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Non-Thermal Strategies in Dairy Processing: Enhancing the Quality of Cow, Sheep, Goat, and Buffalo Milk via Sonication and Thermosonication

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Abstract

This study comprehensively evaluates the effects of pasteurization, sonication, and thermosonication (TS) on the physicochemical, bioactive, and microbiological properties of four milk varieties: cow, sheep, goat, and buffalo. Pasteurization was performed at 90 °C for 1 minute; sonication was applied for 5 to 30 minutes. TS treatments were conducted at temperatures between 40 and 60 °C for varying times. A multidimensional quality assessment included enzymatic activities (polyphenol oxidase [PPO] and pectin methyl esterase [PME]), microbial inactivation targeting Escherichia coli ATCC 25922, total phenolic content (TPC), antioxidant activity (DPPH assay), hydroxymethylfurfural (HMF) content, and turbidity. Thermal pasteurization led to a marked reduction in TPC and antioxidant capacity; for instance, cow milk TPC decreased from 468.19 to 373.18 mg/100 mL, and antioxidant activity declined from 5.99 to 3.48 mg/100 mL. In contrast, sonication enhanced the retention of bioactive compounds, particularly at longer exposure times. Thermosonication further amplified these benefits by significantly reducing enzymatic activity and microbial load. TS at 60 °C demonstrated the highest efficacy, achieving over a 5-log reduction in *E. coli* while reducing PPO and PME activities to 0.02 and 2.11 U/mL, respectively, compared to 1.30 and 7.14 U/mL in untreated sheep milk. Additionally, this condition preserved high antioxidant activity (5.67 mg/100 mL) and produced only moderate HMF levels (1.89 mg/100 mL), indicating minimal thermal degradation. Overall, thermosonication emerged as a superior non-thermal processing technique, effectively ensuring microbial safety and enzymatic deactivation while preserving the functional quality of milk. These findings underscore its potential as an alternative to conventional pasteurization.

Keywords: Bioactive compounds, cavitation, milk quality, microbial inactivation, non-thermal processing, thermosonication

1.INTRODUCTION

Milk is a highly nutritious and perishable liquid food, composed of water, proteins, lipids, lactose, vitamins, and minerals. Due to its favorable composition and neutral pH, it provides conditions conducive to microbial growth, compromising both safety and shelf life (Givens, 2020). To ensure microbiological safety and extend shelf stability, thermal pasteurization remains the most commonly used preservation technique in the dairy industry. Conventional high-temperature short-time (HTST) pasteurization, typically carried out at 72 °C for 15 seconds, or higher-temperature treatments such as 90 °C for 1 minute, effectively reduce pathogenic and spoilage microorganisms (Rabbani et al., 2025). However, these thermal processes can negatively affect milk quality by altering its sensory characteristics, degrading heat-sensitive nutrients, and causing structural changes to proteins (Duan et al., 2025).

In response to consumer demand for minimally processed foods with preserved nutritional and functional properties, alternative processing technologies are being actively explored. Among these, ultrasound, or sonication, has gained interest as a non-thermal processing technique.

Sonication involves the application of sound waves above 20 kHz, which generate a phenomenon known as acoustic cavitation (Yildiz & Feng, 2019). This process involves the formation and collapse of microbubbles in a liquid medium, leading to the release of localized high temperatures (up to 5000 K), high pressures (up to 1000 atm), and intense shear forces. These conditions disrupt microbial cells and inactivate certain enzymes without relying on high temperatures, thereby minimizing thermal degradation of sensitive compounds (Yildiz, 2021).

