



Effect of Ultrasonication on the Properties of Goat Milk Yogurt: Study on Amplitude and Duration

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Abstract—The gel strength of yogurt from goat milk is weak due to the low α_{s1} -casein proportion. According to some studies, treating cow milk via ultrasonication before fermentation produces yogurt that is stiffer than regular yogurt. This study aimed to investigate the influence of amplitude and duration of ultrasonication on the properties of goat milk yogurt (pH, total acid, viscosity and syneresis). Scanning electron microscopy (SEM) was used for additional investigation to evaluate the microstructure of the best-treated sonicated goat milk yogurt and compared to non-sonicated goat milk yogurt. The results demonstrated that these two factors interacted to affect viscosity and that the amplitude and duration of sonication had a significant effect on total acid and syneresis. These two factors, however, had no appreciable impact on the pH. At an amplitude of 80% and duration of 10 minutes, the best treatment was attained and the sample had the following properties: pH of 4.38, total acid of 1.00%, viscosity of 4929.67 cP, syneresis of 13.31%, and 2.36×10^7 CFU/ml for the lactic acid bacteria colonies. In comparison to non-sonicated goat milk yogurt, the sonicated yogurt's microstructure was denser and more complex, which may contribute to the increased gel firmness.

Keywords— Amplitude; Duration; Goat Milk; Ultrasonication, Yogurt

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I. INTRODUCTION

The natural fermentation process can increase the nutritional value of food products such as proteins, essential amino acids and vitamins. Fermentation can increase the sensory value of products related to taste, aroma, and product appearance [1]. One of the fermented products from milk that has been produced commercially is yogurt. Yogurt is widely consumed because it has health benefits, such as improving intestinal microflora, reducing the risk of gastrointestinal disease, and improving lactose intolerance [2]. Yogurt products that are commonly found are made from cow milk, but now many variations of yogurt from other types of milk have been developed, one of which is goat milk [3]. Based on data published by FAO, there were 222 million dairy goats in the world in 2020, yielding 21 million metric tons of milk [4]. The largest increase in goat milk production was in the Asian continent with a percentage of 22% [5]. Therefore, goat milk production was number 3 in the world after cow milk and

buffalo milk [3]. The increase in production and demand for goat milk is due to its health and nutritional benefits, including being superior to cow milk in terms of digestion and lipid metabolism [5].

The curd formed from goat milk yogurt has a weaker consistency and viscosity compared to that of cow milk, resulting in greater syneresis. This may relate to the low amount of α_{s1} -casein, high colloidal calcium, and larger micelle dispersion. To overcome these problems, several approach techniques have been carried out to enhance the goat milk yogurt's texture, including using a simple method that has been widely used, such as the addition of stabilizers and increasing total solids [6]. Additionally, by using new technologies that are safe and environmentally friendly such as ultrasonication. Ultrasonication can increase the coagulation of whey protein in yogurt products which has an impact on increasing the characteristics of the gel. Ultrasonication utilizes the interaction between sound waves in the liquid medium and dissolved gas so that cavitation

occurs which can have a positive influence on the strength of the yogurt gel [7]. The sonication treatment before the fermentation process took place in dairy products was able to improve the gel texture [8]. Several factors that influence the changes concerning the final product's physicochemical characteristics processed by the sonication process including temperature, power, amplitude and duration [9, 10].

Based on the previous research, it showed that amplitude and duration had a noteworthy impact on the characteristics of cow milk yogurt [10]. Research on the effect of ultrasonication on the properties of goat milk yogurt is still limited. Therefore, it is necessary to conduct research to determine the variation of amplitude and duration of sonication to produce goat milk yogurt with the best properties. Furthermore, goat milk yogurt with the best properties will be further analyzed using scanning electron microscopy (SEM) to evaluate the effect of the application of the ultrasonication on the microstructure of goat milk yogurt which may correlate to the properties of yogurt.

II. MATERIAL AND METHODS

A. Materials

Goat milk (obtained from goat farms located in Pakis District, Malang Regency), commercial yogurt (Greenfield yogurt plain), and granulated sugar are the ingredients required to prepare goat milk yogurt. NaOH (Merck, pa), phenolphthalein (PP) indicator (Merck, pa), MRSA medium (Merck), and peptone (Merck), and distilled water were the materials used in the analysis.

