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Impact of ultrasound-assisted fermentation on buffalo yogurt production: Effect on fermentation kinetic and on physicochemical, rheological, and structural characteristics

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A B S T R A C T

This work evaluated the buffalo milk acidification kinetics using partially or fully ultrasound-assisted fermentation and yogurt characteristics without or with sugar (5%). The results showed that assisted fermentation in an ultrasonic bath (25 kHz, 38 W/L) accelerates the fermentation rate (up to 41% increase), reducing the fermentation time (up to 2 h), and, in some conditions, improved the yogurt quality. Samples produced by US-assisted fermentation under 1 or 2 h had higher water holding capacity (up to 35%), consistency index (up to 423%), and apparent viscosity (up to 246%), explained by the formation of a stronger gel, as elucidated by microscopy images. In contrast, no differences in pH, acidity, and lactic acid bacteria viability were observed. Additionally, sucrose contributed positively to the yogurt quality, improving gel formation. Therefore, buffalo milk fermentation under US (up to 2 h) is an interesting strategy that accelerates fermentation and improves the buffalo yogurt quality.

1. Introduction

Buffalo milk has an interesting nutritional profile and technical functional properties to produce fermented milk, due to its high fat, protein, colloidal calcium, and total solids content (Murtaza et al., 2017; Arora & Khetra, 2022). Among fermented milks, yogurt is highlighted by consumers as a natural food, with good nutritional and sensory quality (García-Burgos et al., 2020). In parallel, the production of dairy products from hypoallergenic sources, such as yogurt made from buffalo milk, is a good commercial strategy (Abesinghe et al., 2022).

Although yogurt production involves a simple and established technology (fermentation), it requires long exposure times to relatively high temperatures (42–45 °C) for the growth of lactic acid bacteria (Abesinghe et al., 2022), resulting in considerable energy consumption and, sometimes, limiting the use of yogurt fermentation tank twice in a day, which directly affects the industry productivity. In addition, yogurt often has technological problems such as very low pH, high acidity, high syneresis, and low water retention capacity, which negatively affects the structure and acceptability of the product during its shelf life (Abesinghe et al., 2019).

Ultrasound (US) is a physical process based on the propagation of mechanical waves with a frequency higher than the human audible limit (> 20 kHz) and has advantages, such as ease of use, low cost and eco friendliness (Huang et al., 2017). The main US mechanism is the acoustic cavitation process, characterized by the formation, expansion, and implosion of microbubbles in the medium, generating physical and chemical changes in the food matrix compounds (Xu et al., 2021). It can reduce fat globule size (Capela et al., 2022) and cause partial protein denaturation (Shokri et al., 2022), as well as potentiate the reactions due to higher rates of heat and mass transfer (Magalhães et al., 2022), and the reduction of the diffusion limit barrier between compounds (Wang et al., 2018).

Previous results show that US applied in the pretreatment of milk or during assisted fermentation can increase the nutrient's availability and activate microbial enzymes, facilitating the lactic acid bacteria growth and, consequently, improving the acidification process and yogurt quality (Ojha et al., 2017; Abesinghe et al., 2019, 2022; Akdeniz & Akalın, 2023). However, most of these studies were performed using

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probe ultrasound (Riener et al., 2009; Abesinghe et al., 2019; Delgado et al., 2020, 2022; Akdeniz & Akalın, 2023; Ragab et al., 2023), which exhibit limitations in terms of scalability and durability due to the high wear degree of the probe tip (Soares et al., 2019). Furthermore, US probe system application at the industrial level (scale up) is challenging, since the intensity decreases exponentially with moving away from the probe (Gogate & Kabadi, 2009), which reduces the uniformity of the process and limits its relevance for this purpose. Alternatively, the use of bath ultrasound can be a new strategy to carry out assisted fermentation of buffalo milk yogurt with better cost, scalability, and quality. However, to the best of our knowledge, the US-assisted fermentation using bath ultrasound for buffalo yogurt production has not been studied yet. Therefore, considering the expected benefits, this study evaluated the impact of US-assisted fermentation using bath US at different time intervals on the fermentation kinetics and quality attributes of yogurt produced with buffalo milk with or without sugar during the shelf life, aiming to overcome the previously mentioned limitations.

2. Material and methods

2.1. Buffalo milk and lactic culture

Buffalo milk (5.36% fat, 5.18% lactose, 3.73% protein, 0.75% minerals, and 15.72% total dry extract) was purchased from farmers in Juiz de Fora, MG, Brazil and preserved at 1 °C until processing (48 h). The lyophilized yogurt culture (containing *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, Y472e), was donated by Sacco (Brazil, Campinas, Brazil) and kept at −18 °C.

2.2. Ultrasound-assisted fermentation of buffalo yogurt

Raw buffalo milk was filtered, sucrose added (0 and 5% w/v) and pasteurized at 90 °C/5 min. Afterwards, the samples were cooled to 42 °C and the yogurt culture was added at a concentration of 10^6 CFU/mL of milk.

Subsequently, fermentation was carried out in a thermostatic bath (control process) or in an ultrasonic bath (Unique, model USC 2800 A, Brazil) with temperature control, volumetric capacity of 9.5 L, dimensions of 300×240×150 mm and equipped with five transducers arranged below the tank, at a frequency of 25 kHz, nominal power of 450 W, and volumetric power of 38 W/L, measured according to the calorimetric method (O'Donnell et al., 2010).

US-assisted fermentation was performed at different times: (i) 1 h under US, (ii) 2 h under US, (iii) 3 h under US, and (iv) full time under US. After reaching the determined fermentation time in the ultrasonic bath, fermentation was completed in a thermostatic bath until reaching a pH value of 4.6. The pH monitoring was performed with measurements every 30 min until the end of fermentation. The pH data were modeled using the modified Gompertz equation (Eq. (1)) adapted by De Brabandere and Baerdemaeker (1999) to obtain the time of the latency phase (lag phase - λ(h)) and the maximum pH decrease rate (μ (h^{-1})).

$$pH = pH_0 + (pH_\infty - pH_0)exp\left\{-exp\left[\frac{\mu e}{(pH_0 - pH_\infty)}(\lambda - t) + 1\right]\right\} \quad (1)$$

Where pH_0 = initial pH, pH_∞ = final pH, μ = maximum pH reduction rate (h^{-1}), λ = lag phase time (h), and t = is the time (h).

