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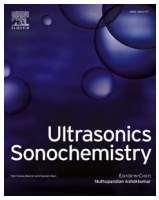

High-intensity ultrasound processing of blueberry pulp: a pathway to improve physicochemical quality and bioactive retention in enriched skyr

Júlia B. Sousa [a], Carolina P.C. Martins [a], Elson Rogério T. Filho [a], Jonas T. Guimarães [a], Mônica M. Pagani [b], Eliane T. Mársico [a], Mônica Q. Freitas [a], Vinicius P. Souza [d], Erico M.M. Flores [d,*], Adriano G. Cruz [c], Erick A. Esmerino [a]

[a] Department of Food Technology, Faculty of Veterinary, Federal Fluminense University (UFF), Niterói, Rio de Janeiro, Brazil
[b] Department of Food Technology, Institute of Technology, Federal Rural University of Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil
[c] Department of Food Technology, Federal Institute of Education, Science and Technology of Rio de Janeiro, Maracanã, Rio de Janeiro, Brazil
[d] Department of Chemistry, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

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ABSTRACT

Concentrated yogurts, such as skyr, are gaining prominence as functional foods, especially when enriched with fruits. However, conventional pulp processing methods, such as pasteurization, may compromise some beneficial properties. In this work, the production of skyr-type yogurt supplemented with blueberry pulp processed by high-intensity ultrasound (HIU) was proposed. The procedure was evaluated at HIU power levels of 160 W, 320 W, 480 W, and 640 W (20 kHz) for 3 min. The dissipated power, acoustic intensity, and acoustic density were calculated for all HIU conditions using the calorimetric method. The obtained products were compared to the products after conventional pasteurization and untreated blueberry pulp in terms of pH, total acidity, color, presence of bioactive compounds, rheology, and profile of volatile compounds. The products obtained by the HIU procedure at power levels of 320 and 480 W presented higher water retention and improved consistency, resulting in higher viscosity and lower syneresis compared to conventional pasteurization. Bioactive compounds, such as phenolics and anthocyanins, were better preserved after conventional pasteurization. However, contrarily to the other ultrasound powers, HIU at 640 W showed better retention of monomeric anthocyanins, suggesting that higher power levels are beneficial for maintaining the bioactivity properties. Electronic nose analysis revealed that HIU treatment and pasteurization generate products with similar volatile compounds profile, but a unique profile was observed while using ultrasonic power levels of 320 and 640 W. Although pasteurization maximizes the extraction of bioactive compounds, HIU treatment enhances the stability of the product and increases the sensorial quality for longer periods, indicating that the processing method can be adapted according to the objective of the product.

1. Introduction

Milk is one of the most important agricultural commodities worldwide, consistently ranking among the top agricultural products in terms of production volume and economic value [1]. In Brazil, dairy is the second most important sector within the food industry, playing a crucial economic and nutritional role [2]. The high nutritional value of dairy products has drawn attention due to the growing search for functional food sources [3,4]. In this context, yogurts are dairy products based on the addition of fermenting agents, which convert the lactose of the milk into lactic acid, providing additional health benefits to the product [3–6]. The development of fermented dairy products stands out due to their potential for better nutritional value, meeting the demands for a functional feeding while maintaining the competitiveness in the market [4,6,7]. Recently, the growth of the consumption of concentrated yogurts, such as skyr yogurt, has gained popularity as a product to increase the nutritional value [8,9].

Traditionally, the recipe for skyr yogurt includes the use of skimmed milk and a concentration step via whey drainage. The final product usually presents relatively low-fat content and high-protein levels, along with a thicker and creamier texture, characteristics that are increasingly sought after by consumers [4,8,9]. Although yogurt is already

* Corresponding author.
E-mail address: ericommf@gmail.com (E.M.M. Flores).

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considered a functional food, the enrichment with other functional ingredients can increase its benefits. In this sense, fruits are a distinctive option offering better sensory aspects, also incorporating nutrients and bioactive compounds [5–7]. The combination of yogurt and fruits has the potential to provide probiotics, prebiotics, high-quality proteins, fatty acids, vitamins, minerals, and antioxidants, which can have a positive impact on consumer health [3–5,10].

Among the wide variety of fruits, the so-called superfruits are particularly noteworthy due to their high concentration of vitamins and antioxidants [10]. The popularity of this category of fruits increased following the publication of the book SuperFoods Rx [11], which highlighted foods with high nutritional value. Blueberries (*Vaccinium myrtillus)* were the first fruit to receive the superfruit distinction due to their exceptional antioxidant properties [11], containing high levels of polyphenols in both skin and pulp [12,13]. The high content of anthocyanins in blueberries particularly stands out because it acts as free radical scavengers, anti-inflammatory agents, blood circulation enhancers, and LDL cholesterol reducers [12–15].

Despite improving the nutritional and functional enhancement, the addition of fruits can adversely impact its physical, chemical, sensory, shelf-life, and microbial characteristics of the yogurt [6,7,16]. Fruit processing is a crucial step to enhance the acceptance, and the conservancy, of concentrated yogurt with added fruit pulp [17]. Various technologies have been used in the food industry for this purpose, including physical preservation methods, such as heating, freezing, refrigeration, and dehydration and chemical preservation methods, such as pH reduction or the use of preservatives [18]. Conventional thermal treatment, such as pasteurization, is the most widely used, effectively reducing the number of microorganisms [18]. However, it can alter physical and chemical properties, mainly because of the degradation of flavors and aromas, impacting nutritional and sensorial quality of the final product [19].

The use of high intensity ultrasound (HIU) as an alternative processing method has been increasingly studied and applied in the food industry [19–22]. Ultrasound are mechanical waves propagating in cycles of compression and rarefaction, at frequencies higher than those audible to humans (>20 kHz) [23]. In a liquid medium, when the acoustic wave has enough energy to overcome the cohesive forces of the medium, it generates cavitation bubbles [22–25]. The HIU is characterized for high-energy densities capable of boosting the disruption of cell walls, the diffusion of the solvents, and, consequently, the extraction of bioactive compounds [22]. Compared to conventional processing techniques, HIU offers advantages such as non-toxicity, high efficiency, and environmental friendliness [20,21,26–29], representing a milder and more efficient alternative to thermal processing techniques such as pasteurization [18,21,28–30]. When applied to fruits in the form of pulp or syrup, HIU enhances juice extraction, reduces microbial activity, improves texture acceptance, and aid in nutrient and bioactive compound preservation, including phenolic compounds [31–33]. This is due to the facilitated disruption of the cells, causing the release of such compounds [34,35].

Considering the current lack of studies evaluating the use of HIU specifically on fruit pulp intended for incorporation into dairy matrices, this study aimed to develop a concentrated yogurt enriched with blueberry pulp previously treated with HIU and to investigate how this technology influences the physicochemical, bioactive, and sensory properties of both the pulp and the final yogurt product. The HIU treatment (20 kHz) was applied to the blueberry pulp at nominal power of 160, 320, 480, and 640 W. Process parameters such as acoustic dissipated power, acoustic intensity, and acoustic density were calculated using the calorimetric method [36–39]. The processed pulp was then incorporated into concentrated yogurt and compared to samples prepared with pasteurized and untreated pulp. This approach allows the assessment of HIU as a non-thermal alternative to improve the quality and functionality of fruit-based yogurt formulations.

2. Material and methods

2.1. Preparation of concentrated yogurt with added blueberry pulp

The yogurt preparation was based on the traditional processing, reported worldwide [40–42]. The ingredients were weighed individually and followed the formulation: 97 % (w w^{-1}) of a commercial skimmed UHT milk (Molico, Rio de Janeiro, Brazil), 3 % (w w^{-1}) of skimmed milk powder (Molico, Rio de Janeiro, Brazil), and 0.005 % (w w^{-1}) of thermophilic bacterial culture (YoFlex L812, Chr. Hansen, São Paulo, Brazil). The yogurt was then drained using cloth bags [42].