B. Methods

Research design

This research was an experimental study with a factorial randomized block design (FRBD). In this study, there were 2 factors which include ultrasonic amplitude (50%, 65%, and 80%) and duration (5 and 10 minutes), which were applied to milk before the fermentation process. Based on these two factors, obtained 6 treatment combinations that will be tested 3 times.

Yogurt starter and sample preparation

A 150 mL of goat milk was filtered to prepare yogurt starter and the filtrate was then heated at 80°C for 5 minutes. Subsequently, the milk was moved into a sterile glass jar and mixed with 9 grams of sugar and homogenized. A 7.5 gram of commercial yogurt starter was added once the milk reached a temperature of 37-40°C. In addition, the milk fermented at 37°C for 24 hours. The back-sloping starter was then allowed to chill in the refrigerator.

A total of 150 ml of goat milk was filtered and heated to a temperature of 80°C for 5 minutes to prepare yogurt sample. The heated milk was put in a glass jar and 9 grams of sugar was added. The milk was then cooled to 60°C and sonicated

using probe with the specified amplitude (50, 65 and 80%) and duration (5 and 10 mins). The milk (with and without ultrasonication) was cooled to drop the temperature to 40°C and then added with 7.5 grams of commercial yogurt culture that has been regenerated. The milk was then fermented for 24 hours at 37°C.

Research analysis

Analysis of goat milk yogurt includes analysis of pH (measured using pH meter), total acid [11], viscosity [12], syneresis [10], total lactic acid bacteria [13] and microstructure [14].

Viscosity

The viscosity was measured using the technique outlined by Gad and Mohamad. Yogurt was allowed to reach room temperature after being chilled in the refrigerator for 24 hours at 4°C. Spindle number 4 and a viscometer (Elcometer 2300 RV) were set up, and the spindle's speed (rpm) was adjusted. For a duration of 1.5 minutes, the viscosity of yogurt was measured every 30 seconds and then the average viscosity (cP) was calculated.

Syneresis

With a minor modification, syneresis was determined using the technique outlined by Kenari and Razavi. The top of the Erlenmeyer flask was covered with the prepared filter paper Whatman No. 1. A thirty-gram sample was put on filter paper and refrigerated for five hours. The weight of the fluid that entered the Erlenmeyer after passing through the filter paper was weighed. Syneresis was calculated using the following formula:

$$\text{Syneresis (\%)} = \frac{\text{weight of fluid (g)}}{\text{weight of sampel (g)}} \times 100 \% \quad \dots \text{eq (1)}$$

Total Lactic Acid Bacteria

With a small modification, the plate counting method was used to determine the total lactic acid bacteria. One milli liter of each peptone solution—which ranged in dilution from 10^{-1} to 10^{-5} —was pipetted and put into a sterile petri dish. After adding 12 to 15 milli liters of MRSA (at a temperature of around 45°C) to each petri dish, the dish was shaken in the pattern of a '8' to ensure that the peptone-rich media was homogenized. The substrate was then allowed to solidify. For 48 hours, the petri dish was inverted and placed in an incubator set to 37°C. The Standard Plate Count (SPC) formula was used to determine the total number of lactic acid bacteria after the colony counter (Stuart) was used to compute the total number of colonies that appeared.

Microstructure

The method for microstructure analysis was modified from that outlined by Pelaes. Samples of yogurt were lyophilized for 24 hours after being frozen at -80°C in a deep freezer (Nuve DF 490). Samples were covered in a layer of gold (Quorum Q150 RS) and affixed to aluminium stubs. A

scanning electron microscope (SEM) (Fei Quanta FEG 650) was used to make the observations.

Statistical Analysis

The chemical and physical properties data obtained were analyzed using the Minitab 19 application with the General Linear Model Analysis of Variance (ANOVA) method. Further tests using the Least Significant Difference test on the Minitab 19 application or the Duncan Multiple Range Test (DMRT) on the SPSS application. Determination of the best treatment based on chemical properties and physical properties using the Multiple Attribute Zeleny method based on 5 parameters, which were pH, total acid, viscosity, syneresis, and LAB count. Then the parameters of best treatment sample were analyzed using paired t-test through Minitab 19 software to be compared to those of control treatment sample. The best sonicated and non-sonicated yogurt samples were analyzed for microstructure using Scanning Electron Microscopy (SEM).