After fermentation, the products were cooled to 7 °C for 24 h and stirred yogurts were obtained by stirring them with a metal spatula (30 times clockwise; 30 times anticlockwise) (Trisbt et al., 2020). For the characterization of the products, the analyzes were carried out with 1 and 21 days of storage at 7 °C.

2.3. Buffalo yogurt characterization

2.3.1. pH, acidity, lactic acid bacteria (LAB) viability and water holding capacity (WHC)

The pH and titratable acidity (% lactic acid) were performed according to AOAC (1999). The viability of lactic acid bacteria was determined according to IDF (2003). For WHC, 15 g of sample was centrifuged at 3000 *g* for 10 min at 5 °C (Hanil Scientific, model combi 514R, Gimpo, Rep. of Korea). The supernatant was carefully drained, and the tubes were weighed. The water holding capacity was defined as the ratio between the weight of the pellet remaining after centrifugation and the initial weight of the yogurt, expressed as a percentage (Ercili-Cura et al., 2013).

2.3.2. Rheological properties

The rheological analyzes were performed using a rotational concentric cylinder rheometer (Brookfield, model R/S plus SST 2000, with an interface coupled to a microcomputer connected to the RHEO-CALC V1.1 software) according to the procedures described by Paula et al. (2018). The flow curves were performed using a CC45 sensor with a shear rate ranging from 0 to 300 s^1, with four flow ramps (ascent, descent, ascent, and descent) for 3 min each and with measurements every 4 s to eliminate the effect of thixotropy. During the experiments, the yogurts were kept at 7 °C±0.1. Data were fitted to the Ostwald-de-Waele model (Power Law, Eq. (2)) using Curve Expert Professional 2.2.0 software). *K* and *n* values were used to calculate the apparent viscosity (η_{app}, Pa·s) of yogurts at shear rates of 10, 50, and 100 s^{-1}.

$$\sigma = K \cdot \dot{\gamma}^n \quad (2)$$

Where σ is the stress (Pa), *K* is the consistency index (Pa·s^n), γ is the strain rate (s^{-1}), and *n* is the flow behavior index (dimensionless).

2.3.3. Optical microscopy

The microstructural analysis was performed using a binocular optical microscope (Anatomic Opton®, Model TIM-18, Campinas, São Paulo, Brazil) with a 20 W lamp and an 8-megapixel portable camera. A drop of each stirred sample was placed on a microscope slide and images were obtained with an objective lens at 10x magnification. For each sample, at least ten images were captured to ensure a representative assessment.

2.4. Experimental design and statistical analysis

The processes and experiments were performed in two repetitions and each experimental unit was carried out in triplicate. Results were expressed as mean ± standard deviation. The data were analyzed using one-way ANOVA followed by post-hoc Tukey test to compare the effects of different treatments at 95% of confidence level using the Statistica software, version 9.2 (StatiSoft INC, USA). Furthermore, Pearson correlation coefficient and principal component analysis (PCA) was employed to analyze the correlation between the data acquired during the yogurt fermentation process, rheological properties, and physicochemical characterization of the yogurts. The analyses were conducted using XLSTAT software, version 2015.2.02 (Addinsoft, Paris, France).

3. Results

Buffalo milk started fermentation at pH 6.6–6.7, with slightly lower values (1%, $p<0.05$) in those with added sugar (Table 1). The overall evaluation of the fermentation curves (Fig. 1) showed that ultrasound-assisted fermentation positively affected the pH drop in formulations independent of sugar addition, reducing the process in 1 h or more. These data were modeled according to the modified Gompertz equation to obtain the parameters of the lag phase (λ, h) and the maximum rate of pH decline (μ, h^{-1}), which were used to determine the time to reach pH

Table 1
Parameters of modified Gompertz Eq. (1) adapted for the ultrasound-assisted fermentation curves of buffalo milk.

Sample	pH_0	λ (h)	μ (h^{-1})	R^2	Time to reach pH 5 (h)	Time from pH 5 to pH_∞ (h)
Control	6.77 ± 0.02^{Aa}	1.08 ± 0.07^{Bb}	−0.45 ± 0.02^{Bb}	0.995	5.7 ± 0.1^{Aa}	2.3 ± 0.1^{Ba}
US-1h	6.77 ± 0.02^{Aa}	1.00 ± 0.04^{Bb}	−0.50 ± 0.06^{Bab}	0.995	5.2 ± 0.4^{Ab}	1.8 ± 0.4^{Ab}
US-2h	6.76 ± 0.01^{Aa}	1.04 ± 0.07^{Bb}	−0.51 ± 0.06^{Bab}	0.995	5.1 ± 0.3^{Ab}	1.9 ± 0.3^{Aab}
US-3h	6.76 ± 0.02^{Aa}	1.15 ± 0.20^{Bab}	−0.52 ± 0.08^{Bab}	0.996	5.2 ± 0.4^{Ab}	1.8 ± 0.4^{Bb}
US-Full	6.77 ± 0.02^{Aa}	1.27 ± 0.07^{Ba}	−0.52 ± 0.04^{Ba}	0.996	5.2 ± 0.2^{Ab}	1.8 ± 0.2^{Ab}
Control + Sugar (5%)	6.69 ± 0.01^{Ba}	2.02 ± 0.06^{Aa}	−0.64 ± 0.05^{Ac}	0.995	5.1 ± 0.2^{Ba}	2.9 ± 0.2^{Aa}
US-1 *h* + Sugar (5%)	6.70 ± 0.01^{Ba}	2.03 ± 0.07^{Aa}	−0.77 ± 0.05^{Ab}	0.996	4.6 ± 0.1^{Bb}	2.0 ± 0.1^{Ab}
US-2 *h* + Sugar (5%)	6.69 ± 0.02^{Ba}	2.00 ± 0.05^{Aa}	−0.83 ± 0.03^{Aab}	0.998	4.4 ± 0.0^{Bb}	2.1 ± 0.0^{Ab}
US-3 *h* + Sugar (5%)	6.69 ± 0.01^{Ba}	1.88 ± 0.06^{Aa}	−0.86 ± 0.05^{Aab}	0.997	4.2 ± 0.1^{Bbc}	2.3 ± 0.1^{Ab}
US-Full + Sugar (5%)	6.70 ± 0.01^{Ba}	1.87± 0.03^{Aa}	−0.90 ± 0.02^{Aa}	0.998	4.1 ± 0.0^{Bc}	1.9 ± 0.0^{Ab}

pH_0: initial pH; λ: lag phase time (h); μ: maximum pH decline rate (h^{-1}); pH_∞: final pH. Significant differences evaluated by the Tukey test ($p<0.05$) among the samples with same sugar content or samples with the same US time are indicated by different superscript lowercase and uppercase letters, respectively.