Numerous studies have evaluated ultrasound's effects in milk and dairy systems, demonstrating its capacity to reduce microbial load (Zupanc et al., 2019), modulate enzymatic activities such as lipase (Manzoor et al., 2021), and maintain or enhance antioxidant properties (Wu et al., 2021). However, sonication alone often falls short of achieving regulatory microbial safety levels in viscous or highly contaminated milk, necessitating combined approaches. To this end, thermosonication — the simultaneous application of ultrasound with moderate heat (40–60 °C) — has emerged as a promising alternative (Abdulstar et al., 2023). Thermosonication synergistically enhances microbial inactivation and enzyme denaturation while better preserving milk's nutritional and sensory qualities compared to conventional heat treatments (Silva et al., 2025; White et al., 2025). Studies have reported effective reductions in spoilage microorganisms, along with retention of total phenolic content and antioxidant activity in thermosonicated dairy products (Manzoor et al., 2021; Wu et al., 2021; Xu et al., 2023; Kalsi et al., 2023).

While polyphenol oxidase (PPO) and pectin methyl esterase (PME) are enzymes predominantly associated with plant tissues and fruits, their potential presence and activity in dairy products have not been extensively documented. It is possible that these enzymes could be introduced into milk through animal feed or may arise due to enzymatic transformations during milk processing and storage. In this study, we take an exploratory approach by measuring PPO and PME activities to assess whether they could serve as novel indicators of enzymatic and oxidative changes affecting milk quality. We acknowledge that there is currently limited information on PPO and PME activity in fresh milk and highlight the need for further research to clarify their roles and significance in dairy systems.

The present study aims to evaluate the effects of three processing methods—thermal pasteurization, sonication, and thermosonication—on the quality attributes of four types of milk: cow, goat, sheep, and buffalo. The investigation includes assessments of enzymatic activity (polyphenol oxidase and pectin methyl esterase), total phenolic content (TPC), antioxidant activity (measured by the DPPH radical scavenging method), hydroxymethylfurfural (HMF) formation, turbidity, and microbial inactivation efficiency against Escherichia coli ATCC 25922. By comparing the outcomes of these treatments, the study seeks to determine the potential of sonication-based techniques as sustainable alternatives to conventional pasteurization, supporting the development of dairy products with improved functional and safety profiles.

2. MATERIALS AND METHODS

2.1. Milk Sample Collection

Fresh raw milk from four animal sources—cow, sheep, goat, and buffalo—was obtained from local producers in Iğdır, Turkey. Upon collection, the samples were promptly transferred to the laboratory in refrigerated conditions and stored at 4 ± 0.5 °C to maintain their inherent physicochemical and microbiological characteristics before processing and analysis.

2.2. Preparation of Escherichia coli Inoculum

The standard bacterial strain Escherichia coli ATCC 25922 was used to assess microbial inactivation performance. The strain was stored

at –80 °C in glycerol stocks and revived by streaking onto tryptic soy agar (TSA) plates. Plates were incubated at 37 °C for 24 hours. A single colony was then inoculated into 10 mL of tryptic soy broth (TSB) and incubated at 37 °C with agitation at 150 rpm until reaching the stationary growth phase, typically after 16–18 hours. Growth phase was confirmed by measuring the optical density at 600 nm (OD_{600}) and by plate counting to estimate colony-forming units per milliliter (CFU/mL), following the standard plate count method (Esua et al., 2022).

For inoculation, milk samples were aseptically inoculated with the prepared *E. coli* culture to achieve an initial microbial load of approximately 10^6 CFU/mL. After inoculation, samples were gently mixed to ensure uniform distribution of the bacteria. Microbial enumeration in milk before and after treatments was conducted by serial dilution in sterile peptone water, followed by spread plating on TSA. Plates were incubated at 37 °C for 24 hours before colonies were counted and results expressed as CFU/mL.

2.3. Conventional Thermal Pasteurization

Thermal treatment was applied using a thermostatically regulated water bath. Milk samples were heated in double-jacketed glass vessels to a target temperature of 90 °C, maintained for 1 minute, and then rapidly cooled in an ice-water bath to halt further thermal impact and preserve quality attributes.