III. RESULT AND DISCUSSION

A. pH

pH is used as one of the parameters that can indicate the quality of yogurt as it is related to the activity of microorganisms during the fermentation. The pH values obtained by the treatment of amplitude and duration of sonication ranged from 4.36 to 4.42. Statistical analysis showed that amplitude and duration of sonication did not significantly affect the pH value. The pH of sonicated goat milk yogurt presented in **Table 1**.

TABLE 1.
 pH OF SONICATED GOAT MILK YOGURT AS THE IMPACT OF AMPLITUDE AND DURATION OF SONICATION

Amplitude (%)	Duration (min)	pH
50	5	4.41 ± 0.12
50	10	4.36 ± 0.11
65	5	4.41 ± 0.14
65	10	4.39 ± 0.13
80	5	4.42 ± 0.11
80	10	4.38 ± 0.10

The decrease in pH value occurred during the fermentation process due to the activity of lactic acid bacteria that produce organic acids so that the pH of yogurt decreased to close to the isoelectric point value of casein at pH 4.6. The absence of a significant effect on pH parameters is supported by the results of research conducted by Pacheco [15] on a buffalo milk yogurt treated with ultrasonication at a frequency of 25 kHz with various duration of sonication. When compared to the control treatment of goat milk yogurt, the pH value of the control was higher (4.48 ± 0.10) than those of sonicated yogurt. This may occur because the ultrasonic treatment of the raw material leads to a decrease in the pH of the milk then results in the lower pH of the sonicated yogurt. The sonication process on milk leads to a decrease of pH as the sonication process results in the reaction between nitrogen and oxygen in

the hot zone of the cavitation bubble so that nitric acid is formed which results in a lower pH value of milk [16].

B. Total Acid

Total acid of goat milk yogurt with sonication treatment ranged from 0.89-1.01%. According to the SNI standard (2981:2009), the total acid in yogurt ranges from 0.7-1.2%. These results indicate that goat milk yogurt with sonication treatment was in accordance with the standards set by SNI. Statistical analysis showed that the amplitude and duration of sonication had a significant effect on the total acid, but there is no interaction between these two factors. The test results for the total acid can be seen in **Table 2** and **Table 3**.

TABLE 2.
 AVERAGE TOTAL ACID OF SONICATED GOAT MILK YOGURT AS THE IMPACT OF AMPLITUDE OF SONICATION

Amplitude (%)	Total Acid (%)
50	0.91 ± 0.03 ^b
65	0.95 ± 0.04 ^{ab}
80	0.98 ± 0.03 ^a

Different superscript letters indicate significant difference (p < 0.05)

TABLE 3.
 AVERAGE TOTAL ACID OF SONICATED GOAT MILK YOGURT AS THE IMPACT OF DURATION OF SONICATION

Duration (min)	Total Acid (%)
5	0.92 ± 0.03 ^b
10	0.97 ± 0.04 ^a

Different superscript letters indicate significant difference (p < 0.05)

Table 2 and **Table 3** showed that the increasing ultrasonic amplitude and duration of sonication on the milk leads to an increase of total acid. The increase in total acid influences the lower pH value of yogurt. The sonication treatment decreased the pH value of goat milk related to the production of nitric acid due to the reaction between nitrogen and oxygen in the hot zone of the cavitation bubble. The increase in the amplitude produces greater vibrations so that the cavitation bubbles formed will be more and more so that more nitric acid is formed and allows the total acid of milk in the high-amplitude treatment to be higher, while the duration of sonication affects the duration of the ultrasonic cavitation phenomenon which also affects the amount nitric acid formed [16]. The measurement of total acid was carried out by the titration method, which allows the measurement of other types of acids other than organic acids so that the amplitude and duration of sonication have a significant effect on the total acid.

C. Viscosity

Viscosity is one of the parameters that can be used in determining the quality of yogurt related to the firmness of the gel. The formation of yogurt viscosity is influenced by a protein network consisting of interactions between casein and fat globules [17]. Amplitude and duration of sonication as a

pre-treatment of milk had a significant effect on the viscosity of goat milk yogurt and there was a significant interaction between these two factors. The viscosity of sonicated goat milk yogurt shown in **Table 4**.