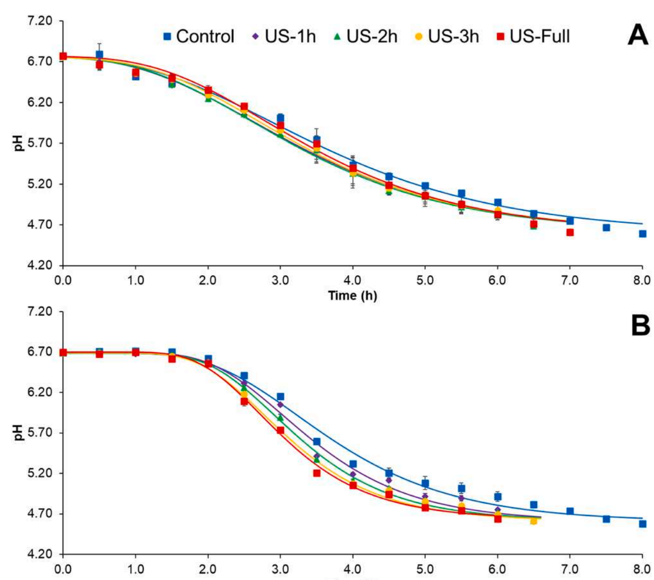

Fig. 1. pH decline during ultrasound-assisted fermentation of yogurt produced from buffalo milk (A) without sugar and (B) with sugar (5%). Dots are experimental data; continuous lines are the predicted data using modified Gompertz Eq. (1).

5.0 and 4.6 (Table 1).

Regarding the λ parameter, which represents the lag phase of microbial growth, the addition of 5% sugar increased this time by up to 100%; whereas, for most of the evaluated conditions, no effect of US-assisted fermentation was verified in the lag phase ($p>0.05$). For the maximum pH decline rate (μ parameter), it was found that the samples with 5% sugar addition, as well as the US-fermented samples, had higher values ($p<0.05$), directly impacting the fermentation time to reach pH 5.0 and 4.6. The highest μ values (101% increase, $p<0.05$) were observed when sugar addition and longer US time were associated, leading to a reduction of 2 h in the total fermentation time ($p<0.05$).

Table 2 shows the values of pH, acidity, and lactic acid bacteria (LAB) count of the yogurts produced with buffalo milk after 1 and 21 days of storage at 7 °C. No difference was observed for pH and acidity among the samples on day 1 ($p>0.05$) (Table 2). After 21 days of storage, a similar behavior was verified; however, post-acidification (increase of up to 0.55% of lactic acid) was observed for all samples during storage, with a pH reduction (up to 0.34) between days 1 and 21 ($p<0.05$). Regarding the LAB count, no difference was verified between the samples, independent on the process of fermentation, sugar content, and storage time ($p>0.05$).

The water holding capacity (WHC) of yogurt samples after 1 (Fig. 2A) and 21 (Fig. 2B) days of storage showed similar behavior. In general, sugar positively affected the WHC, especially for the control sample ($p<0.05$). Regarding the impact of US-assisted fermentation, it was found that samples fermented under US for 1 hour (increase of up to 31%) and 2 h (increase of up to 35%) were the samples with higher values of WHC, regardless of sugar concentration and time evaluated ($p<0.05$). On the other hand, US-assisted fermentation for longer periods (3 h or full fermentation under US) resulted in WHC similar worse than the control (Figs. 2A and B).

Fig. 3 shows the relationship between shear stress (σ, Pa) and shear rate (γ, s^{-1}) of buffalo yogurt samples after 1 and 21 days of storage. All curves showed a pseudoplastic behavior (Rao, 2007), with a non-linear increase in shear stress with the increase in the shear rate and with a decrease in apparent viscosity caused by shear rate increase; which is traditionally observed in this kind of samples due to protein network breakage and chain alignment in the direction of the shear field (Rao, 2007). These experimental data were well fitted to the Ostwald-de-Waele model ($R^2 > 0.99$), and the rheological parameters obtained from the model (consistency index (K) and flow behavior index (n)) for each sample are shown in Table 3. All samples had a consistency index (K) above zero and n below zero, confirming a pseudoplastic behavior during flow (Steffe, 1996). Despite this, samples fermented under US for 1 hour (1h-US) and 2 h (2h-US) showed high values for the K parameter (up to 423%) and for apparent viscosity (η_{app} - up to 246%) compared to control samples, independent of time and sugar addition ($p<0.05$) (Table 3). On the other hand, samples fermented under US for 3 h or more had K and η_{app} similar to/lower than their respective controls. In addition, it was observed that the sweetened samples had greater consistency compared to the unsweetened ($p<0.05$) and that storage caused a reduction in the consistency index and apparent viscosity ($p<0.05$) for most products, being more intense in control samples than in those fermented under sonication for 1 or 2 h ($p<0.05$).

Fig. 4 shows the micrographs of the yogurt samples using optical microscopy with 10x magnification. These micrographs help to explain the difference in the protein network formed in the samples of buffalo yogurt produced by US-assisted fermentation after 1 and 21 days of storage. Based on the images, it was verified that the presence of sucrose (Fig. 4F-J and P-T) contributed to a denser network. Regarding the effect of US-assisted fermentation, it was observed that yogurt samples fermented under US for 1 hour (Fig. 4B, G, L, and Q) and 2 h (Fig. 4C, H, M, and R) showed a greater aggregation of the protein network, with a denser and more compact network compared to their respective control samples (Fig. 4A, F, K, and P), regardless of the time and sugar addition. On the other hand, for the samples produced under US for 3 h or more, the formed network was less compact and showed whey area.