Blueberries at early stages of ripening were purchased in a local store, and cleaned, non-heat-treated and then frozen at −18 °C. The blueberries were blended using a knife-type blender (Zoop Blender, Cadence, Balneário Piçarras, Brazil) and subdivided into sixteen fractions. These samples were treated in triplicate by HIU (ultrasound probe 13 mm of diameter, QR750, Ultronique Disruptor/Sonicator, São Paulo, Brazil) for 3 min at 160 W, 320 W, 480 W, 640 W. For comparison of results, samples were also treated by thermal pasteurization at 85 °C for 3 min, and one sample remained untreated to serve as a control group. After processing, the blueberry pulp was added to the concentrated yogurt at 8 % (w w^{-1}) along with white sugar (União, São Paulo, Brazil) in a proportion of 8 % (w w^{-1}). The finished yogurt was stored for 21 d at 4 °C in a conventional refrigerator and analyzed on days 1 (D1), 7 (D7), 14 (D14), and 21 (D21).

2.2. Measurement of HIU parameters

The delivered power delivered during the HIU treatments were assessed by the determination of the dissipated power (P), acoustic intensity (I) and the acoustic density (D), using the calorimetric method according to previous studies [36–39]. The values were obtained using the Equations 1 to 3.

$$P\ (W) = m\ C_p\ \Delta T\ /\ t \quad (1)$$

$$I\ (W\ cm^{-2}) = 4P\ /\ \pi\ d^2 \quad (2)$$

$$D\ (W\ cm^{-3}) = P\ /\ V \quad (3)$$

where m is the sample mass (kg), Cp is the specific heat capacity of the pulp (4100 J kg^{-1} K^{-1}), ΔT is the temperature increase (°C), t is the sonication time (180 s), d is the probe diameter (0.015 cm), m is the sample mass (g), and V is the sample volume (50 cm^3). The temperature variation (ΔT) was measured based on the difference between the initial and final temperatures, which were measured using a digital thermometer, of the pulp after 3 min of sonication for each power level. All calculations were performed using the pulp as the sonicated medium, considering its specific heat capacity. The ultrasonic parameters for the treatments at nominal powers of 160, 320, 480, and 640 W were, respectively: P = 10.5, 14.1, 17.6, and 20.1 W; I = 22.7, 30.4, 37.9, and 43.3 W cm^{-2}; D = 0.211, 0.283, 0.352, and 0.402 W cm^{-3}.

2.3. Physical-chemical analysis

After preparing the yogurts with added fruit pulp, they were evaluated for their physicochemical properties, phenolic compounds, and antioxidant properties on D0, D7, D14, and D21. Due to the lack of a specific regulation for concentrated yogurts, the analyses described below were carried out based on the current legislation for fermented milks, in accordance with the recommendations of the AOAC Official Methods of Analysis [37], Codex Alimentarius [38], and Manual of Official Methods for Analysis of Foods of Animal Origin [39].

2.3.1. Centesimal composition

The moisture of yogurt was determined according to Bayarri *et al.* [43], using infrared heating on a moisture analysis balance (LJ16,

Mettler Toledo, Columbus, USA), regulated at 105 °C. The other analyses followed the methods described by AOAC Official Methods of Analysis [44]. Mineral residues were determined using the AOAC 945.46 methodology. The protein content was determined using the AOAC 991.20 method, in accordance with Brazilian legislation and the Codex Alimentarius [45,46]. The fat content was assessed using the AOAC 989.05 methodology, in accordance with the Brazilian Technical Regulation for Identity and Quality [46]. Finally, the carbohydrate content was calculated by difference, subtracting the sum of the percentages of moisture, protein, fat, ash, and dietary fiber (if determined) from 100 %, in accordance with the Codex Alimentarius Guidelines on Nutrition Labelling [45].

2.3.2. pH

According to the current Brazilian legislation (Brazilian Ministry of Agriculture, Livestock, and Food Supply, Normative Instuction nr. 46/2007) [46], the pH of yogurt must be in the range of 3.5 to 4.6 after 48 h of the fermentation process. The pH of the yogurt was determined on the D0, D7, D14, and D21 after the yogurt production, using a pH meter (pHS-3E, Satra, Kettering, United Kingdom), previously calibrated at two points of pH, 4.0 and 7.0.

2.3.3. Total acidity

Total acidity was determined on days D0, D7, D14, and D21 after yogurt production during the storage period at a temperature of 4 °C following the methodology AOAC 947.05 of the AOAC Official Methods of Analysis [44]. Results were expressed as grams of lactic acid per 100 g of sample (g/100 g).

2.3.4. Syneresis

The syneresis evaluation was conducted according to the method adapted from Sarker and Siddiqui [47]. Quantities of approximately 5 g of each sample were placed in falcon tubes, then subjected to 1000 G rotation in a centrifuge (Sorvall™ ST 16R, Thermo Scientific, Waltham, USA) for 15 min. The resulting supernatant was removed, and its mass was measured. The calculation of the syneresis index, expressed as a percentage, was performed by dividing the mass of the supernatant (whey) by the total mass of the sample and multiplying the result by by a factor of 100.

2.4. Bioactive compounds

Approximately 1.5 g of the sample was transferred to Falcon tubes (15 mL), 5 mL of a 1:1 methanol/water solution was added, and the mixture was shaken and then let stay without shaking for 1 h. After this time, the sample was centrifuged for 15 min, and the supernatant was collected. Then 5 mL of a 7:3 acetone/water solution was added to the first extraction tube, shaking and leaving the mixture to rest for 1 h. After, the product was centrifuged for 15 min, collecting the supernatant and adding it to the supernatant from the first extraction. The supernatants were then subjected to DPPH determination. To obtain the extract for total phenolics content (TPC) determination, which were also performed in triplicate, approximately 1.5 g of the samples was transferred into Falcon tubes (15 mL), adding 5 mL of acetone and water (in a volume ratio of 70:30, respectively), shaking and leaving to act for 1 h. After resting, the product was centrifuged for 15 min, collecting the supernatant and obtaining the extract for the analysis. For the determination of anthocyanins by the pH differential method, no solvent extraction was performed; instead, the analysis was conducted directly on the sample material, as described in Section 2.4.3.

2.4.1. DPPH (Free radical scavenging capacity) analysis

The antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, adapted from Bontzolis *et al.* [48] and Martins *et al.* [49]. A methanolic solution of DPPH radical (0.06 mmol L^{-1}, $\lambda = 517$ nm) was prepared, and 2850 μL of this solution was added to 150 μL of each extract. The mixture was homogenized in a tube and kept at rest, protected from light, for 60 min. Subsequently, the absorbance was measured in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 517 nm, using methanol as the blank.

2.4.2. Total phenolics

The determination of TPC was evaluated during the storage period, according to Swain and Hillis, Bontzolis *et al.* and Martins *et al.* [48–50]. The absorbance was measured at 725 nm in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan), and the results were expressed in grams equivalent to gallic acid per liter of sample (AG g L^{-1}) from a calibration curve. To perform the analysis, 1 mL of Foulin's solution diluted in distilled water (1:10) was added to 1 mL of the extract and shaken for 1 min. Then, 1.5 mL of a 10 % (w w^{-1}) Na_2CO_3 solution was added, and the tubes were kept protected from light for 2 h before the measurements.

2.4.3. Anthocyanins by pH differential

The determination of anthocyanins was evaluated during the storage period, according to the methodology described by Klopotek *et al.* [51]. The method involves the preparation of two solutions: pH 1.0 and pH 4.5. For pH 1.0, KCl was dissolved in distilled water, followed by the addition of concentrated HCl to adjustment of the pH. For pH 4.5, CH_3COONa was dissolved in distilled water, followed by the addition of concentrated HCl to adjust the pH. Samples were prepared by adding 0.1 and 1.0 g, completed with the corresponding solution, and left to rest. After filtration, spectrophotometric measurements (UV-1800, Shimadzu, Kyoto, Japan) were taken at wavelengths of 510 nm and 700 nm to determine the absorbance of the samples. Calculations to quantify total and monomeric anthocyanins were performed based on the absorbance differences between the wavelengths, using cyanidin-3-glycoside as the main standard.

2.5. Colorimetric analysis

Colorimetric analyses were performed during the storage period. The color parameters were determined using the CIELAB color space system, according to De Campo *et al.* [52] by portable colorimeter (CR-410, Konica Minolta, Osaka, Japan). In the CIELAB color space (Lab *), the L* value represents the brightness of the sample, while the a* and b* values represent the chromaticity coordinates. Specifically, a* represents the color direction between red (+a*) and green (−a*), and b* represents the color direction between yellow (+b*) and blue (−b*). Additionally, the Chroma (C*) and color difference (ΔE), which represent the saturation of the color and the magnitude of color change over time or between treatments, respectively, were calculated based on the L*, a*, and b* values [53]. The instrument was calibrated on a white plate and was equipped with a D65 illuminant and an observation angle of 10°; the readings were performed in reflectance mode with specular included.