2.4. Sonication and Thermosonication Procedures

Ultrasound-based treatments were conducted using an ultrasonic bath (Wise clean, WUC-A10H) operating at a frequency of 53 kHz with a nominal power output of 500 W. Milk samples of 50 mL were placed in sealed glass containers and fully immersed in the ultrasonic bath. For sonication alone, samples were treated at ambient temperature (~25 °C) for 5, 10, 20, or 30 minutes. For thermosonication (TS), milk samples were pre-heated to the target temperatures of 40, 50, or 60 °C by placing the sealed containers in a thermostatically regulated water bath prior to sonication. Temperature was continuously monitored using a digital probe inserted into a control sample container within the ultrasonic bath to ensure uniform heating. The ultrasonic bath water temperature was adjusted as necessary to maintain the desired sample temperature (±0.5 °C) throughout the treatment. Thermosonication treatments were applied for the same time intervals as sonication alone (5, 10, 20, and 30 minutes). Samples were gently stirred manually every 5 minutes to promote uniform exposure to ultrasound and heat.

To systematically investigate the effects of key variables (milk type, treatment time, and temperature) while reducing the number of experimental runs, the Taguchi method was employed as a structured design-of-experiments (DoE) approach. An L16 orthogonal array was selected to ensure a balanced representation of treatment combinations and to efficiently cover the experimental space without requiring a full factorial design. A summary of the selected sample codes and corresponding treatment conditions is presented in Table 1. A complete list of all treatment combinations and experimental conditions is provided in the supplementary material.

Table 1. Experimental Design Based on Taguchi L16 Orthogonal Array

Sample Code	Milk Type	Treatment Description
CM	Cow	Unprocessed cow milk
SM	Sheep	Unprocessed sheep milk
GM	Goat	Unprocessed goat milk
BM	Buffalo	Unprocessed buffalo milk
P-CM	Cow	Thermal pasteurization at 90 °C for 1 min

P-SM	Sheep	Thermal pasteurization at 90 °C for 1 min
P-GM	Goat	Thermal pasteurization at 90 °C for 1 min
P-BM	Buffalo	Thermal pasteurization at 90 °C for 1 min
U-CM1	Cow	Sonication for 5 min at ambient temperature
U-SM1	Sheep	Sonication for 5 min at ambient temperature
U-GM2	Goat	Sonication for 10 min at ambient temperature
U-BM2	Buffalo	Sonication for 10 min at ambient temperature
U-CM3	Cow	Sonication for 20 min at ambient temperature
U-BM3	Buffalo	Sonication for 20 min at ambient temperature
U-SM4	Sheep	Sonication for 30 min at ambient temperature
U-GM4	Goat	Sonication for 30 min at ambient temperature
T-CM1	Cow	Thermosonication at 40 °C for 5 min
T-GM3	Goat	Thermosonication at 40 °C for 20 min
T-BM4	Buffalo	Thermosonication at 40 °C for 30 min
T-SM7	Sheep	Thermosonication at 50 °C for 20 min
T-GM8	Goat	Thermosonication at 50 °C for 30 min
T-CM10	Cow	Thermosonication at 60 °C for 10 min
T-SM10	Sheep	Thermosonication at 60 °C for 10 min
T-BM11	Buffalo	Thermosonication at 60 °C for 20 min

2.5. Turbidity Measurement

Turbidity, used to evaluate the colloidal stability of milk, was determined by centrifuging 6 mL of each sample at 3300 × g for 10 minutes at 25 °C. The supernatant's absorbance was then measured at 600 nm using a UV-Vis spectrophotometer, following the method of Lee et al. (2016).

2.6. Polyphenol Oxidase (PPO) Activity

PPO activity was measured based on a modified method by Abid (2014). Milk samples were centrifuged at 10,000 × g for 10 minutes at 4 °C. A 3 mL reaction mixture was prepared, consisting of 100 mM phosphate buffer (pH 6.5) and 0.07 M catechol as the substrate. The absorbance of the reaction was monitored at 410 nm. PPO activity was expressed as the percentage of residual activity relative to untreated control samples.