Table S1 (Supplementary material - Table S1) shows the Pearson correlation matrix representing the relationship between the quality parameters of buffalo yogurt produced through ultrasound-assisted fermentation after 1 and 21 days of storage. The outcomes of this

Table 2
pH, acidity, and lactic acid bacteria count of the stirred yogurt with or without sugar produced by ultrasound-assisted fermentation after 1 and 21 days of storage at 7ºC.

Sample		pH		Acidity (Expressed in% lactic acid)		Lactic acid bacteria count (log CFU/mL)	
		1 day	21 day	1 day	21 day	1 day	21 day
Control	without sugar	4.57 ± 0.03^{Aa}	4.23 ± 0.03^{Ba}	0.93 ± 0.04^{Ba}	1.36 ± 0.12^{Aa}	8.32 ± 0.35^{Aa}	7.93 ± 0.10^{Aa}
US-1h		4.55 ± 0.05^{Aa}	4.21 ± 0.04^{Ba}	0.94 ± 0.03^{Ba}	1.42 ± 0.04^{Aa}	8.53 ± 0.40^{Aa}	7.90 ± 0.15^{Aa}
US-2h		4.54 ± 0.04^{Aa}	4.25 ± 0.03^{Ba}	0.93 ± 0.04^{Ba}	1.41 ± 0.02^{Aa}	8.58 ± 0.38^{Aa}	8.01 ± 0.26^{Aa}
US-3h		4.55 ± 0.04^{Aa}	4.24 ± 0.02^{Ba}	0.95 ± 0.05^{Ba}	1.40 ± 0.04^{Aa}	8.46 ± 0.33^{Aa}	7.86 ± 0.20^{Aa}
US-Full		4.52 ± 0.03^{Aa}	4.22 ± 0.05^{Ba}	0.96 ± 0.06^{Ba}	1.39 ± 0.14^{Aa}	8.47 ± 0.35^{Aa}	7.95 ± 0.16^{Aa}
Control	with sugar (5%)	4.55 ± 0.04^{Aa}	4.25 ± 0.05^{Ba}	0.94 ± 0.01^{Ba}	1.39 ± 0.06^{Aa}	8.40 ± 0.31^{Aa}	8.06 ± 0.23^{Aa}
US-1h		4.53 ± 0.02^{Aa}	4.24 ± 0.05^{Ba}	0.93 ± 0.03^{Ba}	1.48 ± 0.05^{Aa}	8.49 ± 0.35^{Aa}	7.95 ± 0.20^{Aa}
US-2h		4.54 ± 0.03^{Aa}	4.27 ± 0.03^{Ba}	0.98 ± 0.04^{Ba}	1.40 ± 0.04^{Aa}	8.50 ± 0.39^{Aa}	8.09 ± 0.30^{Aa}
US-3h		4.51 ± 0.03^{Aa}	4.24 ± 0.03^{Ba}	0.94 ± 0.04^{Ba}	1.42 ± 0.04^{Aa}	8.56 ± 0.34^{Aa}	7.99 ± 0.19^{Aa}
US-Full		4.50 ± 0.02^{Aa}	4.28 ± 0.02^{Ba}	0.92 ± 0.05^{Ba}	1.35 ± 0.11^{Aa}	8.51 ± 0.38^{Aa}	8.03 ± 0.18^{Aa}

Different uppercase letters in the same line or lowercase letters in the same column for the samples differ according to Tukey's test ($p < 0.05$).

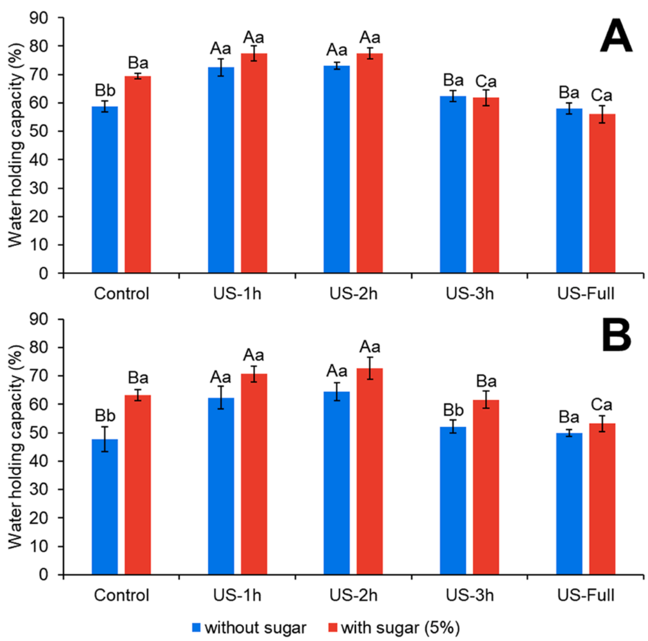

Fig. 2. Water holding capacity (%) of the stirred yogurt with or without sugar produced by ultrasound-assisted fermentation after 1 (A) and 21 (B) days of storage. Different capital letters mean significant differences between samples for the same sugar concentration. Different lowercase letters indicate a significant difference between the same samples with different sugar concentrations according to Tukey's test ($p < 0.05$).

