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Original Article

Innovative Ultrasonic Treatment for Enhancing Physicochemical and Foaming Properties of Raw Milk: Toward Sustainable Food and Agriculture

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Abstract

Raw cow's milk is highly perishable and traditionally processed using thermal treatments, which can degrade its nutritional and functional properties. This raises a need for alternative methods that can ensure the safety and quality of raw cow's milk. Ultrasound-treated cow's milk is an emerging, innovative non-thermal technology that supports sustainable food processing by improving milk quality without high heat. This study aimed to analyse the effects of sonication on the microbial load, physicochemical, and foaming properties of raw cow's milk, highlighting its potential in sustainable dairy innovation. The treated milk reduced fat (3.28%) and protein (3.08%) compared to untreated raw milk. Microbial analysis revealed that sonicated milk had <3 cfu/g of *Escherichia coli*, 0 cfu/g of *Staphylococcus*, and no detectable *Salmonella spp.* Depending on amplitude and time settings, the total coliform count was significantly reduced to 1100 cfu/g. Physicochemical assessments included pH, viscosity, specific gravity, particle size distribution, zeta potential, titratable acidity, and colour. Statistical analysis using two-way ANOVA and post hoc Tukey's test showed that milk treated at 40% amplitude for 6 minutes exhibited the most favourable quality outcomes across multiple

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parameters. These findings support using ultrasound as an innovative, energy-efficient processing method that enhances milk quality and contributes to sustainable food and agricultural practices.

1. Introduction

The natural dairy product known as milk is made from the mammary gland secretions of lactating mammals [1]. Cow's milk is an example of a dairy product and acts as a superfood, as it is generated from healthy cows fed on grass. In 2012, the Food and Agriculture Organisation (FAO) estimated that about 85% of all milk worldwide was produced from cows, about 11% by buffaloes, 2% by goats, 1.4% by sheep, and 0.2% by camels. Thus, cow's milk has become one of the highest commercial productions in the dairy industry. This is because cow's milk is also known as a highly nutritious dairy food for human consumption. Due to its abundant nutrients, it is frequently considered the ideal food in the dairy sector. Cow's milk does play a significant role in health protection, especially for humans [2].

During milk production, cows are exposed to various biological, chemical, and physical risks, which can directly interfere with production. Raw milk is also a favourable substrate for microbial development, which decreases milk quality and shelf life due to its high nutritional value. The ageing duration in these trials was shorter than the milk's shelf life, and the milk used was pasteurised and homogenised. Furthermore, the scope of this research is restricted to the assessment of foamability and foam stability. Therefore, more research is needed on how the foaming characteristics of cow's milk vary during storage over their shelf life. The heat treatment in milk processing modulates the flavour, the microbial content, and the milk proteins through ultrasound processing [3]. Therefore, there is a growing demand for novel food processing technologies that preserve milk's quality while ensuring microbial safety.

To prepare high-quality dairy products, producing good-quality raw milk is considered due to its substantial contribution to the dairy industry. As a result, there is growing interest in developing novel food processing technologies that provide effective microbiological inactivation at temperatures lower than thermal pasteurisation, keeping the raw milk's physicochemical, nutritional, and sensory qualities. Since raw milk has easy access to microbial activity, which can affect human health, ultrasound is one of the nonthermal treatments used in recent decades for dairy products [4–6]. In food science and technology, the impact of milk processing with ultrasound has gained significant attention. Due to its function in enhancing safety and postponing food decomposition, its effects on microbes have been the subject of substantial research as a preservation technique [4].

High-intensity ultrasound (HIU) is a promising recent technology created with simplicity, economy, and energy efficiency in mind, and it applies to milk processing [5,6]. Due to its many uses in processing and product evaluation, HIU has generated much attention in food science and technology, especially in milk processing [5,7]. HIU could improve the physicochemical characteristics and has already done so. However, knowledge gaps have only been partially explored in recent years, such as comparisons and combinations with other procedures (such as microfluidisation or pasteurisation), or applications on novel items. However, no studies have been done on milk processing using only high-intensity ultrasound applications. The use of HIU in milk and other milk-based drinks has demonstrated some benefits in terms of quality physicochemical metrics. Recently, in addition to bovine milk, the effects of HIU treatment on buffalo milk, sheep milk, and supplemented milk have also been studied [4]. HIU

has been shown to have favourable effects, particularly on the size of fat globules or fat crystals, fat droplets, and other milk particles [3,5,6].

Ultrasound processing is a promising method for treating raw milk, as traditional thermal treatments often cause significant physicochemical changes, including alterations in pH, colour, viscosity, and microstructure [5,6]. In addition to the natural variability in milk quality caused by environmental and biological factors, applying novel food technologies, such as ultrasonic homogenisation, can enhance foam quality by reducing particle size. This innovative technique aids in preserving the fresh cow's milk's physicochemical, nutritional, and microbiological activity. This study aims to address existing knowledge gaps by investigating the effects of ultrasound treatment on raw cow's milk. It explores how different ultrasound amplitudes and treatment durations affect microbial load, physicochemical properties, and foaming behaviour. The findings are expected to demonstrate how this innovative, energy-efficient processing method can improve milk quality while contributing to the sustainability of food systems and agricultural resilience.