2.6. Rheological analysis

The rheological properties of yogurt were obtained at room temperature (25 ± 1 °C) 24 h after the manufacturing. A rotational viscometer (DV-II, Brookfield Engineering Laboratories, Stoughton, USA) was used according to Le Ba *et al.* [54]. A cylindrical spindle (LV-4, Brookfield Engineering Laboratories, Stoughton, USA) was used inside an adapted cylindrical container. The measurements of the spindle (5.8 cm in height, 1.8842 cm in diameter and 0.9421 cm in radius) and the container (4.9 cm in height, 3.1 cm in diameter, 1.55 cm in radius) were obtained to calculate the shear rate, shear stress and viscosity parameters according to the Equations 4 to 6:

$$\gamma = 2\ \omega\ Rc_2\ Rb_2 / x_2\ (Rc_2 - Rb_2) \quad (4)$$

$$\sigma = M / 2\ \pi\ Rb_2\ L \quad (5)$$

$$\eta = \sigma / \gamma\ 100 \quad (6)$$

where σ is the shear stress (dynes cm^{-2}), γ is the shear rate (s^{-1}), η is the viscosity (mPa), Rc is the radius of the vessel (cm), Rb is the radius of the spindle (cm), x is the radius at which the shear rate is being calculated (cm), M is the torque applied by the instrument (dyne cm), L is the effective length of the spindle (cm), and ω is the angular velocity of the spindle (rad s^{-1}).

The average torque was measured as the spindle speed increased from 0 to 250 rpm (2 rpm s^{-1}). Flow curves were obtained in three sequential flow steps: up-down-up cycles. Data from the third flow curve were fitted to the power law model, based on the Ostwald-Waele equation, presented in Equation 7. Measurements were made in triplicate at 25 °C.

$$\sigma = k\ \gamma\ n \quad (7)$$

where k is the consistency index (mPa s^{-1}), n is the flow behavior index (dimensionless), σ is the shear stress (mPa), and γ is the shear rate (s^{-1}).

2.7. Electronic nose (e-nose) analysis

The e-nose analysis was performed using the fast GC E-nose (Heracles II, Alpha MOS, Toulouse, France) adapted from the methodology of Kovacs *et al.* [55]. The identification of volatile compounds was performed based on the Kovats index, using a standard solution of C6–C16 alkanes and the Aro ChemBase library (Alpha MOS, France) to perform the identification of the compounds. For the E-nose test, the samples were kept frozen at − 18 °C until the day of analysis and subsequently thawed for 24 h in refrigeration before performing the analysis.

Two grams of yogurt were transferred into a 20 mL glass vessel. Before injection, each sample was incubated in the autosampler oven at 50 °C for 10 min to volatize the compounds. After headspace generation, a gas sample was withdrawn and injected into the GC injector port. A dry run (air-only) was performed between samples of different treatments. In total, six yogurt samples were analyzed in triplicate.

For the data handling of the results obtained with the e-nose, the compounds were refined to a minimum peak area of 100, and only those with known relevance to food aroma and sensory quality were included in the final list. This selection was based on compounds typically associated with odor-active substances in food matrices. After signal acquisition, a principal component analysis (PCA) was performed to evaluate the differences in the volatile profile of the samples regarding their processing method. A threshold of minimum peak area of 500 was applied, without additional filtering regarding the functional role of the compounds, allowing the inclusion of all detected volatiles to ensure a more comprehensive and representative analysis of the volatile profile.

2.8. Data analysis

The results from the physical–chemical, colorimetric, and rheological analyses were subjected to analysis of variance (One-way ANOVA) and Tukey's mean test with a $p < 0.05$, which allows the identification of specific groups that present significant differences from each other.

3. Results and discussion

3.1. Centesimal composition

The results of the centesimal composition, including lipids, proteins, carbohydrates, ash, and moisture, are presented in Table 1. The results indicated that HIU treatments differed significantly from the control treatment, especially at powers of 320 W and 480 W, which presented higher moisture values. This effect can be attributed to the rupture of the cell walls of the blueberry pulp caused by the ultrasound-induced cavitation phenomenon, which enhances the release of water and hydrophilic compounds [32].

The control sample presented the lowest moisture value, not differing significantly from the pasteurized sample, which in turn did not present a significant difference in relation to the 160 W and 640 W treatments.

Table 1
Centesimal composition of concentrated skyr-type yogurt with added blueberry pulp (*Vaccinium myrtillus*) processed by high-intensity ultrasound (20 kHz).

Treatments	Moisture (%, m/m)	Ash (%, m/m)	Protein (%, m/m)	Lipids (%, m/m)	Carbohydrate (%, m/m)
Control	83.3 ± 0.1^{a}	1.28 ± 0.01^{a}	7.03 ± 0.08^{a}	0.20 ± 0.00^{a}	8.17 ± 0.15^{a}
Pasteurization	84.1 ± 0.1^{ab}	1.27 ± 0.01^{a}	7.07 ± 0.08^{a}	0.20 ± 0.00^{a}	7.39 ± 0.82^{ab}
US 160 W	84.7 ± 0.1^{bc}	1.26 ± 0.01^{a}	6.93 ± 0.08^{a}	0.20 ± 0.00^{a}	6.87 ± 0.28^{bc}
US 320 W	85.4 ± 0.1^{c}	1.25 ± 0.01^{a}	7.03 ± 0.08^{a}	0.20 ± 0.00^{a}	6.13 ± 0.48^{c}
US 480 W	85.3 ± 0.1^{c}	1.23 ± 0.01^{a}	7,00 ± 0.08^{a}	0.20 ± 0.00^{a}	6.22 ± 0.21^{bc}
US 640 W	85.2 ± 0.1^{bc}	1.23 ± 0.01^{a}	7.07 ± 0.08^{a}	0.20 ± 0.00^{a}	6.28 ± 0.25^{bc}

*Results were expressed as mean ± standard deviation (n = 3). Different letters (a-d) in the same column indicate the difference by Tukey's test ($p < 0.05$).

However, the results demonstrate that applying 320 W or 480 W of HIU power maximizes the release of water and hydrophilic compounds, while other conditions presented reduced effects. The moisture content of fruit pulp added to yogurt can change depending on the type and quantity of fruit, in addition to the production process. Studies by Ghasempour *et al.* [56] indicate that the addition of fruit pulp generally maintains yogurt moisture between 80 % and 85 %. Similarly, Barbosa Junior *et al.* [57] reported values close to those obtained in this study for natural yogurts with the addition of blueberry pulp, which supports this trend.

Although Brazilian legislation does not establish specific values for the ash content in yogurts, this metric is essential to assess the total mineral content of the product. According to the United States Department of Agriculture (USDA) [58,59], the ash content in nonfat Greek yogurt is 0.75 g, while in blueberries this value is 0.23 g. In this study, the average ash content found was 1.25 %, a value higher than that reported by Leite *et al.* [60], who identified an average of 0.910 % in concentrated yogurts added with 10 % juçara (*Euterpe edulis*) pulp. The difference observed in the results can be attributed to the variations in the ingredients used in the production of yogurts. The difference in ash content can be explained by the fact that the treatments applied to blueberry pulp, such as HIU and pasteurization, do not significantly affect the mineral content in the final product.

Concentrated yogurts, such as skyr, have a significantly higher protein content compared to traditional yogurt [51]. As a concentrated yogurt, skyr must meet the specifications of the Codex Alimentarius [45], which establishes a minimum of 5.6 % protein. The samples analyzed in this study presented an average of 7 % protein w w^{-1} (14 g in 200 g), a value in agreement with such regulation. This high-protein content is attributed to the production process used, as described by Tamime and Robinson [60], which includes the addition of milk powder and the removal of whey, methods that concentrate the proteins. The protein values in the samples analyzed did not show significant differences between them. According to data from the USDA [58], 100 g of blueberry pulp contains approximately 0.7 g of protein, a relatively low value.