2.7. Pectin Methylesterase (PME) Activity

PME activity was quantified by titrating a 0.5% (w/v) citrus pectin solution with 0.05 N NaOH while maintaining the pH at 7.7. The release of carboxyl groups was recorded and expressed as mmol of carboxyl groups released per minute per milliliter of milk sample, based on the method reported by Abid (2014).

2.8. Total Phenolic Content (TPC)

The total phenolic content of milk was assessed using the Folin–Ciocalteu colorimetric method described by Mtaoua et al. (2017). Aliquots of centrifuged milk were mixed with Folin–Ciocalteu reagent and sodium carbonate solution. After incubation, absorbance was measured at 725 nm. Results were expressed as mg gallic acid equivalents (GAE) per 100 mL of milk.

2.9. Antioxidant Activity (DPPH Assay)

The antioxidant capacity was evaluated using the DPPH radical scavenging method described by Maisuthisakul et al. (2007). Samples were

reacted with a DPPH methanolic solution, and the decrease in absorbance at 515 nm was recorded. The antioxidant activity was expressed as mg Trolox equivalents (TE) per 100 mL of milk.

2.10. Hydroxymethylfurfural (HMF) Content

HMF content, an indicator of thermal degradation, was determined following the procedure of Cohen et al. (1998). Milk samples were mixed with ethanol, centrifuged, and reacted with thiobarbituric acid and trichloroacetic acid. The absorbance was measured at 443 nm, and concentrations were calculated using a standard calibration curve. Results were reported as mg HMF per 100 mL of milk.

2.11. Statistical Analysis

All measurements were conducted in triplicate. Statistical analyses were performed using JMP Statistical Discovery Software (Version 7.0). One-way analysis of variance (ANOVA) was applied to determine significant differences ($p < 0.05$), and mean comparisons were conducted using the Least Significant Difference (LSD) test.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Properties of Raw and Treated Milk Samples

The physicochemical, enzymatic, and bioactive properties of milk samples subjected to pasteurization, sonication, and thermosonication treatments are summarized in Table 2.

Table 2. Enzymatic activities (PPO and PME), total phenolic content, antioxidant activity, hydroxymethylfurfural (HMF), and turbidity of milk samples from cow (CM), sheep (SM), goat (GM), and buffalo (BM) before (raw), pasteurized (P-), ultrasound-treated (U-), and thermosonicated (T-) at various conditions

Sample Name	PPO (U/mL)	PME (U/mL)	Total Phenolic Content (mg/100 mL)	Antioxidant Activity (mg/100 mL)	HMF (mg/100 mL)	Turbidity
CM	0.2 ± 0.02 (a)	2.66 ± 0.47 (a)	468.19 ± 21 (b)	5.99 ± 0.82 (a)	–	0.55 ± 0.03 (b)
SM	1.3 ± 0.02 (a)	7.14 ± 0.63 (a)	548.04 ± 77 (a)	7.55 ± 0.41 (a)	–	0.44 ± 0.12 (c)
GM	0.5 ± 0.01 (a)	3.57 ± 0.09 (a)	492.65 ± 12 (a)	6.23 ± 0.12 (a)	–	0.21 ± 0.09 (d)
BM	0.80 ± 0.03 (a)	5.69 ± 0.51 (a)	507.16 ± 23 (a)	6.88 ± 1.11 (a)	–	0.15 ± 0.16 (c)
P-CM	0.15 ± 0.05 (a)	1.95 ± 0.42 (b)	373.18 ± 11 (d)	3.48 ± 0.14 (e)	1.65 ± 0.43 (a)	0.56 ± 0.06 (b)
P-SM	0.5 ± 0.03 (b)	5.48 ± 0.55 (b)	419.23 ± 14 (e)	5.23 ± 0.15 (e)	3.72 ± 0.25 (a)	0.47 ± 0.12 (c)
P-GM	0.3 ± 0.01 (b)	2.72 ± 0.68 (b)	420.25 ± 22 (d)	3.15 ± 0.22 (e)	2.88 ± 0.69 (a)	0.23 ± 0.02 (d)
P-BM	0.21 ± 0.03 (b)	3.65 ± 0.17 (b)	500.12 ± 27 (b)	4.72 ± 0.14 (d)	2.20 ± 0.27 (a)	0.46 ± 0.06 (a)
U-CM1	0.10 ± 0.03 (b)	2.02 ± 0.05 (b)	434.67 ± 14 (c)	4.65 ± 0.33 (d)	1.12 ± 0.09 (b)	0.62 ± 0.07 (a)
U-SM1	0.20 ± 0.01 (c)	5.25 ± 0.09 (b)	527.74 ± 13 (b)	6.14 ± 0.49 (c)	2.23 ± 0.14 (b)	0.55 ± 0.11 (b)
U-GM2	0.15 ± 0.02 (c)	3.88 ± 0.26 (a)	469.19 ± 19 (b)	5.73 ± 0.21 (b)	2.48 ± 0.21 (b)	0.24 ± 0.06 (d)
U-BM2	0.18 ± 0.02 (b)	3.20 ± 0.23 (b)	485.34 ± 13 (c)	5.06 ± 0.17 (b)	1.86 ± 0.19 (b)	0.50 ± 0.14 (a)
U-CM3	0.04 ± 0.03 (c)	1.79 ± 0.33 (c)	464.55 ± 11 (b)	5.16 ± 0.15 (c)	0.89 ± 0.09 (c)	0.63 ± 0.15 (a)
U-BM3	0.15 ± 0.02 (b)	3.91 ± 0.30 (b)	442.18 ± 13 (d)	4.92 ± 0.09 (c)	1.42 ± 0.12 (c)	0.34 ± 0.06 (b)