2. Methodology

2.1. Sample preparation

The cow's raw milk was collected from Dairy Farm at Kota Kinabalu, Sabah, Malaysia. Raw milk samples were stored at 4°C at the Faculty of Food Science and Nutrition of University Malaysia Sabah (FSMP, UMS) until needed. The compositions in the milk samples were analysed by the Department of Veterinary Services at Kepayan, Kota Kinabalu, Sabah, Malaysia, using the FOSS FT 120 Milkoscan.

2.2. Ultrasound Processing

200 mL raw milk samples were treated using an ultrasonic processor with a maximum nominal power of 500 W. The cow's raw milk sample was subjected to ultrasonic processing for 3 minutes, 6 minutes, and 9 minutes, respectively, at different amplitudes. Sonication was performed using *Vibra-Cell VCX500* (500 W, 20 kHz) with 13 mm horns and 3 mm micro tips (Sonics & Materials Inc., Danbury, USA). The sonic amplitude was set at 30% and 40% (50 W Joule). Larger quantities could be sonicated at 100% to shorten processing times. Based on this type of ultrasonicator, *Vibra-cell VCX500* has a maximum limit of 40% to prevent overheating. Micro-tips were soaked about 1 cm into the milk sample, which was contained in a 1000 mL beaker. To avoid the overheated milk, the sample was placed in a 24°C iced-steamed tub for the processing time of up to 9 minutes, or 19°C for a longer processing time, which was stated by Karlović et al. [8] and Postelmans et al. [9] with modification.

2.3. Milk Composition Analysis

The protein and fat content were determined in the milk composition analysis. 50 mL of control and ultrasonicated milk samples were analysed using Foss FT 120 Milkoscan [10]. The fat and protein content were displayed on the digital analyser when the analysis was completed.

2.4. Microbial Analysis

Determining colony-forming units (CFUs) of total and coliform bacteria using suitable media is part of the microbiological analysis of milk samples. All microbiological analysis media were sterilised before use according to the manufacturer's instructions.

1 g of sample was pipetted into 9 mL sterile 0.1 % peptone water and shaken 25 times to homogenise. A 10-fold serial dilution was prepared in 0.1 % peptone water. Then, a 3M™ Petrifilm™ RYM Count Plate was placed on a flat surface for each dilution and evaluated. The top of the film was

lifted, and 1 mL of each dilution was dispensed onto the centre of the bottom film of each plate. The film was then rolled down onto the sample. The 3M™ Petrifilm™ Flat Spreader was placed at the centre of the plate and pressed gently to distribute the sample evenly. Before forming the gel, the inoculum was spread over the entire 3M™ Petrifilm™ RYM Count Plate growth area. After removing the spreader, the plate was left undisturbed for at least 1 minute to permit the gel to form. The 3M™ Petrifilm™ RYM Count Plates were incubated horizontally at 25 or 28 °C with the clear side up in stacks of no more than 40 plates. The plates were enumerated after 48 hours of incubation. An additional 12 hours of incubation time were extended for better interpretation if the colonies appear faint. 3M™ Petrifilm™ RYM Count Plates were counted using a standard colony counter with a back light or an illuminated magnifier to assist with the estimated enumeration [11].

2.5. Physicochemical Properties

2.5.1. pH

The pH of the raw and ultrasonicated cow's milk samples was measured using a digital pH meter (OHAUS, Starter 3100). 10mL of ultrasonicated cow's milk was used as a sample and placed in a beaker for measurement. A glass electrode of the pH meter was then neutralised first with distilled water until it reached a pH of 7 before it was immersed in the samples. The pH of the samples was measured during the 0^{th}, 1^{st}, and 2^{nd} weeks after the ultrasonication period of the cow's milk [12]. The measurement was applied to untreated and ultrasonicated milk, and repeated three times.

2.5.2. Titratable Acidity

The titratable acidity of the raw and ultrasonicated milk samples was tested following the standard methods of [13]. Milk samples of 10 mL were added to a 10 mL measuring cylinder. 3-4 drops of 0.5% phenolphthalein indicator were added and left for two minutes. After two minutes, it was titrated with standard 0.1 N sodium hydroxide (NaOH) until a faint pink colour persisted for 10 seconds. The titratable capacity of the sample was calculated using Equation (1) as follows:

$$\%\ \text{Titratable acidity} = \frac{(\text{mL of } 0.1\ \text{N NaOH} \times 0.0009\text{ml}) \times 100}{(\text{mL of sample used})} \quad (1)$$

2.5.3. Measurement of Colour

The milk samples' colour measurements' L*, a*, and b* values were determined using a colourimeter (Konica Minolta). The instrument was first calibrated with a white reference tile before the measurements were taken. 5 mL of the milk samples was taken out, and the colour was measured [14]. This measurement was repeated for ultrasonicated milk, and triplicate measurements were made.