According to the Brazilian legislation (Normative Instruction No. 46) [46], the maximum fat content permitted for skimmed yogurts is 0.5 %. Therefore, to be classified as skimmed yogurt in Brazil, the product must contain a maximum of 0.5 g of fat per 100 g. The samples analyzed in this study presented an average of 0.2 % fat, as shown in Table 1, a value that is in accordance with those described for yogurt skyr by Gudmundsson and Kristbergsson [60]. Furthermore, according to the USDA

[58], 100 g of blueberry pulp contain approximately 0.31 g of total lipids (that is considered a low lipids content).

In this sense, both the low protein and lipid content in blueberries contribute to the absence of significant differences between samples, regardless of the type of treatment applied. This indicates that the addition of blueberries, by itself, does not contribute significantly to the increase in protein in the product, although it does not cause the increase in the lipid content. However, the processing method does not promote the decomposition of these components, indicating that the treatments did not impact the final lipid or protein content of the product.

The average carbohydrate content obtained in this study was 6.84 %. Since it was calculated using the difference method, an inversely proportional relationship with moisture content was observed. In samples with higher moisture content, such as those treated with 320 W and 480 W, a reduction in carbohydrate content was observed. The control treatment, with lower moisture content, presented a higher carbohydrate concentration.

3.2. pH and titratable acidity

The analysis of pH and titratable acidity data of blueberry yogurt was performed four times: at D0, day D7, D14 and D21. Although Brazilian legislation does not specify pH values for yogurts, it is recognized that pH plays an important role in inhibiting microbiological growth [61]. Brandão [62] defines that the ideal pH for yogurt ranges between 4 and 4.6, since pH values higher than 4.6 enhances the separation of the serum of the yogurt. As the gel was not sufficiently formed at pH lower than 4.0, it causes the compression of the clot due to the reduction of protein hydration, also causing the product to drain, indicating a loss of quality. The pH values of the samples vary between a minimum of 4.1 and a maximum of 4.5. The results are shown in Table 2.

On D0, the control sample, which did not undergo any treatment, presented the highest pH (4.51) and differed significantly from all the treated samples. The pH of treated samples did not differ from each other, indicating an initial impact of the treatment on the acidity of the yogurt. Bermudez-Aguirre and Niemira [61] demonstrated that HIU can cause a reduction in pH, mainly by breaking intermolecular bonds, promoting a greater release of H^+ ions and, consequently, decreasing the pH of the samples. Mandha *et al.* [63] studied the effects of pasteurization on fruit juices and observed that, in some cases, the thermal process can lead to the formation of acids, resulting in a decrease in pH.

From D7, some differences in pH values occurred, but no correlation with the type of treatment. Suggesting that the initial effect of the treatments on pH reduced over time. This variation indicates that the fermentation process and production of organic acids, which initially reduce pH, tend to overcome the effect of processing on acidity.

The pH differences observed in relation to the days of storage can be attributed to the increase in fermenting bacteria, which convert lactose into lactic acid, resulting in the decrease in pH [64–66]. The absence of specific control over which bacteria proliferate more in each sample may have been one of the reasons for the differences observed, especially on the last day of storage. Although the HIU and pasteurization treatments had an initial impact on pH, especially on D0, suggesting that fermentation associated with storage was the predominant factor in pH changes over time. Bacterial activity during fermentation appears to have been the main driver of the drop and stabilization of pH from the second week onwards, overcoming the effect of the treatments.

Titratable acidity measures the total number of acids present in a sample, providing a more comprehensive picture of the impact of these acids on texture and flavor, while pH measures only the concentration of H^+ and is more susceptible to immediate variations during fermentation and food handling processes [67]. This difference is relevant because pH can change more rapidly in response to processing, while titratable acidity is less sensitive to small variations, explaining the constancy in acidity even though pH presents variations. The results obtained for titratable acidity are presented in Table 3.

Titratable acidity values ranged from a minimum of 0.95 g to a maximum of 1.48 g of lactic acid per 100 g, all within the limits established by Brazilian legislation (0.6 to 1.5 g/100 g) [46]. Significant differences were observed for all samples over the storage period. However, no significant differences were found among treatments during the first two weeks (D0, D7, and D14). These differences became evident only in D21, when samples containing blueberry pulp processed with HIU at 320 W and 640 W exhibited significantly higher titratable acidity (1.48 g/100 g) compared to the control (1.44 g/100 g).

These findings suggest that the application of HIU to the blueberry pulp enhanced the release or transformation of bioactive compounds capable of stimulating microbial metabolism during storage, thereby increasing acid production over time. This effect was not immediate but became more pronounced by the fourth week, indicating a delayed influence of the HIU-processed pulp on yogurt acidification. These results are consistent with the findings of Pacheco *et al.* [67], who reported similar behavior in buffalo yogurt produced with ultrasound-assisted fermentation. Furthermore, a clear inverse correlation was observed between pH and titratable acidity: as the pH decreased, acidity increased across all treatments during storage [66,67].

Table 2
Ph of concentrated skyr-type yogurt with added blueberry pulp (*Vaccinium myrtillus*) processed by high-intensity ultrasound (20 kHz). D0 to D21 means the number of days that the finished yogurt was stored at 4 °C in a conventional refrigerator.

Treatments	D0	D7	D14	D21
Control	4.51 ± 0.01^{bC}	4.42 ± 0.01^{bcB}	4.21 ± 0.01^{aA}	4.20 ± 0.01^{dA}
Pasteurization	4.45 ± 0.01^{aBC}	4.44 ± 0.01^{cC}	4.24 ± 0.01^{bB}	4.18 ± 0.01^{cdA}
160 W	4.44 ± 0.01^{aD}	4.34 ± 0.01^{aC}	4.21 ± 0.01^{aB}	4.13 ± 0.01^{abA}
320 W	4.45 ± 0.01^{aD}	4.36 ± 0.01^{aC}	4.21 ± 0.01^{aB}	4.14 ± 0.01^{abA}
480 W	4.44 ± 0.01^{aD}	4.37 ± 0.01^{bC}	4.20 ± 0.01^{aB}	4.13 ± 0.01^{aA}
640 W	4.44 ± 0.01^{aC}	4.40 ± 0.01^{bC}	4.21 ± 0.01^{aB}	4.16 ± 0.01^{bcA}

*Results were expressed as mean ± standard deviation (n = 3). Lowercase superscript letters (a-d) in the same column indicate difference by Tukey's test ($p < 0.05$) for processing. Superscript capital letters (A-D) in the same row indicate difference by Tukey's test ($p < 0.05$) as a function of days.

Table 3
Acidity of concentrated skyr-type yogurt with added blueberry pulp (*Vaccinium myrtillus*) processed by high-intensity ultrasound (20 kHz). D0 to D21 represent the number of days that the yogurt was stored at 4 °C.

Treatments	D0	D7	D14	D21
Control	0.96 ± 0.03^{aA}	1.09 ± 0.01^{aB}	1.30 ± 0.02^{aC}	1.45 ± 0.01^{bD}
Pasteurization	0.96 ± 0.03^{aA}	1.10 ± 0.01^{aB}	1.39 ± 0.02^{aC}	1.38 ± 0.01^{aC}
160 W	1.00 ± 0.03^{aA}	1.11 ± 0.01^{aB}	1.33 ± 0.02^{aC}	1.48 ± 0.01^{cD}
320 W	0.98 ± 0.03^{aA}	1.10 ± 0.01^{aB}	1.34 ± 0.02^{aC}	1.48 ± 0.01^{cD}
480 W	0.96 ± 0.03^{aA}	1.10 ± 0.01^{aB}	1.35 ± 0.02^{aC}	1.47 ± 0.01^{bcD}
640 W	1.02 ± 0.03^{aA}	1.11 ± 0.01^{aB}	1.31 ± 0.02^{aC}	1.48 ± 0.01^{cD}

*Results were expressed as mean ± standard deviation (n = 3). Different letters (a-c) in the same column indicate the difference by Tukey's test ($p < 0.05$) for processing. Different letters (A-D) in the same row indicate the difference by Tukey's test ($p < 0.05$) as a function of days.

3.3. Syneresis

The results for syneresis analysis (separation of whey from yogurt gel) are shown in Table 4.

The control treatment presented the highest syneresis value, differing statistically from all samples treated with HIU. The pasteurized sample also showed a high syneresis value. not differing from the control sample and those treated with HIU at 160 W, 320 W and 480 W. The HIU treatments at 160 W, 320 W and 480 W resulted in a reduction in syneresis, with values of 0.34, 0.34 and 0.34, respectively. The lowest syneresis was observed in the sample treated at 640 W, which differed significantly from the control and pasteurized samples. The moisture results correlated with syneresis indicate that HIU was able to release water more effectively from the blueberry pulp, probably through the release of pectin, which helped to retain water in the matrix, improving syneresis in this processing.