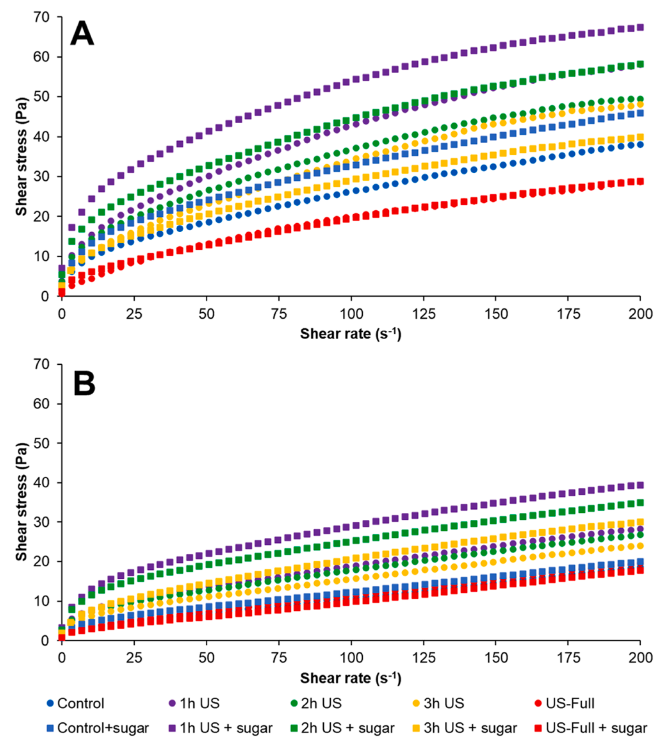

Fig. 3. Shear stress (σ, Pa) versus shear rate (γ, S^{-1}) at 7 °C of stirred yogurt with or without sugar produced by ultrasound-assisted fermentation after 1 (A) and 21 (B) days of storage.

correlation reveal a suitable correlation among the fermentation kinetic parameters (positive correlation was determined between λ parameter and μ values: $r = 0.870$, $p<0.05$), and similarly, a robust correlation was observed between the WHC values and the rheological parameters of the samples (K parameter after 1 and 21 days of storage: $r = 0.888$ and 0.836, respectively and η_{app} after 1 and 21 days of storage: $r = 0.921$ and 0.837, respectively $p<0.05$ - Table S1). Moreover, Figure S1 (Supplementary material - Fig. S1) presents the scree plot with the eigenvalue and cumulative variability (%) of Principal Components (PCs) with the elbow occurring at PC2 of the samples. These findings confirm the appropriate correlation among the parameters for the characterization of the different samples. The PCA, able to explain 91.97% of the total variation among the samples, was used to summarize the impacts of US on the parameters evaluated in this work (Fig. 5). The results of pH, acidity and LAB viability were not inserted, because there were no differences between the samples for these parameters at each evaluated time. Results of PCA clearly showed that the characteristics of samples produced by US-assisted fermentation for 1 and 2 h were similar (higher WHC, K, and η_{app}), whereas the control and samples fermented in US for times longer than 3 h had similar characteristics with respect to structure, i.e. lower WHC and consistency (parameters with the main component on the abscissa axis) but had different behavior regarding pH decline during fermentation (parameters with the main component on the ordinate axis), in which the longer US time potentiated the pH decline rate. Furthermore, sugar addition increased WHC, K, η_{app}, and leaded a fast pH decline.

4. Discussion

Yogurt is a dairy product with high commercial interest (Salama & Bhattacharya, 2022). Its production, although involving a well-established, simple, and relatively cheap technology (Tamime & Robinson, 2007), requires long exposure to mild temperatures (42

Table 3
Rheological parameters obtained by the Power Law (Ostwald-de-Waele) model and apparent viscosity (η_{app}, Pa·s) of stirred yogurt with or without sugar produced by ultrasound-assisted fermentation after 1 and 21 days of storage.

Sample	Ostwald–de-Waele Model			Apparent viscosity (η_{app} - Pa·s)		
	K (Pa·s^n)	*n*	R^2	$\gamma = 10$ s^{-1}	$\gamma = 50$ s^{-1}	$\gamma = 100$ s^{-1}
			1 day			
Control	$2.71 \pm 0.51^{d*}$	$0.50 \pm 0.02^{abc*}$	0.998	$0.85 \pm 0.13^{d*}$	$0.38 \pm 0.04^{e*}$	$0.27 \pm 0.03^{d*}$
US - 1h	$4.91 \pm 1.20^{c*}$	0.47 ± 0.04^{bc}	0.998	$1.44 \pm 0.22^{c*}$	$0.61 \pm 0.05^{bc*}$	$0.43 \pm 0.02^{b*}$
US - 2h	$4.68 \pm 1.23^{c*}$	0.45 ± 0.03^{c}	0.998	$1.31 \pm 0.25^{c*}$	$0.54 \pm 0.07^{cd*}$	$0.37 \pm 0.04^{bc*}$
US - 3h	2.98 ± 1.16^{d}	0.54 ± 0.06^{ab}	0.998	$1.00 \pm 0.25^{cd*}$	$0.47 \pm 0.07^{de*}$	$0.34 \pm 0.04^{cd*}$
US - Full	$1.40 \pm 0.12^{e*}$	$0.57 \pm 0.03^{a*}$	0.999	$0.52 \pm 0.04^{e*}$	$0.26 \pm 0.02^{f*}$	$0.20 \pm 0.02^{e*}$
Control + Sugar	$4.31 \pm 1.17^{c*}$	$0.45 \pm 0.03^{c*}$	0.998	$1.20 \pm 0.23^{c*}$	$0.49 \pm 0.06^{cd*}$	$0.33 \pm 0.04^{cd*}$
US - 1 *h* + Sugar	$10.43 \pm 1.04^{a*}$	0.36 ± 0.01^{d}	0.998	$2.36 \pm 0.18^{a*}$	$0.84 \pm 0.05^{a*}$	$0.54 \pm 0.03^{a*}$
US - 2 *h* + Sugar	$7.00 \pm 0.82^{b*}$	0.40 ± 0.02^{d}	0.997	$1.76 \pm 0.14^{b*}$	$0.67 \pm 0.04^{b*}$	$0.44 \pm 0.02^{b*}$
US - 3 *h* + Sugar	3.41 ± 1.62^{cd}	0.48 ± 0.06^{abc}	0.999	0.99 ± 0.36^{cd}	0.42 ± 0.11^{de}	0.29 ± 0.06^{cd}
US - Full + Sugar	$1.49 \pm 0.53^{e*}$	0.59 ± 0.09^{a}	0.998	$0.53 \pm 0.12^{e*}$	$0.26 \pm 0.04^{f*}$	$0.20 \pm 0.03^{e*}$
			21 days			
Control	0.51 ± 0.14^{d}	0.67 ± 0.05^{a}	0.994	0.24 ± 0.05^{cd}	0.14 ± 0.02^{ef}	0.11 ± 0.01^{de}
US - 1h	2.01 ± 1.04^{bc}	0.51 ± 0.08^{b}	0.995	0.62 ± 0.18^{b}	0.28 ± 0.07^{bc}	0.19 ± 0.04^{bc}
US - 2h	1.91 ± 1.16^{bc}	0.52 ± 0.09^{b}	0.995	0.59 ± 0.26^{bc}	0.26 ± 0.06^{bc}	0.18 ± 0.05^{bcd}
US - 3h	1.48 ± 0.71^{bc}	0.54 ± 0.08^{b}	0.994	0.48 ± 0.16^{bc}	0.22 ± 0.05^{cd}	0.16 ± 0.03^{cd}
US - Full	0.54 ± 0.11^{d}	0.67 ± 0.05^{a}	0.995	0.25 ± 0.04^{cd}	0.15 ± 0.02^{def}	0.12 ± 0.01^{de}
Control + Sugar	0.89 ± 0.09^{c}	0.58 ± 0.03^{b}	0.992	0.34 ± 0.07^{c}	0.17 ± 0.02^{de}	0.13 ± 0.01^{de}
US - 1 *h* + Sugar	4.67 ± 1.36^{a}	0.41 ± 0.04^{c}	0.998	1.17 ± 0.25^{a}	0.45 ± 0.07^{a}	0.29 ± 0.04^{a}
US - 2 *h* + Sugar	4.00 ± 1.31^{a}	0.41 ± 0.04^{c}	0.996	1.01 ± 0.25^{a}	0.39 ± 0.07^{ab}	0.26 ± 0.04^{ab}
US - 3 *h* + Sugar	2.04 ± 0.28^{b}	0.51 ± 0.04^{b}	0.997	0.65 ± 0.13^{b}	0.29 ± 0.03^{bc}	0.21 ± 0.02^{bc}
US - Full + Sugar	0.31 ± 0.17^{d}	0.76 ± 0.06^{a}	0.994	0.18 ± 0.04^{d}	0.12 ± 0.02^{f}	0.10 ± 0.01^{e}