2.5.4. Specific gravity

The raw milk sample was filled sufficiently into a glass cylinder (100 mL capacity), where it was mixed well and poured into the lactometer jar up to its brim. The lactometer was placed in the jar at a rotating speed, and the reading was taken at a stationary phase. The temperature of the milk was recorded with the help of a thermometer. Corrected Lactometer Reading (CLR) was calculated by adding 0.2 to Lactometer Reading (LR) for each degree Fahrenheit above 80.6°F or by subtracting 0.2 for each degree Fahrenheit below 80.6 °F, as shown in Equation (2) [15]. The measurement was conducted on ultrasonicated milk three times in total. The specific gravity of the milk samples was determined using Equation (3).

$$CLR = LR \pm (\Delta) \quad (2)$$

$$\text{Specific gravity} = \left(\frac{CLR}{1000}\right) + 1 \quad (3)$$

2.5.5. Measurement of Viscosity

Viscosity of milk samples was measured using an AR 1500 Rheometer (TA Instruments, UK), with a cone and plate geometry (cone diameter 40 mm, angle 0, gap 0.2 mm) [16]. For each measurement, 2.0 mL of the raw milk samples was carefully deposited over the plateau of the rheometer. Steady state flow measurements were performed at 25 ± 0.1 °C in the 0–1000 s^{-1}. This measurement was repeated using ultrasonicated milk. Triplicate measurements were done.

2.6. Emulsification Properties

2.6.1. Determination of Particle Size Distribution

The distribution of particle size in the milk samples was determined by the method reported by [17] using Zetasizer Nano S (Malvern Instruments Ltd., Worcestershire) and water as a diffuser. The refractive index of milk and water fat is 1.462 and 1.330, respectively. The time was recorded at 3, 6, and 9 minutes. The measurement was applied to raw and ultrasonicated milk and repeated three times.

2.6.2. Zeta Potential

A zetasizer Nano (Malvern Instruments Ltd., Worcestershire) was used to determine the zeta-potential of the ultrasonically treated and raw milk (sample). The samples were diluted with deionised water at 1:100 and placed inside a disposable polycarbonate cuvette (ATA Scientific, DTS1061). Measurements were repeated 10 times per run with a minimum of 3 runs [17].

2.7. Foam Preparation

2.7.1. Mechanical Foaming

According to its user manual, milk was foamed using an electrical milk foamer (Biolomix, China) with a nominal power of 500 W. 115 mL of milk was heated to 65°C and automatically foamed for 130 seconds [16].

2.7.2. Foam Structure

Foam surface at 0 and 10 min of destabilisation process was imaged using an optical light microscopy (E400 Eclipse, Nikon, Japan) integrated with a 5.0 MP camera system [16]. During imaging, an Olympus LG-PS2 lamp was employed to illuminate the foam. The diameter of air bubbles in the milk was taken, and images were manually measured using Image-Pro Plus 6.0 software. However, if the air bubbles are not spherical, the longest length is considered the diameter of an air bubble.

2.7.3. Statistical Analysis

All analysis was performed in triplicate, and the data generated from the study were expressed as the mean ± standard deviation of three repetitions. Two-way ANOVA was used to analyse the data for the physicochemical analysis. Using IBM SPSS statistical software version 28.0.0, a one-sample T-test with a 95% confidence interval was performed on the data to determine the mean and standard deviations.

3. Results

3.1. Composition of Fat and Protein in Milk

The fat and protein content of the milk samples treated with ultrasound processing under two amplitudes (30% and 40%) and the three durations (3, 6, and 9 minutes) are shown in Table 1. Milk treated with an amplitude of 40% with 6 minutes of ultrasonication recorded the lowest percentage of fat content, 3.28%. In contrast, the highest fat content, 3,85%, was found in the ultrasonicated milk with an amplitude of 30% in 3 minutes. The control sample (raw milk) recorded the highest fat content (4.00%). The reduction in fat content upon ultrasonication may be attributed to the disruption of the milk fat globule membrane, which reduces the size of fat globules larger than 1 μm and changes the granular surface of the fat globules due to the interactions with casein micelles [6,18].

Table 1. Fat and protein content of control and ultrasonicated milk with two different amplitudes.

Amplitude (%)	Time Taken for Ultrasound Processing (min)	Fat Content (%)	Protein Content (%)
0 (Control)	0	4.00 ± 0.02	**3.28 ± 0.01**
30	3	3.85 ± 0.01	**3.08 ± 0.02**
	6	3.83 ± 0.03	**3.24 ± 0.01**
	9	3.48 ± 0.01	**3.22 ± 0.03**
40	3	3.60 ± 0.02	**3.17 ± 0.02**
	6	3.28 ± 0.04	**3.20 ± 0.02**
	9	**3.70 ± 0.02**	**3.18 ± 0.01**

Under two amplitudes, the lowest percentage of protein content recorded was 3.08% at the amplitude of 30% with 3 minutes of ultrasonic treatment, while the highest protein content, 3.24%, was found in the ultrasonicated milk treated with an amplitude of 30% with 6 minutes. Likewise, raw milk contained higher protein content than the ultrasonically treated milks. According to Thirunavookarasu et al. [19], the ultrasonic cavitation can break apart protein molecules into smaller pieces by interfering with hydrophobic and electrostatic interactions, like hydrogen bonds and van der Waals forces among protein three-dimensional complex structures. Shokri et al. [20] reviewed the ultrasound (US) treatment and indicated that either controlled or moderate US treatment caused structural disruption of the proteins and reduced milk protein particle size.