TABLE 4.
 VISCOSITY OF SONICATED GOAT MILK YOGURT AS THE IMPACT OF AMPLITUDE AND DURATION OF SONICATION

Amplitude (%)	Duration (min)	Viscosity (cP)
50	5	3002.22 ± 250.25 ^c
50	10	3145.00 ± 119.53 ^c
65	5	3007.78 ± 121.85 ^c
65	10	3353.33 ± 183.51 ^c
80	5	4195.56 ± 353.49 ^b
80	10	4929.67 ± 424.61 ^a

Different superscript letters indicate significant difference ($p < 0.05$)

The higher the amplitude and the longer the duration of sonication on the milk, the higher the viscosity of goat milk yogurt. The increase in viscosity due to sonication treatment is caused by the homogenization effect that occurs due to the ultrasonic cavitation phenomenon. Ultrasonic cavitation is the phenomenon of the formation of micro-sized bubbles due to a decrease in pressure in the liquid. The homogenization of milk fat globules occurs due to the rupture of cavitation bubbles which produce shear and turbulence forces so that they can break down milk fat globules into smaller sizes. As the ultrasonic amplitude increases, the resulting vibration will be greater and the longer the duration of sonication applied, the cavitation phenomenon lasts longer so that more cavitation bubbles are formed to be able to break down fat globules into smaller ones and increased surface area [18]. An increased quantity of casein micelles absorbed on the fat globule membrane surface coincided with the increased fat membrane surface area. The process of acidification during fermentation enables fat globules to play a role in aggregation and subsequent network development. This indicates that a considerable increase in casein will enhance firmness [19]. The increased viscosity of yogurt from milk which treated with ultrasonication also could be linked to the denaturation of whey protein due to ultrasonication. A stronger yogurt coagulum is produced when the whey proteins are denatured because they are more likely to associate with casein and casein micelles [20, 21].

D. Syneresis

Syneresis in yogurt is a condition for the separation of whey liquid from the gel that occurs due to the instability and weakening of the yogurt gel network. Syneresis occurs during the storage process and can affect the acceptability of the final yogurt product. Statistical analysis showed that the amplitude and duration of sonication had a significant effect on the syneresis value, but there is no interaction between these two factors. Syneresis value of sonicated goat milk yogurt can be seen in **Table 5** and **Table 6**.

TABLE 5.
 AVERAGE SYNERESIS OF SONICATED GOAT MILK YOGURT AS THE IMPACT OF AMPLITUDE OF SONICATION

Amplitude (%)	Syneresis (%)
50	20.12 ± 0.11 ^a
65	18.01 ± 1.11 ^b
80	14.44 ± 1.60 ^c

Different superscript letters indicate significant difference ($p < 0.05$)

TABLE 6.
 AVERAGE SYNERESIS OF SONICATED GOAT MILK YOGURT AS THE EFFECT OF DURATION OF SONICATION

Duration (min)	Syneresis (%)
5	20.12 ± 2.37 ^a
10	18.01 ± 3.38 ^b

Different superscript letters indicate significant difference ($p < 0.05$)

There was a decrease in the syneresis as the ultrasonic amplitude and duration of sonication increased. This is related to the cavitation effect which increases as the amplitude and duration of sonication were applied [18]. Ultrasonic cavitation affects the size of fat globules in milk to become smaller. The greater the ultrasonic amplitude, the more cavitation bubbles formed lead to shear and turbulence forces will be greater so that more fat globules are split with a very small size. The smaller fat globule size will increase the surface of the fat membrane which is also affected by the presence of fat dispersion [7]. The increase in the surface area of the fat membrane was followed by an increase in the number of casein micelles attached and adsorbed to the surface of the fat globule membrane. Acidification during fermentation will allow the fat globules to contribute to the aggregation, thus in building the network. This suggests that significant rise in casein will improve the firmness and stability against whey separation and reduce syneresis [19].

E. Total lactic acid bacteria

Lactic acid bacteria (LAB) is a type of bacteria used in the process of making yogurt. The amount of lactic acid bacteria contained in yogurt determines the suitability of the product for consumption. The growth of lactic acid bacteria is influenced by the availability of nutrients in the medium which include protein and carbohydrates. The higher the available nutrients, the higher the activity of lactic acid bacteria in fermented products. The total lactic acid bacteria of sonicated goat milk yogurt presented in **Table 7**.