Syneresis was significantly lower in blueberry pulp samples treated with HIU when compared to pasteurized and control samples, especially at higher power levels. The cavitation process generated by HIU promotes cell lysis of blueberry pulp, releasing intracellular compounds such as pectins and other soluble fibers. Pectins, polysaccharides with gelling and thickening properties, contribute to water retention, decreasing syneresis in yogurt [68].

In addition to acoustic cavitation-related pectin extraction, Kumar *et al.* [32] identify pH as a crucial parameter that affects both extraction yield and bioactive compound properties during HIU-assisted extraction. Pectin extraction from fruit and vegetable by-products is most efficient in a pH range of 1 to 5. Blueberry pulp pH has a relatively high acidity range, with pH values ranging from 3.1 to 3.6 [67,68] a value compatible with the optimal pH for pectin extraction. The pH value during ultrasonic treatment may have further optimized pectin extraction, enhancing the beneficial effects of HIU in reducing syneresis.

3.4. Bioactive compounds

The values of bioactive compounds, antioxidant activity by DPPH radical scavenging, total phenolics, and total and monomeric anthocyanins are presented in Table 5 (blueberry pulp) and Table 6 (yogurt with added pulp).

The control sample showed a DPPH reduction value of 46.2 %, significantly lower than all other treatments. Pasteurization exhibited the highest value (82.8 %) and was statistically superior to the HIU treatments. The 160 W HIU treatment also significantly differed from the control, although it remained lower than pasteurization. Bermúdez-Aguirre and Barbosa-Cánovas [53,61] highlight that while ultrasound may facilitate the extraction of bioactive compounds, higher power levels can lead to antioxidant degradation, which explains the reduction observed in the 320 W, 480 W, and 640 W treatments.

Although these power levels did not show significant differences among themselves, they were all significantly higher than the control. Corroborating with the findings of De Araújo *et al.* [69], that both ultrasound and pasteurization were more effective than the control in enhancing the antioxidant capacity of araçá-boi (*Eugenia stipitata*) pulp. However, it differs in the sense that HIU showed greater efficacy in improving antioxidant activity, likely due to the combination of heat and sonication (thermosonication) [69].

Regarding TPC, pasteurization also showed the highest result (3.46 mg GAE/100 g). All other treatments, including the control, did not statistically differ from each other. This outcome may be explained by the impact of thermal and non-thermal treatments on bioactive compounds. Chemat *et al.* [69] reported that moderate heat, as used in pasteurization, can enhance the extraction efficiency of bioactive compounds, such as polyphenols, by breaking down cell walls and improving solubility.

Following the trend of antioxidant capacity and total phenolics, pasteurization was the most effective in extracting anthocyanins, presenting the highest values for total anthocyanins (19.8 mg/100 g) and monomeric anthocyanins (18.3 mg/100 g). The low reduction percentage (7.64 %) confirms that pasteurization preserved most of the anthocyanins in their bioactive form, suggesting that thermal treatment

Table 4
Syneresis of concentrated skyr-type yogurt with added blueberry pulp (*Vaccinium myrtillus*) processed by high-intensity ultrasound.

Treatments	Syneresis
Control	0.37 ± 0.00^{c}
Pasteurization	0.35 ± 0.00^{bc}
US 160 W	0.34 ± 0.00^{ab}
US 320 W	0.35 ± 0.00^{b}
US 480 W	0.34 ± 0.00^{ab}
US 640 W	0.33 ± 0.00^{a}

*Results were expressed as mean ± standard deviation (n = 3). Different letters (a-d) in the same column indicate the difference by Tukey's test ($p < 0.05$).

Table 5
Bioactive compounds of blueberry pulp (*Vaccinium myrtillus*) processed by pasteurization and high-intensity ultrasound.

Treatment	% DPPH Reduction	Total Phenolics (mg GAE/100 g)	Total Anthocyanins (mg/100 g)	Monomeric Anthocyanins (mg/100 g)
Control	46.2 ± 0.6^{d}	1.80 ± 0.22^{b}	27.1 ± 0.6^{d}	16.5 ± 0.8^{c}
Pasteurization	82.3 ± 0.4^{a}	3.46 ± 0.08^{a}	197 ± 4^{a}	182 ± 16^{a}
US 160 W	54.8 ± 2.2^{b}	1.93 ± 0.08^{b}	41.5 ± 2.9^{c}	32.0 ± 5.7^{bc}
US 320 W	50.2 ± 0.3^{c}	1.63 ± 0.08^{b}	53.1 ± 0.9^{b}	44.3 ± 5.4^{b}
US 480 W	49.8 ± 0.9^{c}	1.72 ± 0.24^{b}	52.5 ± 0.8^{b}	39.7 ± 8.8^{bc}
US 640 W	50.7 ± 1.1^{c}	1.86 ± 0.00^{b}	57.0 ± 2.2^{b}	54.9 ± 2.7^{b}

*Results are expressed as mean ± standard deviation (n = 3). Different letters (a-c) in the same column indicate the differences according to Tukey's test ($p < 0.05$).

Table 6
Bioactive compounds of skyr yogurt added with blueberry pulp (*Vaccinium myrtillus*) processed by pasteurization and high-intensity ultrasound (20 kHz).

Treatment	% DPPH Reduction – Yogurt	Total Phenolics (mg GAE/100 g) – Yogurt	Total Anthocyanins (mg/100 g) – Yogurt	Monomeric Anthocyanins (mg/100 g) – Yogurt
Control	7.19 ± 0.31^{a}	0.768 ± 0.011^{b}	21.3 ± 0.4^{c}	3.58 ± 0.16^{b}
Pasteurization	6.34 ± 0.83^{a}	0.939 ± 0.064^{a}	29.0 ± 0.1^{b}	2.15 ± 0.10^{b}
US 160 W	4.52 ± 1.36^{ab}	0.766 ± 0.056^{b}	27.9 ± 0.5^{b}	0.945 ± 0.029^{b}
US 320 W	6.32 ± 2.56^{a}	0.756 ± 0.011^{b}	30.9 ± 0.5^{ab}	3.30 ± 0.39^{b}
US 480 W	5.57 ± 0.87^{ab}	0.741 ± 0.032^{b}	30.5 ± 2.1^{ab}	0.073 ± 0.006^{b}
US 640 W	5.70 ± 1.24^{ab}	0.701 ± 0.038^{bc}	32.6 ± 1.7^{a}	23.9 ± 5.7^{a}
Plain yogurt	2.63 ± 0.68^{b}	0.659 ± 0.018^{c}	N.D.**	N.D.**

*Analysis performed in triplicate. Results are expressed as mean ± standard deviation. Different letters (a-c) in the same column indicate the differences according to Tukey's test ($p < 0.05$).
**ND: not determined.

effectively maintained anthocyanin stability. Among the HIU treatments, the condition using 640 W showed the smallest difference between them and the lowest reduction percentage (3.74 %), despite not differing from the other HIU treatments in terms of total and monomeric anthocyanin content. Indicating minimal degradation and better preservation of monomeric anthocyanins. Tiwari *et al.* [70] also reported that sonication was effective in preserving anthocyanins, demonstrating significant retention during juice processing. On the other hand, the control (untreated sample) showed the lowest values for total (2.71 mg/100 g) and monomeric anthocyanins (1.65 mg/100 g), with a high reduction percentage (38.3 %), demonstrating that processing is essential for preserving or enhancing anthocyanin extraction. Compared to pure yogurt, the inclusion of 8 % pulp resulted in an average increase of 129 % in antioxidant capacity and 15.5 % in total phenolic content.

Among the treatment conditions, 640 W exhibited the highest total anthocyanin content (32.6 mg/100 g) and monomeric anthocyanins (23.9 mg/100 g), significantly differing from the other treatments. The other treatments showed no significant differences among themselves, presenting lower levels of bioactive and stable anthocyanins in the yogurt.

Consistent with the results for pure pulp, the 640 W treatment had the lowest anthocyanin degradation (0.545 mg/100 g) representing a 25 % reduction, indicating better retention of bioactive anthocyanins. The 320 W and 480 W conditions had the highest differences between total and monomeric anthocyanins, but did not statistically differ from 160 W and pasteurized samples.