3.2. Microbial Count

Table 2 depicts the total bacteria count (TBC), total coliform count, counts for *Escherichia coli*, *Staphylococcus aureus,* and *Salmonella spp*. in the milk samples. Based on the findings, Salmonella spp. and *Staphylococcus aureus* were not detected in raw milk and ultrasonicated samples. The *Escherichia coli* count in all milk samples was below 3 CFU/g.

The control and ultrasonically-treated milk samples under 30% amplitude, with 3, 6, and 9 minutes and samples under 40% amplitude with 3 minutes showed more than 2400 CFU/g of the total coliform count. As opposed to this, samples treated under 40% amplitude with 6 and 9 minutes had total coliform counts of 1100 CFU/g, which were lower than those of other samples. The findings were consistent with those of Li et al. [21], who also noted that extended sonication times resulted in a higher elimination of microbial count, especially for *E. coli* and *Staphylococcus aureus*. However, the control raw milk recorded the lowest total bacterial count (58000 CFU/g) compared to the treated samples. Cross-contamination may have occurred during the sample preparation.

3.3. Physicochemical Properties

A few physicochemical properties of the milk samples were measured, such as pH, titratable acidity, colour, specific gravity, viscosity, and particle size measurement.

3.3.1. pH

Table 3 presents the pH of control and ultrasonicated milk samples. The raw milk sample showed a pH value of 6.76 ± 0.04, the highest of the samples. A standard pH of milk is 6.6 and 6.8, which is slightly acidic but close to neutral. This is because the bacteria (lactic acid bacteria) present in the milk convert the lactose into lactic acid [22]. It can be deduced that the high intensity of ultrasound treatment on the raw milk decreased the pH under different amplitudes and with different durations. Milk treated with ultrasonication, under amplitude 30% for 9 minutes, obtained the lowest pH, 6.62 ± 0.02. among the other samples. However, there were no significant differences in the pH of the ultrasound-treated milk with raw milk ($p>0.05$), as the pH obtained was still within the range of the standard milk pH. Apart from this, samples treated with an amplitude of 30% and 40% in 6 and 9 minutes are not significantly different from each other.

Table 2. Microbial count of control and ultrasonicated milk.

Amplitude (%)	Time (min)	Total Bacteria Count (CFU/g)	Total Coliform Count (CFU/g)	*Escherichia coli* Count (CFU/g)	*Staphylococcus aureus* Count (CFU/g)	*Salmonella* spp.
0 (Control)	0	58000	>2400	<3	0	Negative
30	3	78000	>2400	<3	0	Negative
	6	76000	>2400	<3	0	Negative
	9	70000	>2400	<3	0	Negative
40	3	68000	>2400	<3	0	Negative
	6	60000	1100	<3	0	Negative
	9	60000	1100	<3	0	Negative

Table 3. pH of the Ultrasonicated Milk with Control.

Amplitude (%)	Time (min)	pH
0 (Control)	0	**6.76 ± 0.04^{b}**
30	3	**6.68 ± 0.03^{ab}**
	6	**6.65 ± 0.05^{a}**
	9	**6.62 ± 0.02^{a}**
40	3	**6.67 ± 0.03^{ab}**
	6	**6.64 ± 0.04^{a}**
	9	**6.64 ± 0.04^{a}**

*Value represents mean ± standard deviation for which n=3.
*Different letters of the alphabet (a, b, c) in the same column indicate a significant difference ($p<0.05$) between samples.

3.3.2. Titratable acidity

The titratable acidity of milk samples treated with ultrasonication is presented in Table 4. The control milk sample recorded the lowest acidity, 0.13%, among the samples. Typically, raw milk has a 0.14% to 0.16% initial natural acidity and contains traces of lactic acid. According to Tona et al. [23], a crucial test for evaluating the quality of raw milk is the measurement of titratable acidity (lactic acid). The

percentage (%) of lactic acid in raw milk is the titratable acidity. The titratable acidity of the milk samples with 30% amplitude for 9 minutes and 40% amplitude for 3 minutes was 0.15%, respectively, significantly higher than that of other milk samples (p<0.05). There was no significant difference between the other four ultrasonicated samples (p>0.05).