Different lowercase letters in the column at the same time indicate a significant difference among samples according to Tukey's test ($p < 0.05$). *: indicates statistical difference by Tukey's test ($p<0.05$) for the same sample between different times (1 and 21 days).

−45ºC) for LAB growth, which results in considerable energy consumption (Abesinghe et al., 2022) and limits the number of batches processed daily. In addition, although yogurt produced from buffalo and sheep milk has a higher consistency compared to yogurt produced with milk from other species (cow, goat, and camel) (Terzioğlu et al., 2023), this product commonly shows technological problems during storage, such as post-acidification and syneresis due to protein network contraction (Gawai, Mudgal & Prajapati, 2017).

Ultrasound applied before (on milk) or during fermentation has been described as able to reduce the fermentation time and alter the microstructure of yogurt produced with milk from cow (Akdeniz & Akalın, 2023), buffalo (Abesinghe et al., 2022) and goat (Delgado et al., 2020). However, in most published works, this impact was determined using probe ultrasound (Delgado et al., 2020; Abesinghe et al., 2022; Akdeniz & Akalın, 2023; Ragab et al., 2023) instead of batch ultrasound. Although the probe models are able to reach higher power than bath ultrasound, it has lower scalability and durability (Soares et al., 2019) that limits the technology application for commercial proposes. Thus, obtaining data from bath US is mandatory for scaling this technology and allows the performance comparison between the two ultrasonic systems (Jambrak et al., 2014). Moreover, the small amount of data available covering non-bovine milk processed by emerging technologies needs to be overcome in order to develop alternatives to process different milk sources considering their particularities (Deshwal et al., 2021a).

With respect to the fermentation, US affected mainly the maximum pH decline rate (μ), while sugar addition also affected the lag phase (λ) of microbial growth (Table 1). This means that yogurt culture had difficulty to adapt in sweetened buffalo milk, possibly due to the increase in milk osmotic pressure caused by the presence of sucrose (Sodini et al., 2004) while the disturbance caused by US (Akdeniz & Akalın, 2023) had only minor effects on yogurt microorganisms' adaptation. On the other hand, during the maximum pH decline rate, which occurs between pH 5.7 and 5.0 as consequence of the symbiotic growth of cultures of *S. salivarius* subsp. *thermophilus* and L. *delbrueckii* subsp. *bulgaricus* (De Brabandere & Baerdemaeker, 1999), mainly sugar but also US had a positive impact.

Results from samples added with sucrose suggested that the presence of this disaccharide, which increases the availability of fermentative substrate, has favored the metabolic activity of lactic acid bacteria (Gomes et al., 2022) after their adaptation to a higher osmotic pressure environment. Complimentarily, the longer US exposure time during fermentation contributed to an increase in the acidification rate (μ), which, consequently, resulted in a shorter time to reach pH 5.0 and the final pH (4.6). This result demonstrates that ultrasound can enhance the rate of microbial fermentation due to the structural modification of substrates by sonication (Capela et al., 2022; Shokri et al., 2022), facilitating the access of microbial enzymes, as well as by the acceleration of mass and energy transfer (Wang et al., 2018) during US-assisted fermentation that probably improved biochemical and enzymatic reactions during fermentation (Akdeniz & Akalın, 2023). According to the literature, fermentation under sonication can improve the membrane permeability of bacteria, allowing the release of intracellular enzymes, such as β-galactosidase (Abesinghe et al., 2019). Interestingly, the effect of sonication was more pronounced in samples with 5% of sugar, suggesting a kind of synergism between both growth promoters, such as US ability to improve the bacteria access to sugar (Carrillo-Lopez et al., 2021).

With respect to the final stage of fermentation, pH 5.0 and 4.6 are considered important (Tribst et al., 2018). At pH 5.0 occurs the beginning of gel formation in the samples due to the solubilization of colloidal calcium phosphate (CCP) and electrostatic repulsion reduction caused by the proximity to the casein isoelectric point (Oliveira et al., 2014; Tribst et al., 2018). The pH 4.6 is considered the end of fermentation, with the formation of three-dimensional network interactions between casein–casein due to the increased hydrophobic and electrostatic charge interactions (Lee & Lucey, 2010; Oliveira et al., 2014). The final consequences of μ increase were observed in the reduction of the time spent to reach pH 5.0 and 4.6, with sweetened and/or sonicated samples being 30 to 120 min faster (Table 1 and Fig. 1), representing up to 25% saving time and heating in the production of buffalo yogurt. Furthermore, these results highlighted that the negative impacts of sugar at the beginning of the fermentation (Sodini et al., 2004) were overcome by the positive effects during the symbiotic growth (Gomes et al., 2022).