Finally, the interaction between bioactive compounds (such as phenolics and anthocyanins) and milk components, particularly proteins, was not fully considered. This interaction may have masked the potential additional antioxidant benefits that HIU treatments could have offered, suggesting the importance of considering protein–polyphenol interactions in future studies on yogurt enrichment with bioactive compounds.

3.5. Colorimetric analysis

The colorimetric analysis was performed to assess the impact of the treatment procedure on the color of the final product. The colorimetry results can be seen in Table 7. The analysis of luminosity (L*) showed that the pasteurized treatment did not present statistically significant differences in relation to the 160 W, 320 W, and control treatments on D0. This indicates that, at this initial stage, the different processing methods, pasteurization and HIU at lower power, resulted in similar luminosity levels for yogurt. The 480 W and 640 W treatments, although they did not present significant differences in relation to the control, 160 W and 320 W, showed a significant difference when compared to the pasteurized treatment.

Furthermore, it is possible to notice that the heat generated by pasteurization may have influenced the values of luminosity. During the pasteurization process, considering that anthocyanins are sensitive to heat, the moderate temperature used may not have been enough to fully degrade the phenolic compounds but may have caused structural modifications that affected the color of the product, making it lighter [71,72]. After the second week of storage, none of the samples showed statistically significant differences in the luminosity values (L*), indicating that there was a stabilization of this parameter.

In addition to L*, a*, and b*, the ΔE and chroma (C*) parameters were also evaluated to better understand the visual differences between treatments during storage. The ΔE parameter quantifies the overall color difference compared to the control sample, values between 2 and 10 are considered visually perceptible at first glance. In the present study, all treated samples showed ΔE values above 2.0 at some point during the storage period, indicating noticeable chromatic changes to the human eye [73]. The sample treated at 640 W presented a ΔE value of 5.39 on D0, the highest observed, suggesting that HIU promoted immediate visual modifications. Other power levels also resulted in perceptible differences, such as the 480 W sample on D7 (ΔE = 5.10) and the 320 W sample at the same time point (ΔE = 3.22). Samples treated at 160 W and by conventional pasteurization showed intermediate variations, with ΔE ranging from 2.2 to 3.8. These results indicate that ultrasound

Table 7
Color attributes of concentrated skyr-type yogurt with added blueberry pulp (*Vaccinium myrtillus*) processed by high-intensity ultrasound (20 kHz) according to the type of processing during the storage period. D0 to D21 means the number of days that the finished yogurt was stored at 4 °C in a conventional refrigerator.

Treatments	D0	D7	D14	D21
Control	L*: 80.7 ± 0.0^{abA}	L*: 81.4 ± 0.0^{aB}	L*: 85.3 ± 0.0^{aD}	L*: 85.1 ± 0.0^{aC}
	a*: 5.7 ± 0.0^{bC}	a*: 4.1 ± 0.0^{abA}	a*: 4.3 ± 0.0^{bcB}	a*: 4.3 ± 0.0^{bcB}
	b*: 3.3 ± 0.0^{abcB}	b*: 1.2 ± 0.0^{aA}	b*: 6.3 ± 0.0^{bC}	b*: 6.4 ± 0.0^{bC}
	C*: 7.7 ± 0.0^{bC}	C*: 7.7 ± 0.0^{bC}	C*: 6.6 ± 0.0^{abB}	C*: 4.3 ± 0.0^{aA}
	ΔE: 0.0 ± 0.0	ΔE: 0.00 ± 0.0	ΔE: 0.0 ± 0.0	ΔE: 0.0 ± 0.0
Pasteurization	L*: 83.4 ± 0.6^{bA}	L*: 82.2 ± 1.5^{aA}	L*: 81.8 ± 2.5^{aA}	L*: 81.9 ± 2.5^{aA}
	a*: 5.1 ± 0.2^{abB}	a*: 3.6 ± 0.2^{cA}	a*: 4.5 ± 0.4^{cB}	a*: 4.6 ± 0.4^{abB}
	b*: 2.6 ± 0.6^{abA}	b*: 1.4 ± 0.9^{aA}	b*: 6.1 ± 0.1^{bB}	b*: 6.0 ± 0.18^{bB}
	C*: 7.6 ± 0.0^{bC}	C*: 7.6 ± 0.0^{bC}	C*: 5.8 ± 0.0^{aB}	C*: 3.9 ± 0.0^{aA}
	ΔE: 3.5 ± 0.5^{aA}	ΔE: 2.9 ± 0.5^{aA}	ΔE: 3.3 ± 0.5^{abA}	ΔE: 1.5 ± 0.5^{abA}
160 W	L*: 79.5 ± 2.0^{abA}	L*: 80.6 ± 2.5^{aA}	L*: 81.5 ± 2.1^{aA}	L*: 81.5 ± 2.0^{aA}
	a*: 5.0 ± 0.4^{abA}	a*: 4.3 ± 0.4^{bA}	a*: 4.5 ± 0.6^{cA}	a*: 4.5 ± 0.6^{cA}
	b*: 2.0 ± 1.3^{aA}	b*: 1.0 ± 0.4^{aA}	b*: 6.4 ± 0.5^{bB}	b*: 6.4 ± 0.5^{bB}
	C*: 7.8 ± 0.0^{bB}	C*: 7.8 ± 0.0^{bB}	C*: 5.5 ± 0.0^{aA}	C*: 4.4 ± 0.0^{aA}
	ΔE: 3.8 ± 0.5^{aA}	ΔE: 2.2 ± 0.5^{aA}	ΔE: 3.6 ± 0.5^{abA}	ΔE: 2.2 ± 0.5^{abA}
320 W	L*: 81.0 ± 2.2^{abA}	L*: 82.7 ± 2.7^{aA}	L*: 82.9 ± 2.8^{aA}	L*: 82.9 ± 2.8^{aA}
	a*: 4.2 ± 1.0^{aA}	a*: 3.4 ± 0.4^{aA}	a*: 3.5 ± 0.1^{abA}	a*: 3.5 ± 0.2^{abA}
	b*: 3.5 ± 2.6^{abcA}	b*: 1.6 ± 0.5^{aA}	b*: 5.1 ± 1.5^{bA}	b*: 5.0 ± 1.5^{bA}
	C*: 6.2 ± 0.2^{bA}	C*: 6.2 ± 0.2^{bA}	C*: 5.6 ± 0.2^{aA}	C*: 3.8 ± 0.2^{aB}
	ΔE: 2.9 ± 0.9^{aA}	ΔE: 3.2 ± 0.9^{aA}	ΔE: 2.8 ± 0.9^{bA}	ΔE: 2.6 ± 0.9^{abA}
480 W	L*: 76.7 ± 2.0^{aA}	L*: 81.6 ± 2.3^{aB}	L*: 82.8 ± 1.0^{aB}	L*: 82.9 ± 1.0^{aB}
	a*: 5.9 ± 0.6^{bB}	a*: 3.9 ± 0.4^{abA}	a*: 4.0 ± 0.5^{abcA}	a*: 4.1 ± 0.5^{abcA}
	b*: 6.0 ± 1.3^{bcB}	b*: 1.2 ± 0.9^{aA}	b*: 4.5 ± 1.0^{abB}	b*: 4.5 ± 1.0^{abB}
	C*: 6.1 ± 0.0^{bA}	C*: 6.1 ± 0.0^{bA}	C*: 4.4 ± 0.0^{aB}	C*: 4.1 ± 0.0^{aB}
	ΔE: 3.2 ± 0.0^{aAB}	ΔE: 5.1 ± 0.0^{aB}	ΔE: 3.0 ± 0.0^{bAB}	ΔE: 1.9 ± 0.0^{A}
640 W	L*: 77.3 ± 3.4^{aA}	L*: 81.4 ± 0.9^{aAB}	L*: 83.8 ± 0.7^{aB}	L*: 83.7 ± 0.7^{aB}
	a*: 6.3 ± 0.3^{bB}	a*: 3.6 ± 0.4^{abA}	a*: 3.2 ± 0.2^{aA}	a*: 3.2 ± 0.2^{aA}
	b*: 6.8 ± 0.9^{cC}	b*: 0.2 ± 0.1^{aA}	b*: 2.4 ± 0.4^{aB}	b*: 2.5 ± 0.4^{aB}
	C*: 4.4 ± 0.0^{bA}	C*: 4.3 ± 0.0^{bA}	C*: 4.1 ± 0.0^{aB}	C*: 3.6 ± 0.0^{aB}
	ΔE: 5.4 ± 0.0^{aB}	ΔE: 4.3 ± 0.0^{aB}	ΔE: 4.3 ± 0.0^{bB}	ΔE: 1.4 ± 0.0^{abA}

Results were expressed as mean ± standard deviation (n = 3). Different letters (a-d) in the same column indicate difference by Tukey's test ($p < 0.05$) for processing. Different letters (A-D) in the same row indicate difference by Tukey ($p < 0.05$) as a function of days. (L) luminosity, (a*) scale between red (+a) and green (−a) colors, (b*) scale between yellow (+b) and blue (−b) colors.

processing, particularly at higher power levels, significantly affects product color, and such changes are potentially noticeable to consumers.