3.3.3. Colour

Based on Table 5, all the milk samples recorded a high L* value of 93, indicating a light colour of the samples (white). Although ultrasonic processing of raw milk minimally impacted the lightness of the milk, significant differences in L* value were shown between the control and treated samples. The highest L* value was recorded for the sample with an amplitude of 40% for 9 minutes, 95.63 ± 0.01, significantly different from the other samples (p<0.05). Similarly, raw milk (control) obtained the lowest L* value of 93.24 ± 0.03, noticeably lower than the treated samples. For a* value, all the samples had negative values, indicating that they all were showing a weak redness. The highest a* value recorded was -1.50 ± 0.01, with 30% for 3 and 6 minutes, respectively, higher than other samples (p<0.05). Likewise, all the samples recorded a positive b* value, indicating that all the ultrasonically treated milk samples had a yellowish-white appearance. All the treated samples had a higher b* value than the raw milk (10.07 ± 0.01) and were significantly different from each other (p<0.05). The exception was exhibited for the sample treated with 30% amplitude for 6 minutes, which had no significant difference from the control (p>0.05).

Table 4. Titratable acidity of the control and ultrasonicated milk.

Amplitude (%)	Time (min)	Titratable Acidity[1]
0 (Control)	0	**0.13 ± 0.01[a]**
30	3	**0.14 ± 0.01[b]**
	6	**0.14 ± 0.02[b]**
	9	**0.15 ± 0.01[d]**
40	3	**0.15 ± 0.01[c]**
	6	**0.14 ± 0.02[b]**
	9	**0.14 ± 0.02[b]**

*Value represents mean ± standard deviation for which n=3.
*Different letters of the alphabet (a, b, c) in the same column indicate a significant difference (p<0.05) between samples.
[1] Expressed as % of lactic acid.

3.3.4. Specific gravity

Table 6 illustrates the specific gravity of raw and ultrasonicated milk samples. Based on Table 6, the milk sample with 30% amplitude in 6 minutes recorded the highest specific gravity, 1.04 ± 0.01. It was comparable to the specific gravity of raw milk and another treated sample (amplitude 40%, 6 minutes) (p>0.05), but significantly different from the other samples (p<0.05).

Since fat is the lighter component, it lowers specific gravity. Milk's specific gravity varies with temperature. It gets smaller as the temperature rises, as hot temperatures cause the proteins to hydrate [18]. The specific gravity of the ultrasound-treated milk varies from one another, maybe due to the surrounding temperature change and the handling method.

Table 5. Colour (L*, a* and b*) of the control and ultrasonicated milk.

Amplitude (%)	Time (min)	Color		
		L*	a*	b*
0 (Control)	0	93.24 ± 0.03^{a}	-1.42 ± 0.01^{c}	**10.07 ± 0.01^{a}**
30	3	93.24 ± 0.03^{b}	-1.50 ± 0.01^{a}	**10.11 ± 0.01^{c}**
	6	93.44 ± 0.01^{b}	-1.50 ± 0.01^{a}	**10.05 ± 0.01^{a}**
	9	93.41 ± 0.00^{b}	-1.43 ± 0.01^{b}	**10.08 ± 0.01^{b}**
40	3	93.43 ± 0.01^{b}	-1.45 ± 0.01^{b}	**10.18 ± 0.01^{d}**
	6	93.59 ± 0.01^{c}	-1.34 ± 0.01^{d}	**10.27 ± 0.01^{f}**
	9	**93.63 ± 0.01^{d}**	**-1.36 ± 0.01^{e}**	**10.21 ± 0.01^{e}**

*Value represents mean ± standard deviation for which n=3.
*Different letters of the alphabet (a, b, c) in the same column indicate a significant difference (p<0.05) between samples.

Table 6. Specific gravity of the control and ultrasonicated milk.

Amplitude (%)	Time (min)	Specific Gravity
0 (Control)	0	**1.02 ± 0.01^{a}**
30	3	**1.02 ± 0.01^{b}**
	6	**1.04 ± 0.01^{a}**
	9	**1.02 ± 0.02^{c}**
40	3	**1.02 ± 0.02^{b}**
	6	**1.03 ± 0.02^{a}**
	9	**1.02 ± 0.02^{c}**

*Value represents mean ± standard deviation for which n=3.
*Different letters of the alphabet (a, b, c) in the same column indicate a significant difference (p<0.05) between samples.

3.3.5. Viscosity

Based on Table 7, the highest viscosity was recorded for the raw milk with a value of 542.33 ± 2.52. It significantly differed from all the ultrasonicated milk samples (p<0.05). In addition, the viscosity of treated milk reflected that the viscosity gradually increases when the amplitude increases with the treatment time. All the viscosities of milk samples were significantly different from each other (p<0.05). Based on the results, the viscosity of ultrasonicated milk was associated with milk protein since there was a decrease in value compared to the control, the raw milk, but eventually increased with increasing time and amplitude. The increase in viscosity may be due to the decrease in milk fat globule size and greater dispersity of particles caused by ultrasonic treatment [5,6]. This outcome signifies that the smaller fat droplets agglomerate and emulsify, having higher resistance to flow [5,24].

3.3.6. Particle size distribution

Table 8 demonstrates the diameter of milk fat globules, reflecting milk samples' particle size distribution. The smallest particle size distribution of the fat globules obtained in ultrasonicated milk samples was 322.5 nm, treated with 30% amplitude for 6 minutes, while the largest particle size was 596.1 nm under 40% amplitude for 6 minutes. The changes in interfacial and electrostatic properties can be used to explain why the milk fat globule diameter increased even after ultrasonic treatment with a longer time and higher amplitude [5]. As a result, it may have a more profound impact on the stability and characteristics of milk, making it more susceptible to integration and consolidation [5,25].

