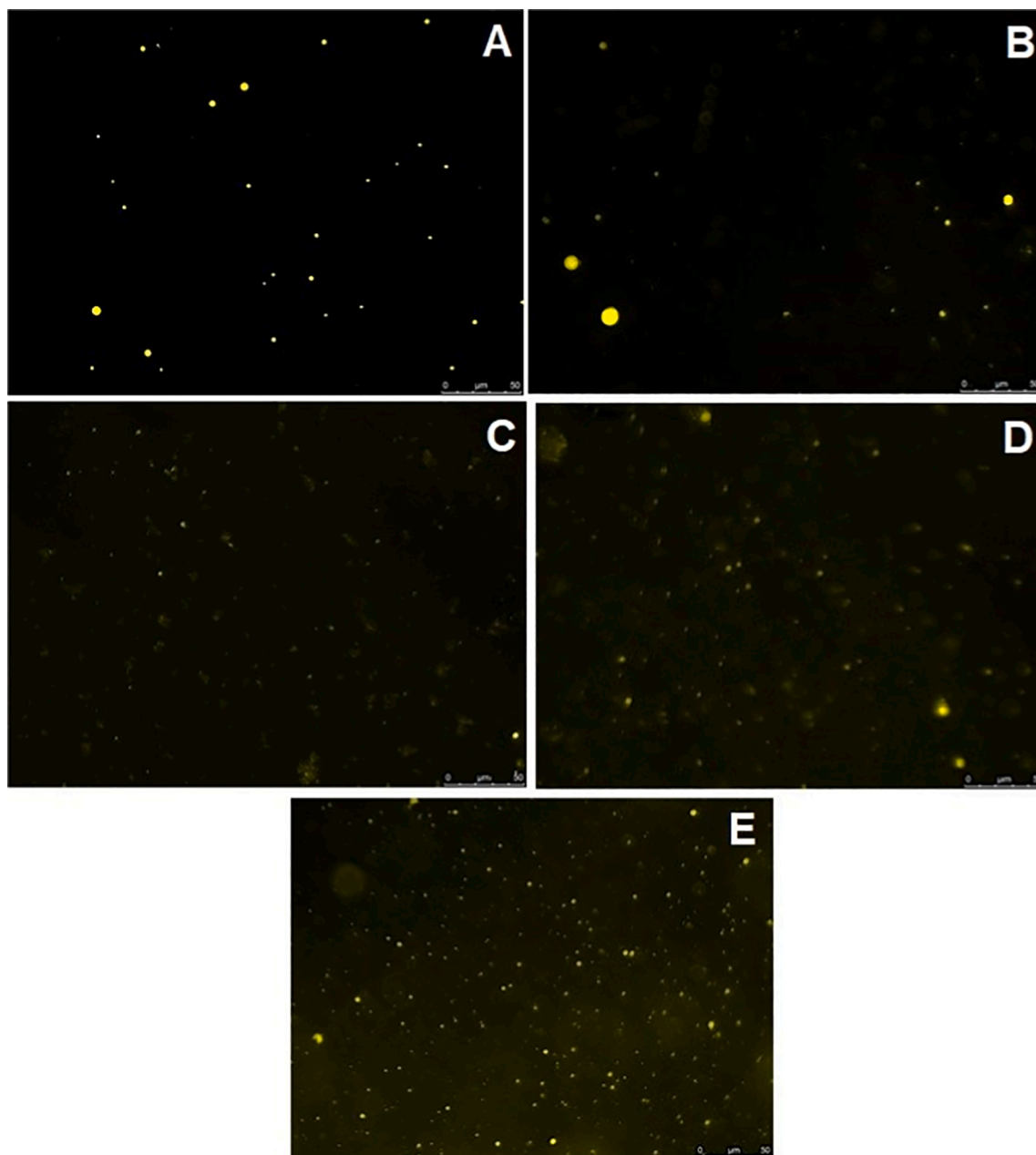


Fig. 9. Confocal laser scanning microscopy of Jamun fruit dairy dessert samples. A: Control sample. B: HTST (high-temperature short-time treatment (90 °C/20 s)). C: TS200 (Thermosonication at 90 °C/200 W). D: TS400 (Thermosonication at 90 °C/400 W). E: TS600 (Thermosonication at 90 °C/600 W).

fat globules in dairy products [61,62,63]. Acoustic vibration causes the fat droplets to disperse in the aqueous phase of the milk, and further cavitation causes the fat droplets to break down. In addition, the ultrasound treatment can disintegrate the fat globule membrane, causing a reduction in its size and an alteration in its granular surface due to the interaction between the casein micelles and the disintegrated membrane [10].

4. Conclusion

This study was the first to evaluate the application of TS in dairy desserts. TS treatments led to more significant microbial inactivation and microbiological stability during storage of the Jamun fruit dairy dessert compared to the conventional treatment. In addition, TS enabled satisfactory results in rheological tests and functional activities related to bioactive compounds (higher anti-hypertensive, antioxidant, and

anti-diabetic activities, and lower anthocyanins degradation). The utilization of lower TS powers (200 W) is advised when better fatty acid profiles are required (higher monounsaturated and polyunsaturated fatty acid contents), and it could keep a better volatile profile (higher number of terpenes). On the other hand, higher TS powers (600 W) may be used for better functional properties (anti-hypertensive, antioxidant, and anti-diabetic). Thermosonication is an efficient emerging technology for dairy desserts, as synergistic effects of temperature with cavitation were observed. Therefore, extending the scientific literature with the advancement of the study of process parameters associated with TS treatment and its application in different food matrices becomes important.

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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