TABLE 7.
 TOTAL LACTIC ACID BACTERIA OF SONICATED GOAT MILK YOGURT AS THE EFFECT OF AMPLITUDE AND DURATION OF SONICATION

Amplitude (%)	Duration (min)	Total LAB (CFU/ml)
50	5	2.67 x 10 ⁷
50	10	2.13 x 10 ⁷
65	5	6.1 x 10 ⁷
65	10	1.6 x 10 ⁷
80	5	1.83 x 10 ⁷
80	10	2.36 x 10 ⁷

The analysis on total lactic acid bacteria in yogurt samples was aimed to determine the availability of lactic acid bacteria

in yogurt samples. The total lactic acid bacteria in sonicated goat milk yogurt ranged from 1.6×10^7 to 2.67×10^7 CFU/ml. The total lactic acid bacteria in sonicated goat milk yogurt showed various results. This was influenced by the implementation of the ultrasonic method which was carried out before the addition of lactic acid bacteria culture. The presence of sonication treatment as a pre-treatment did not affect the chemical composition of sonicated milk, which is a nutrient for the growth of lactic acid bacteria [22]. Several factors that can affect the number of lactic acid bacteria colonies include pH, water activity, temperature, oxygen, heat treatment, and most importantly the number of starters. Based on the minimum bacterial colony standard set by SNI (2981:2009) which is 107 CFU/ml or 7 log CFU/ml, all samples meet the SNI standard.

F. Best Treatment

The best treatment was selected using the Zeleny method which will then be analyzed for microstructure using the SEM method. The selection of the best treatment is based on the ideal value or expected value of each parameter consisting of pH, total acid, viscosity, syneresis, and total LAB. In this analysis, the expected value of the viscosity parameter is the higher one, while the value of the expected syneresis is the lower one. Ideal values for pH parameters and total acid using reference from best treatment from research conducted by Kenari and Razavi [10]. The data in **Table 8** is the ideal value for each parameter.

TABLE 8.
 IDEAL VALUE FOR BEST TREATMENT DETERMINATION

Parameter	Ideal value
Viscosity (cP)	4929.67
Syneresis (%)	13.31
pH	4.36
Total acid (%)	0.92
Total LAB (CFU/ml)	$6,1 \times 10^7$

TABLE 9.
 RANK OF TREATMENT BASED ON ZELENY METHOD

Treatment		Result	Rank
Amplitude (%)	Duration (min)		
50	5	0.271	6
50	10	0.250	5
65	5	0.244	4
65	10	0.202	3
80	5	0.117	2
80	10	0.022	1

By calculating the degree of density (dk) and density distance (Lp) using the optimum value found in **Table 8**, the best treatment was determined. The best treatment was determined based on the lowest value of the sum between L1, L2, and L∞. Based on the calculations that have been carried out, the best

treatment of goat milk yoghurt samples was attained at amplitude of 80% and duration of 10 minutes (**Table 9**).