Table 7. Viscosity of the control and ultrasonicated milk.

Amplitude (%)	Time (min)	Viscosity (mPa.s)
0 (Control)	0	**542.33 ± 2.52^{g}**
30	3	**87.13 ± 1.42^{a}**
	6	**183.83 ± 0.15^{b}**
	9	**300.43 ± 0.86^{c}**
40	3	**320.33 ± 0.59^{d}**
	6	**408.43 ± 0.61^{f}**
	9	**377.73 ± 0.57^{e}**

*Value represents mean ± standard deviation for which n=3.
*Different letters of the alphabet (a, b, c) in the same column indicate a significant difference (p<0.05) between samples.

Table 8. Diameter of milk fat globule for milk samples after ultrasound treatment for 3, 6 and 9 minutes.

Amplitude (%)	Time (min)	Diameter of Milk Fat Globule (nM)
0 (Control)	0	**709.7 ± 0.05**
30	3	**446.5 ± 0.04**
	6	**322.5 ± 0.03**
	9	**358.4 ± 0.01**
40	3	**411.4 ± 0.03**
	6	**596.1 ± 0.02**
	9	**472.6 ± 0.01**

*Value represents mean ± standard deviation for which n=3.
*Different letters of the alphabet (a, b, c) in the same column indicate a significant difference (p<0.05) between samples.

However, from the results, the particle size of the fat globule in the control sample was recorded as the largest among the samples, which was 709.7nm. As natural emulsifying agents, hydrophobic and hydrophilic radicals on the surface of milk fat globules (MFGs) are crucial in avoiding globule flocculation and coalescence and maintaining the charge balance between milk protein and fat globules [6,24]. Because of the charge difference between MFGs and serum protein, acid caused milk protein to adhere to MFG surfaces with greater tenacity [26].

3.3.7. Zeta Potential

According to Table 9, all the milk samples that underwent ultrasonic treatment, including the raw milk (control), were categorised as flocculation or coagulation since all the zeta potential values were between 0 and ±5 mV. One of the reasons for showing these results was due to the agglomeration. The nanoparticles with the large surface area create high total surface energy, which is thermodynamically unfavourable [27]. Following that, the particles tend to agglomerate to minimise the surface energy. Agglomeration can cause various issues for nanosuspensions, including rapid settling/creaming, crystal growth and inconsistent dosing [5,24].

3.3.8. Foam Structure

Based on Table 10, at time t = 0 minutes, for all the samples, air bubbles were round, and as the destabilisation process progressed, their shape gradually changed [5]. After 10 minutes of foaming, the air bubbles were polygonal. Due to disproportion, coalescence, and draining, the size of the air bubble increased (the size distribution curve shifted to the right side) [28]. Based on the results above, the milk sample treated with an amplitude of 40% for 6 minutes and 9 minutes recorded the highest value of

foam destabilisation after 10 minutes compared to the other samples, including the control raw milk. The findings were similar to those of Binti Maklin et al. [5], in which the larger fat globules present in milk samples cannot maintain the air bubbles at the air-liquid interface, causing a rapid destabilisation.

Table 9. Zeta potential of milk samples treated with ultrasonication under 3, 6 and 9 minutes with amplitudes of 30% and 40%.

Amplitude (%)	Time (min)	Zeta Potential (mV)
0 (Control)	0	**-0.57 ± 219**
30	3	**-1.45 ± 216**
	6	**-0.33 ± 207**
	9	**-0.30 ± 210**
40	3	**-0.47 ± 196**
	6	**0.28 ± 209**
	9	**-0.54 ± 207**

Table 10. Foam structure of each milk sample was treated with ultrasonication under 3, 6, and 9 minutes with amplitudes of 30% and 40%.

Amplitude (%)	Time (min)	Foam Structure	
		0 minute	**10 minutes**
0 (Control)	0	448.64 ± 388.87	**1087.67 ± 1149.36**
30	3	1005.54 ± 1459.19	**978.29 ± 243.81**
	6	97.56 ± 280.10	**412.75 ± 865.08**
	9	125.83 ± 192.73	**181.15 ± 405.17**
40	3	167.15 ± 95.82	**6082.13 ± 2039.23**
	6	216.00 ± 228.47	**4583.61 ± 5144.82**
	9	**62.27 ± 107.78**	**716.41 ± 629.08**

4. Conclusion

This study demonstrates the potential of ultrasound treatment as an innovative, non-thermal method for enhancing raw milk's physicochemical and microbial quality. The results showed that higher amplitude and longer sonication time, particularly at 40% amplitude for 6 minutes, significantly improved microbial safety by reducing *E. coli, Staphylococcus spp.,* and coliform counts, indicating effective microbial inactivation. Although changes in pH, titratable acidity, and colour were minimal, significant differences were observed between samples. Most ultrasonically treated samples achieved the standard range for specific gravity between 1.028 and 1.030, except for milk processed at 30% amplitude for 6 minutes.