Regarding chroma (C*), which reflects color intensity and saturation, a slight decrease was observed across all samples during storage, indicating a gradual fading of color intensity over time. This trend aligns with the findings of Ścibisz, Ziarno, and Mitek (2019) [74], who also reported a reduction in chroma values in fruit-flavored yogurts over the storage period, suggesting pigment degradation and a decrease in color saturation as common phenomena in such matrices.

During the storage period, there was a progressive increase in the luminosity of the samples, indicating that they became lighter, tending towards whiter tones. The control sample showed significant variations in luminosity, indicating that the absence of treatment impacted the stability of the white tone. In contrast, the pasteurized, 160 W and 320 W treatments did not show statistically significant differences throughout the period, suggesting greater stability in luminosity. The HIU treatments at 480 W and 640 W showed changes only at the beginning of storage but maintained stability in luminosity values.

Under specific pH conditions, anthocyanins can undergo hydrolysis and convert to chalcones, which are colorless and less stable structures. This process occurs due to the chemical reconfiguration of the flavonoid ring of anthocyanins [75]. The a* and b* values observed in this study were positive, indicating a tendency towards red and yellow yogurts, as found by Mior *et al.* [76], who evaluated sheep's milk yogurt, natural and flavored with blueberry.

Regarding the a* parameter, the control, 480 W and 640 W ultrasound treatments did not show significant differences on the D0. These treatments also did not differ from the pasteurized and 160 W treatments, which showed similar values to the 320 W treatment. Suggesting that although different processes were applied, they had a similar impact on the preservation of the red color. Thus, all compared groups maintained a very similar color intensity, reflecting the stability of the compounds responsible for the color. In addition, it was possible to observe a direct correlation between the levels of monomeric anthocyanins and the intensity of the red hue: the higher the concentration of these anthocyanins, the more pronounced the red color. This behavior was evident on the D0, which was not influenced by the storage time. At this stage, the 640 W treatment, which presented the highest content of monomeric anthocyanins, also exhibited the most intense shade of red.

Regarding changes during storage, the 160 W and 320 W treatments maintained stability in the a* value, while 480 W and 640 W maintained a tendency to differentiate at the beginning of the treatment and then reach color stability. The pasteurized treatment showed changes only in the second week, stabilizing soon after. The control, in turn, presented marked differences on all days, as well as in luminosity.

The storage period has been shown to exert a more significant influence on the stability of the red coloration compared to the type of processing, especially due to the gradual oxidation of anthocyanins. Which are highly sensitive to factors such as pH, light, temperature, and processing leading to the degradation of anthocyanins during storage, especially under oxidative conditions, resulting in noticeable changes in the color of yogurt [61].

Regarding the b* on the first day of storage, the 160 W treatment did not present significant differences in relation to the pasteurized treatment, which was also similar to the control and 320 W. These treatments did not differ from 480 W, which presented results similar to 640 W. There was a gradual differentiation in the b* values, with 160 W presenting the lowest intensity of yellow, while 640 W exhibited the highest intensity, indicating a more pronounced yellow coloration.

As storage progressed, most treatments showed an increase in b* values, indicating a tendency towards a more yellowish coloration. This behavior may also be related to anthocyanin degradation. All samples, except the one treated with 320 W, showed significant differences in the first two weeks of storage, stabilizing from the third week onwards. However, the treatment with 320 W remained stable throughout the period, suggesting better stability in the yellow coloration. These results suggest that, while the type of processing played an important role in the initial storage phase, the storage time was decisive for the stabilization of the color parameters.

3.6. Rheological analysis

The applied treatments, including pasteurization and HIU, revealed different impacts on the rheological properties of the samples, as shown in Table 8. The control sample showed a moderate viscosity (831 ± 72 mPa s), with no difference in relation to the pasteurized samples (872 ± 25 mPa s) and the 160 W treatment (819 ± 49 mPa s). This suggests that both pasteurization and HIU at low power (160 W) did not substantially modify the viscosity when compared to the control. However, as the HIU power increased, a significant increase in viscosity was observed. Table 9..

The 320 W treatment resulted in an increase in viscosity to 959 ± 79 mPa s, being significantly higher than the control and the lower power treatments (160 W and pasteurized). This increase was even more pronounced with the 480 W treatment, which reached the highest observed viscosity (1152 ± 91.0 mPa s), being statistically different from all other treatments except 320 W. This behavior is consistent with the literature, which suggests that moderate HIU powers promote changes in colloidal structures, increasing the viscosity of the product [77,78].

The viscosity of the 640 W treatment (705 ± 98 mPa s) was significantly different from that of the 480 W and 320 W treatments, suggesting that HIU may cause structural degradation, reducing viscosity. This phenomenon may be attributed to excessive particle breakage or destabilization of the colloidal matrix [77]. The value of the consistency index (k) also followed this trend, with the highest values observed for the treatments of 320 W (16.0 ± 1.0 mPa s) and 480 W (18.2 ± 0.4 mPa s), both significantly different from the others. The lowest consistency was observed at 640 W, which did not differ statistically from the control.

Rheological analyses indicated that all samples exhibited pseudoplastic behavior, characteristic of non-Newtonian fluids, as evidenced by the flow index (n) value lower than 1, results that corroborate those found by Şengül *et al.* [79], who analyzed the effect of adding blueberries to concentrated yogurt. This behavior means that the viscosity of the samples decreases with increasing shear rate, a common property in many food products such as yogurts and emulsions [78].

A correlation between syneresis, moisture content, and viscosity suggests that HIU treatments at intermediate powers (320 W and 480 W) were effective in improving water retention and maintaining viscosity. These treatments allowed efficient pectin release without compromising the integrity of the colloidal matrix, which explains the higher viscosity values. On the other hand, the 640 W treatment, despite having shown

Table 8
Rheological properties of concentrated skyr-type yogurt enriched with blueberry (*Vaccinium myrtillus*) pulp processed by high-intensity ultrasound.

Treatments	Viscosity 40 s^{-1} (mPa s)	Consistency index (mPa s)	Flow behavior index (−)	Deviation modulus (%)
Control	831.0 ± 72.0^{bc}	12.0 ± 1.0^{bc}	0.61 ± 0.01^{a}	2.0 ± 0.2
Pasteurized	872.0 ± 25.0^{bc}	14.0 ± 1.0^{b}	0.60 ± 0.01^{a}	3.0 ± 1.0
160 W	819.0 ± 49.0^{bc}	13.6 ± 0.3^{b}	0.59 ± 0.01 ^{a}	3.0 ± 1.0
320 W	959.0 ± 79.0^{ab}	16.0 ± 1.0^{a}	0.59 ± 0.01 ^{a}	2.8 ± 0.4
480 W	1152.0 ± 91.0^{a}	18.2 ± 0.4^{a}	0.61 ± 0.02 ^{a}	3.0 ± 1.0
640 W	705.0 ± 98.0^{c}	11.0 ± 1.0^{c}	0.60 ± 0.01 ^{a}	2.0 ± 1.0

*Results are expressed as mean ± standard deviation (n = 3). Different letters (a-c) in the same column indicate differences according to Tukey's test ($p < 0.05$).

Table 9
Volatile compounds identified by E-nose of concentrated skyr −type yogurt with added blueberry pulp (*Vaccinium myrtillus*) processed by high-intensity ultrasound according to the type of processing during the storage period.