Finally, the comparison of the results obtained by bath US-assisted fermentation with probe US showed that both ultrasonic systems are able to improve the efficiency of the fermentation process in yogurt production, as well as improve the technical functional properties of the gels obtained (Abesinghe et al., 2019). However, considering the flow rate and volume of milk processed on an industrial scale, bath ultrasound allows a better feasibility and homogeneity of the process, since the ultrasonic intensity of the tip US decreases exponentially with the

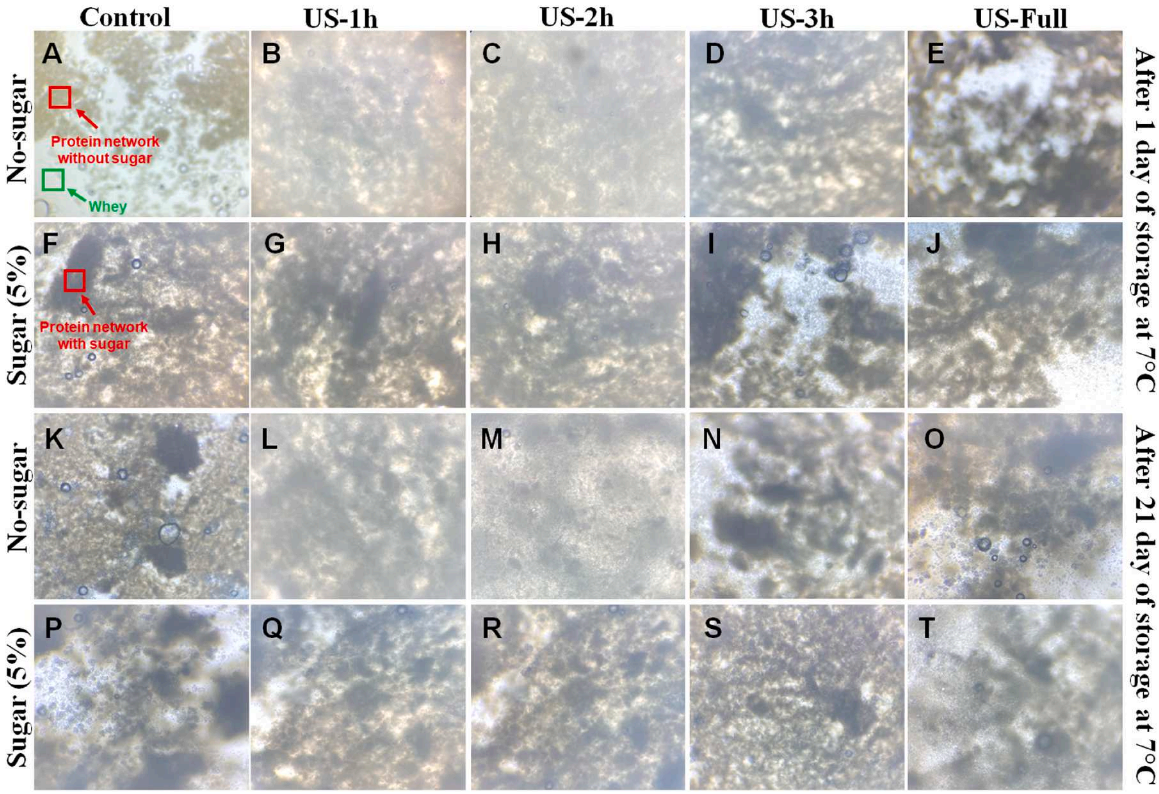

Fig. 4. Optical microscopy images of stirred yogurt with (F-J and P-T) or without (A-E and K-O) sugar produced by ultrasound-assisted fermentation (control (A, F, K, P); US-1 h (B, G, L, Q); US-2 h (C, H, M, R); US-3 h (D, I, N, S); US-Full (E, J, O, T)) after 1 (A-J) and 21 (K-T) days of storage.

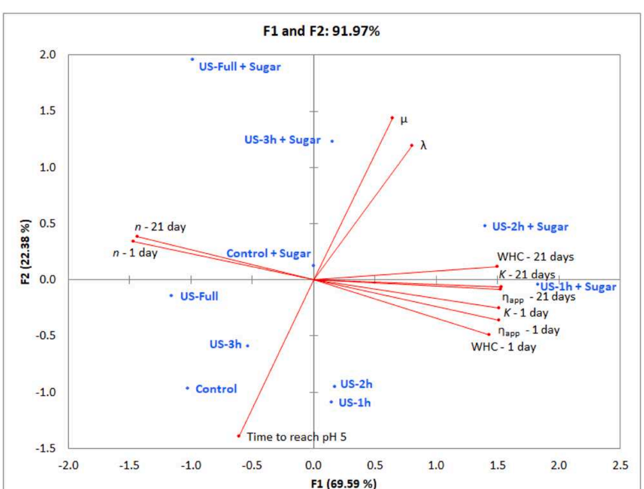

Fig. 5. Principal component analysis (PCA) of the parameters of fermentation kinetics, WHC and rheological behavior of the stirred yogurt with or without sugar produced by ultrasound-assisted fermentation after 1 and 21 days of storage. US: ultrasound; λ: lag phase time (h); μ: maximum pH decline rate (h^{-1}); WHC: Water holding capacity (%); K: consistency index (Pa·s^n); η_{app}: apparent viscosity (Pa·s); n: flow behavior index (dimensionless).

increase in the distance between the probe and the processed material (Gogate & Kabadi, 2009). Furthermore, the high wear linked to the limited tip resistance of the probe ultrasound makes the industrial production difficult (Soares et al., 2019).