Furthermore, sonication at 40% amplitude and 6 minutes produced milk with the highest viscosity among the treated samples and demonstrated improved particle size distribution and zeta potential, highlighting better colloidal stability. These improvements support the application of ultrasound as a sustainable and innovative approach in dairy processing, aligning with current goals for safer, higher-quality milk products with minimal environmental impact.

However, the study faced several limitations. The short shelf-life of raw milk required freezing (>10°C), which may have affected protein structure and microbial behaviour during defrosting. Additionally, potential hygiene lapses during sonication may have contributed to environmental

contamination. Future work should focus on optimising cold chain handling and process hygiene to ensure consistent, scalable application of ultrasound technology in sustainable dairy production.

Declaration of Conflict of Interest

The authors declared no conflict of interest with any other party in the publication of the current work.

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References

[1] Z. Asefa, Physical Properties and Chemical Compositions of Raw Cow Milk in Milk Shades around Addis Ababa, Ethiopia, Asefa. Journal of Natural Sciences Research 9(19) (2019) 33–37. https://doi.org/10.7176/JNSR/9-19-04.

[2] A. Merwan, N. Amza, and M. Tamiru, Review on Milk and Milk Product Safety, Quality Assurance and Control. International Journal of Livestock Production 9(4) (2018) 67–78. https://doi.org/10.5897/IJLP2017.0403.

[3] S.B. Gregersen, L. Wiking, and M. Hammershøj, Acceleration of Acid Gel Formation by High Intensity Ultrasound is Linked to Whey Protein Denaturation and Formation of Functional Milk Fat Globule-Protein Complexes. Journal of Food Engineering 254 (2019) 17–24. https://doi.org/10.1016/j.jfoodeng.2019.03.004.

[4] C.F. Balthazar, A. Santillo, J.T. Guimarães, A. Bevilacqua, M.R. Corbo, M. Caroprese, R. Marino, E.A. Esmerino, M.C. Silva, R.S.L. Raices, M.Q. Freitas, A.G. Cruz, and M. Albenzio, Ultrasound Processing of Fresh and Frozen Semi-Skimmed Sheep Milk and its Effects on Microbiological and Physical-Chemical Quality. Ultrasonics Sonochemistry 51 (2018) 241–248. https://doi.org/10.1016/j.ultsonch.2018.10.017.

[5] S.N.B. Maklin, N.B. Julmohammad, S.B. Saallah, S.B. Saalah, N.B.M. Yaakub, M.D.B. Sintang, S.N.B.A. Rahman, and N.B. Julmohamad, Effect of Sonication Time on Physical and Foaming Properties of Pasteurized Milk. International Dairy Journal 161 (2024) 106137. https://doi.org/10.1016/j.idairyj.2024.106137.

[6] N.M. Yaakub, S.M.M. Kamal, N.L. Chin, N. Julmohammad, N. Therdthai, and A. Sulaiman, Optimisation of Non-Thermal Ultrasonication Process for Retaining Nutritional Quality in Cow milk: Effects of Fat Percentage and Treatment Parameters. Advances in Agricultural and Food Research Journal 5 (2024) a0000531. https://doi.org/10.36877/aafrj.a0000531.

[7] L.M. Carrillo-Lopez, I.A. Garcia-Galicia, J.M. Tirado-Gallegos, R. Sanchez-Vega, M. Huerta-Jimenez, M. Ashokkumar, and A.D. Alarcon-Rojo, Recent Advances in the Application of Ultrasound in Dairy Products: Effect on Functional, Physical, Chemical, Microbiological and Sensory Properties. Ultrasonics Sonochemistry 73 (2021) 105467. https://doi.org/10.1016/j.ultsonch.2021.105467.

[8] S. Karlović, T. Bosiljkov, M. Brnčić, D. Semenski, F. Dujmić, B. Tripalo, and D. Ježeka, Reducing Fat Globules Particle-Size in Goat Milk: Ultrasound and High Hydrostatic Pressures Approach. Chemical and Biochemical Engineering Quarterly 28(4) (2014) 499–507. https://doi.org/10.15255/CABEQ.2014.19400.

[9] A. Postelmans, B. Aernouts, J. Jordens, T. Van Gerven, and W. Saeys, Milk Homogenization Monitoring: Fat Globule Size Estimation from Scattering Spectra of Milk. Innovative Food Science & Emerging Technologies 60 (2020) 102311. https://doi.org/10.1016/j.ifset.2020.102311.

[10] T.M. Ho, P. Dhungana, B. Bhandari, and N. Bansal, Effect of the Native fat Globule Size on Foaming Properties and Foam Structure of Milk. Journal of Food Engineering 291 (2020) 110227. https://doi.org/10.1016/j.jfoodeng.2020.110227.

[11] AOAC SMPR® 2014.003 - AOAC INTERNATIONAL, AOAC INTERNATIONAL (2024). https://www.aoac.org/resources/smpr-2014-003-2/.