Compounds	Control	Pasteurized	160 W	320 W	480 W	640 W
(−)-beta-pinene			●	●		●
(z)-4-heptenal				●		●
1-hexanol	●					
1-propanol-2-methyl	●		●		●	
2,3-pentanedione				●	●	●
2,5-dimethylpyrazine				●		●
2-heptanone	●			●	●	●
3-heptanone				●		●
3-methylbutanal	●					
acetic acid					●	●
anethole			●			
butan-2-one	●	●	●	●	●	●
butanal	●			●		●
butane-2,3-dione			●	●		●
butyl acetate			●	●	●	
decanal			●			
ethyl acetate	●	●	●	●	●	●
ethyl butyrate	●		●	●	●	●
ethyl isobutyrate		●			●	●
heptanal				●		●
hexanoic acid	●		●			●
n-nonanal	●	●	●			
octanal			●			
pentanal				●	●	●
pentanoic acid			●			
propanal			●		●	

good results in terms of water retention (lower syneresis and high moisture content), had a lower performance in terms of viscosity. This suggests that while intermediate powers maintained the balance between pectin release and colloidal network integrity, the highest power exceeded this limit, resulting in degradation of the colloidal structure and a drop in viscosity, even with higher water retention.

3.7. Electronic nose (e-nose) analysis

When analyzing the sample compounds, it is possible to notice that the volatile compounds 1-hexanol, anethole, and decanal, present in the control sample, were completely lost in all treatments applied. These compounds have chemical structures that make them prone to volatilization or degradation under certain temperature and processing conditions. Safwa *et al.* [80] demonstrated that some systems, such as ultrasound, microwave, and high pressure, better preserved the aromatic compounds, and, in contrast, pasteurization inactivated essential enzymes and reduced the release of volatile compounds, causing significant losses in the aromatic quality of the products.

These results corroborate those found in this study, where among the treatments analyzed, pasteurization was the technique that presented the lowest amount of volatile compounds. The control sample presented 11 compounds, of which 8 were lost after pasteurization. The only volatile compound formed after this treatment was ethyl isobutyrate, an ester associated with sweet, fruity aromas [81].

HIU, in addition to inducing the formation of several compounds associated with fresh, fruity, sweet, and citrus aromas, such as beta-pinene, (Z)-4-heptenal, 2,5-dimethylpyrazine, butyl acetate, octanal and pentanal. It also promoted the formation of compounds characteristic of dairy and fermented products, such as 2,3-pentanedione, 3-heptanone, butane-2,3-dione, and 3-methylbutanal. However, at higher power levels, HIU also led to the formation of acetic acid, the presence of which is associated with a more acidic and vinegar aroma, potentially undesirable depending on the sensory context of the product [79].

Zhu *et al.* [82] obtained divergent results from this study when analyzing the treatment of apple juice with pasteurization and HIU. They observed that neither pasteurization nor high-power HIU resulted in significant differences, but both treatments differed from untreated juice. It can be explained by the variation in the processing methodologies used. In the study by Zhu, HIU was applied directly to apple juice at 975 W for 12 min. In contrast, in the present study, HIU was applied to blueberry pulp before its incorporation into yogurt, using a shorter treatment time (3 min) and lower power levels (160–640 W). Furthermore, the food matrices involved are fundamentally different, which also directly influences the release and formation of volatile compounds during storage. Zheng *et al.* [81] observed that HIU when applied at the appropriate power and time, can significantly improve volatile compounds, corroborating the ability of this technology to modulate volatile profiles depending on the treatment conditions.

Fig. 1 presents the PCA (Principal Component Analysis) plot generated from the e-nose analysis, illustrating the distribution of samples treated with different methods. The axes (PC1 and PC2) represent the two principal components that together explain most of the variation in the data.

The samples treated by pasteurization and HIU at 480 W showed similar volatile profiles, as evidenced by the proximity of their points on the graph. This suggests that, although pasteurization is a thermal process and HIU is non-thermal, both caused comparable modifications in the volatile compounds present in the yogurt. It is possible that the HIU power at 480 W was sufficient to cause structural and chemical changes that mimic the impact of heating in pasteurization. Furthermore, the control and 160 W ultrasound treatments also showed significant proximity on the graph, indicating that HIU at low power did not significantly alter the volatile profile of the yogurt compared to the control.

The samples treated with HIU at 320 W and 640 W were further away from the other treatments, with emphasis on the 640 W treatment, which was positioned alone in the graph. This indicates that HIU at higher powers had a much greater impact on the volatile profile of the samples. High HIU power, especially at 640 W, may have caused greater degradation or transformation of the volatile compounds due to the intense cavitation generated during processing.

4. Conclusions

The developed product maintained desirable nutritional

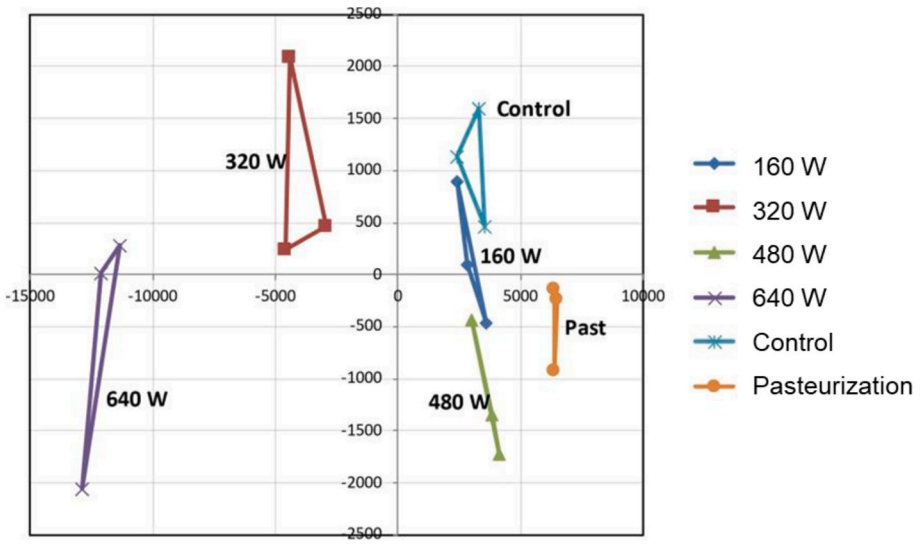

Fig. 1. The PCA (Principal Component Analysis) plot generated from the e-nose analysis, illustrating the distribution of samples treated with different methods.

characteristics, exhibiting a high-protein content and low-fat content, aligning with consumer expectations for healthy and functional foods. Additionally, the addition of blueberry pulp led to an average increase of 129 % in antioxidant capacity and 15.5 % in total phenolics, highlighting the effectiveness of the pulp in enhancing the functional profile of yogurt, making it even more appealing to consumers. The use of ultrasound positively impacted the blueberry pulp, with the highest power level standing out for its retention of monomeric anthocyanins, which are essential for yogurt functionality. Intermediate ultrasound power levels (320 W and 480 W) were effective in preserving color and enhancing volatile compounds associated with fresh and fruity aromas, whereas pasteurization resulted in greater degradation of volatile compounds. Furthermore, the use of HIU also brought benefits to the texture and stability of the yogurt, reducing syneresis and increasing water retention.

Finally, although HIU has proven to be a promising alternative to pasteurization, conducting microbiological analyses and sensory tests with consumers are important to confirm product acceptance. In addition to the technological improvements achieved, the development of this product contributes to more sustainable and health-focused food systems. Future research should explore the combination of ultrasound with other technologies, as well as assess different intensities within the optimal power range, to enhance the observed benefits, broaden the industrial applicability of this approach, and identify the most suitable conditions to maximize both technological functionality and.

CRediT authorship contribution statement

Júlia B. Sousa: Validation, Methodology, Investigation, Formal analysis, Data curation. **Carolina P.C. Martins:** Methodology, Investigation, Formal analysis. **Elson Rogério T. Filho:** Validation, Formal analysis. **Jonas T. Guimarães:** Validation, Investigation, Formal analysis, Conceptualization. **Mônica M. Pagani:** Investigation, Formal analysis, Data curation. **Eliane T. Mársico:** Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Mônica Q. Freitas:** Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Vinicius P. Souza:** Validation. **Erico M.M. Flores:** Validation. **Adriano G. Cruz:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Erick A. Esmerino:** Writing – original draft, Supervision, Project administration, Investigation, Conceptualization.

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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