With respect to the characteristics of yogurt produced, it was observed that partial or full fermentation under sonication was unable to modify the viability of LAB, final acidity and the occurrence of post-acidification during yogurt storage. These results can be explained by considering that the end of fermentation was determined when the samples reached the same pH (pH=4.6), resulting in similar LAB growth despite the difference in fermentation time (Tribst et al., 2018). The occurrence of post-acidification is a well-described phenomenon as a consequence of LAB metabolism during storage at low temperatures (Tamime & Robinson, 2007). Although the pH reduction and acidity increase may have a sensory impact (Shori et al., 2022), it did not alter the viability of LAB, which is explained by commercial starters developed to tolerate these levels of pH reduction (Deshwal et al., 2021b).

Conversely, the time of sonication applied, and sugar addition impacted the microstructure (Fig. 4) and, consequently, the stability (Figs. 2 and 5) and rheological behavior (Table 3 and Figs. 3 and 5) of the yogurt produced. In general, samples sonicated for 1 or 2 h had a structural improvement due to the formation of a denser and compact protein network (Fig. 4), with greater water holding capacity (Figs. 2 and 5), higher consistency and lower consistency loss after storage (Table 3 and Fig. 5). These results can be explained by considering that the cavitation effect caused by the US process leads to the rupture of the fat globule membrane with a consequent reduction in its diameter (Capela et al., 2022). Furthermore, US can induce molecular unfolding or aggregation, as well as protein defragmentation (Wu et al., 2018; Zhang et al., 2018) by disrupting hydrophobic interactions and hydrogen bonds of milk proteins, including casein and whey proteins (Wu et al., 2018; Zhang et al., 2018; Magalhães et al., 2022). These changes may increase protein surface hydrophobicity through exposure of sulfhydryl and hydrophobic groups (Wu et al., 2018), contributing to improvements in gel formation (Abesinghe et al., 2019). Consequently, a better interaction between protein and fat occurs during gel development (Tribst et al., 2020), increasing the cohesiveness of the system (Tribst et al., 2018) and its ability to retain water entrapped in the network (Tribst et al., 2020). It is important to note that, up to 2 h of fermentation, all samples remained at pH > 6.3; therefore, all effects promoted by US in these samples were exclusively before gel formation.

On the other hand, for samples sonicated during 3 h or during full fermentation, negative alterations on the structure of the samples, with less compact/weak network and whey areas, were observed (Fig. 4), directly impacting their consistency (Table 3 and Fig. 5) and stability (Figs. 2 and 5). This suggests that, although US can be positive for fermentation due to improvements in LAB growth and interactions between milk macromolecules (Abesinghe et al., 2019; Capela et al., 2022; Magalhães et al., 2022), if the process is applied during the phase that the network starts to form (Tribst et al., 2018) or even at a slightly higher pH (pH< 6.0) - when calcium solubilization is expressive and increase in the micelle size is expected (Lee & Lucey, 2010; Oliveira et al., 2014) - the sonication will negatively affect the network structure due to its disturbance caused by cavitation wave (Körzendörfer et al., 2017, 2018). This effect overcomes the benefits observed in the first hours of sonication, resulting in yogurts with a similar/weaker structure than those obtained using unprocessed buffalo milk (control samples).

Complementarily, the impact of sugar addition was clearly observed, resulting in a denser network (Fig. 4) that increased yogurt WHC (Figs. 2 and 5) and consistency during flow (Figs. 3 and 5 and Table 3). This result was expected since sucrose has a good interaction with water and milk protein through the formation of hydrogen bonds, favoring the water entrapment in the protein network structure (Damodaran et al., 2007). In addition, storage negatively impacted all samples, forming whey areas in the microstructure (Fig. 4) that led to the reduction of the sample's consistency (Table 3) and WHC (Fig. 2). This result can be justified by changes in the structure of the protein network, due to syneresis, proteolysis caused by residual proteases, as well as the pH reduction to values below the isoelectric point of the casein weakening the protein network (Tamime & Robinson, 2007). Despite these negative effects had been observed in all samples, their magnitudes were different, being less intense in samples fermented under sonication for 1 or 2 h. This suggests that, in addition to the gains observed in the structure of buffalo yogurt immediately after production, these processes were able to minimize the occurrence of undesirable changes during yogurt storage.

Comparing the yogurt stability observed in the present study with the obtained in others researches that applied US using probe, it is verified that both processes can minimize the loss of strength of acid gels (Körzendörfer et al., 2017, 2018; Abesinghe et al., 2019). However, the process conditions, such as power, time, and frequency must be carefully selected to avoid defects in yogurt quality such as syneresis, weak gels, and the presence of large particles (Körzendörfer et al., 2017; Abesinghe et al., 2019).

In summary, the results highlighted that ultrasonic bath-assisted fermentation below 2 h can accelerate the fermentation process and improve the gel characteristics of buffalo milk yogurt. This result is of great industrial interest in order to improve the fermentation efficiency of buffalo yogurt and the stability of the product during storage. In this context, bath ultrasound can be easily incorporated into the processing of buffalo milk yogurt, with the advantage of being simpler, more durable, and easier to be scaled than probe ultrasound.

5 Conclusions

US-assisted fermentation for 1 or 2 h applied at the beginning of the fermentation process was effective to enhance the fermentation rate, as well as to improve the quality attributes (gel consistency and water holding capacity) of buffalo yogurt due to the formation of a strong protein-fat network. On the other hand, fermentation assisted by US for 3 h or more had a negative impact on the yogurt structure, probably due to the disturbance caused by US waves in the milk gel network during the acidification. Furthermore, these samples did not show significant variations in pH, acidity, and lactic acid bacteria viability when compared to the control sample. Finally, the sucrose addition contributed positively to the quality attributes of the yogurt, facilitating the formation of the protein network and improving the final consistency of the gel. These results are important to expand the use of ultrasonic bath in food processing and help to optimize the production of buffalo yogurt on an industrial scale.

Ethical statement: studies in humans and animals

This manuscript has no work with humans and animals.

CRediT authorship contribution statement

Flaviana Coelho Pacheco: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Eliane de Fátima Teixeira:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Ana Flavia Coelho Pacheco:** Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Paulo Henrique Costa Paiva:** Methodology, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Alline Artigiani Lima Tribst:** Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Bruno Ricardo de Castro Leite Júnior:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2023.100338.

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