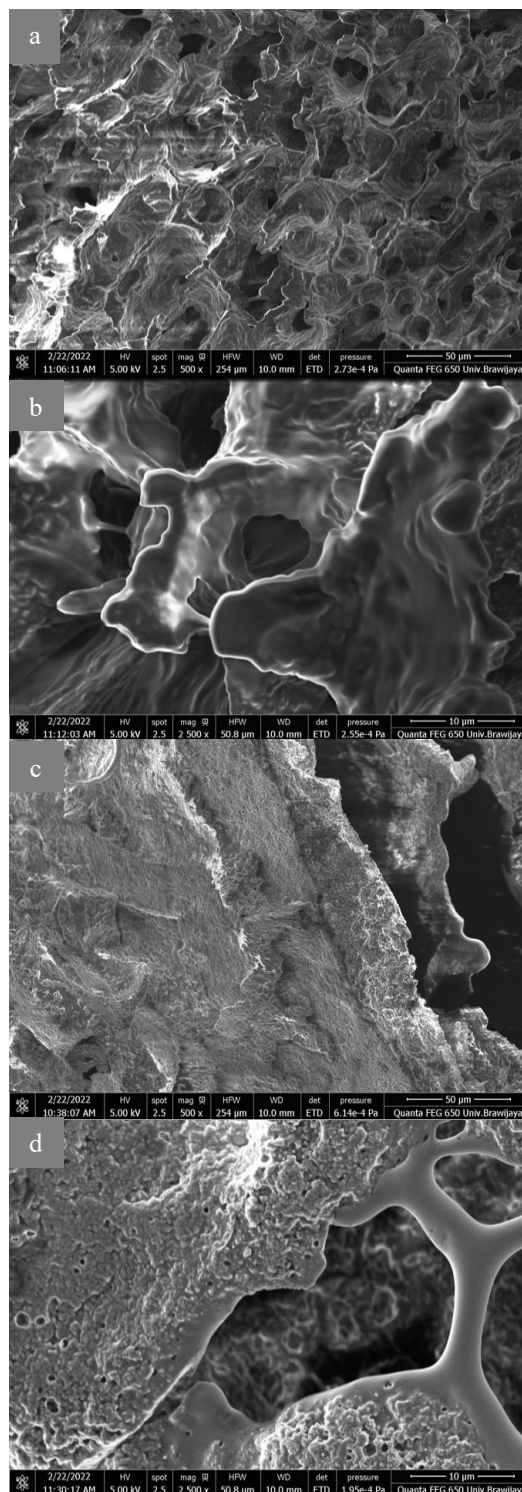


Fig. 1. Microstructure of non-sonicated goat milk yogurt with magnification of 500x (a) and 2500x (b) and best sonicated goat milk yogurt with magnification of 500x (c) and 2500x (d)

TABLE 10.
 COMPARISON OF PARAMETERS BETWEEN
 BEST TREATMENT SAMPLE AND CONTROL TREATMENT SAMPLE

Parameters	Best Treatment	Control Treatment	Significance
Viscosity (cP)	4929.67	2382.22	*
Syneresis (%)	13.31	29.49	*
pH	4.38	4.48	-
Total Acid (%)	1.01	0.99	-
Total LAB (CFU/ml)	2.36×10^7	2.87×10^7	

Symbol * indicate significant difference ($p < 0.05$)

The parameters of best treatment sample were compared to those of control treatment using paired t-test to show the effectiveness of ultrasonication (**Table 10**). These results revealed that ultrasonication treatment gave significant impact on viscosity and syneresis of goat milk yogurt, but did not give significant effect on other parameters.

G. Microstructure

The Scanning Electron Microscopy (SEM) was utilized for microstructure analysis on the best treated sonicated goat milk yogurt (amplitude 80% and duration of 10 minutes) compared to the non-sonicated one. Microstructure analysis was carried out at 500x and 2500x magnification.

The results of the SEM analysis of the non-sonicated goat milk yogurt presented in **Figure 1.a** and **1.b** showed the number of large voids or empty spaces with varying sizes resembling a honeycomb on the surface of the yogurt. The presence of amplitude and duration of sonication applied to milk resulted in a tighter and more compact yogurt structure. This can be seen from the reduced cavities on the surface of the yogurt as shown in **Figure 1.c** and **1.d**. The results of the SEM analysis on the non-sonicated goat milk yogurt which has a lot of empty space and resembles a honeycomb are similar to the results obtained by Gürbüz et al. [23]. Large cavities in the non-sonicated goat milk yogurt leads to the formation of a weak gel structure, so that syneresis will be greater [24]. According to the research conducted by Tian et al. [25], yogurt that has higher viscosity and lower syneresis revealed more arranged and dense protein clusters with a highly connected network microstructure.

IV. CONCLUSION

The variation of amplitude and duration of sonication did not have significant effect on the pH of goat milk yogurt, while on the total acid and syneresis parameters, each factor had a significant effect and gave significant interactions on the viscosity parameter. The sonication treatment applied before fermentation was able to enhance the firmness of the goat milk yogurt gel. The increase in gel rigidity is due to the homogenizing effect caused by ultrasonic cavitation. The best treatment of sonicated yogurt was obtained at 80% amplitude

and 10 minutes of duration of sonication with the pH of 4.38, total acid of 1.00%, viscosity of 4929.67 cP, and syneresis of 13.31%. The number of colonies of lactic acid bacteria contained in the treatment amounted to 2.36×10^7 CFU/ml. Microstructure analysis conducted on the best treatment and non-sonicated goat milk yogurt showed that yogurt with sonication treatment had a more intricate and dense structure than non-sonicated goat milk yogurt which may correlate to an increase in gel firmness due to sonication treatment.

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CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

USE OF ARTIFICIAL INTELLIGENCE (AI) TOOLS STATEMENT

We used Grammarly (Grammarly Inc., 2025) to improve the clarity and grammar of the manuscript. The authors reviewed and approved all changes.

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