[12] P. Mudgil, B. Jumah, M. Ahmad, F. Hamed, and S. Maqsood, Rheological, Micro-Structural and Sensorial Properties of Camel Milk Yogurt as Influenced by Gelatin. LWT 98 (2018) 646–653. https://doi.org/10.1016/j.lwt.2018.09.008.

[13] Association of Official Analytical Chemists, Van Nostrand's Encyclopedia of Chemistry, 2005. https://doi.org/10.1002/0471740039.vec0284.

[14] D. Sert, N. Akin, and E. Dertli, Effects of Sunflower Honey on the Physicochemical, Microbiological and Sensory Characteristics in Set Type Yoghurt during Refrigerated Storage. International Journal of Dairy Technology 64 (2010) 99–107. https://doi.org/10.1111/j.1471-0307.2010.00635.x.

[15] J.W. Fuquay, Encyclopedia of Dairy Sciences. Academic Press, Elsevier Ltd, 2011.

[16] T.M. Ho, T.H.A. Le, A. Yan, B.R. Bhandari, and N. Bansal, Foaming Properties and Foam Structure of Milk during Storage. Food Research International 116 (2018) 379–386. https://doi.org/10.1016/j.foodres.2018.08.051.

[17] Y. Yang, N. Nakada, R. Nakajima, M. Yasojima, C. Wang, and H. Tanaka, pH, Ionic Strength and Dissolved Organic Matter Alter Aggregation of Fullerene C60 Nanoparticles Suspensions in Wastewater. Journal of Hazardous Materials 244–245 (2012) 582–587. https://doi.org/10.1016/j.jhazmat.2012.10.056.

[18] M. Kaci, S. Meziani, E. Arab-Tehrany, G. Gillet, I. Desjardins-Lavisse, and S. Desobry, Emulsification by High Frequency Ultrasound using Piezoelectric Transducer: Formation and Stability of Emulsifier Free Emulsion. Ultrasonics Sonochemistry 21 (2013) 1010–1017. https://doi.org/10.1016/j.ultsonch.2013.11.006.

[19] N. Thirunavookarasu, S. Kumar, A. Anandharaj, and A. Rawson, Effect of Ultrasonic Cavitation on the Formation of Soy Protein Isolate – Rice Starch Complexes, and the Characterization and Prediction of Interaction Sites using Molecular Techniques. Heliyon 8 (2022) e10942. https://doi.org/10.1016/j.heliyon.2022.e10942.

[20] S. Shokri, F. Javanmardi, M. Mohammadi, and A.M. Khaneghah, Effects of Ultrasound on the Techno-Functional Properties of Milk Proteins: A Systematic Review. Ultrasonics Sonochemistry 83 (2022) 105938. https://doi.org/10.1016/j.ultsonch.2022.105938.

[21] W. Li, T.S.H. Leong, M. Ashokkumar, and G.J.O. Martin, A Study of the Effectiveness and Energy Efficiency of Ultrasonic Emulsification. Physical Chemistry Chemical Physics 20 (2017) 86–96. https://doi.org/10.1039/c7cp07133g.

[22] H. Sharma, F. Ozogul, E. Bartkiene, and J.M. Rocha, Impact of Lactic Acid Bacteria and their Metabolites on the Techno-Functional Properties and Health Benefits of Fermented Dairy Products. Critical Reviews in Food Science and Nutrition 63 (2021) 4819–4841. https://doi.org/10.1080/10408398.2021.2007844.

[23] G.O. Tona, I.C. Oladipo, and S.O. Oseni, Assessment of Physicochemical and Microbiological Quality of Jersey Cattle Raw Milk at Different Stages of Lactation. International Journal of Science and Research 5(6) (2016) 2610–2613. https://doi.org/10.21275/v5i6.28061602.

[24] S.N. Maklin, N. Julmohammad, S. Saallah, S. Saalah, M.J.H. Akanda, S.N.A. Rahman, and N. Julmohamad, Techniques for Nanoemulsion in Milk and its Application: A Review. International Food Research Journal 32(1) (2025). https://doi.org/10.47836/ifrj.32.1.01.

[25] Y. Sun, Y.H. Roos, and S. Miao, Comparative Study of Interfacial Properties and Thermal Behaviour of Milk Fat Globules and Membrane Prepared from Ultrasonicated Bovine Milk. Ultrasonics Sonochemistry 102 (2024) 106755. https://doi.org/10.1016/j.ultsonch.2024.106755.

[26] Y. Sun, Y.H. Roos, and S. Miao, Changes in Milk Fat Globules and Membrane Proteins Prepared from pH-Adjusted Bovine Raw Milk. Foods 11 (2022) 4107. https://doi.org/10.3390/foods11244107.

[27] L. Wu, J. Zhang, and W. Watanabe, Physical and Chemical Stability of Drug Nanoparticles. Advanced Drug Delivery Reviews 63 (2011) 456–469. https://doi.org/10.1016/j.addr.2011.02.001.

[28] S. Damodaran, Protein Stabilization of Emulsions and Foams. Journal of Food Science 70 (2006) R54–R66. https://doi.org/10.1111/j.1365-2621.2005.tb07150.x.