U-SM4	0.18 ± 0.02 (c)	3.08 ± 0.45 (c)	488.19 ± 11 (c)	5.87 ± 0.19 (d)	2.11 ± 0.13 (b)	0.27 ± 0.02 (d)
U-GM4	0.11 ± 0.01 (c)	2.12 ± 0.16 (c)	444.18 ± 29 (c)	5.26 ± 0.53 (d)	1.67 ± 0.21 (c)	0.36 ± 0.24 (b)
T-CM1	0.08 ± 0.02 (b)	1.15 ± 0.12 (d)	443.24 ± 11 (c)	5.25 ± 0.67 (c)	1.14 ± 0.25 (b)	0.64 ± 0.31 (a)
T-GM3	0.12 ± 0.05 (c)	2.35 ± 0.25 (c)	476.18 ± 9 (b)	5.58 ± 0.11 (c)	1.55 ± 0.17 (c)	0.29 ± 0.28 (c)
T-BM4	0.06 ± 0.01 (c)	1.84 ± 0.30 (c)	487.19 ± 14 (c)	5.16 ± 0.15 (b)	1.14 ± 0.10 (d)	0.40 ± 0.08 (b)
T-SM7	0.02 ± 0.01 (d)	1.01 ± 0.15 (d)	494.65 ± 17 (c)	6.64 ± 0.87 (b)	2.08 ± 0.28 (b)	0.33 ± 0.08 (d)
T-GM8	0.08 ± 0.03 (d)	1.15 ± 0.12 (d)	455.46 ± 17 (c)	5.25 ± 0.67 (d)	1.14 ± 0.25 (d)	0.64 ± 0.31 (a)
T-CM10	0.06 ± 0.02 (c)	1.03 ± 0.15 (d)	496.05 ± 14 (a)	5.58 ± 0.11 (b)	1.55 ± 0.17 (a)	0.35 ± 0.03 (c)
T-SM10	0.02 ± 0.01 (d)	2.11 ± 0.09 (d)	455.46 ± 17 (d)	5.67 ± 0.18 (d)	1.89 ± 0.33 (c)	0.72 ± 0.16 (a)
T-BM11	0.02 ± 0.01 (c)	1.42 ± 0.15 (c)	488.72 ± 11 (c)	4.81 ± 0.12 (c)	0.95 ± 0.11 (e)	0.48 ± 0.06 (a)

a-e: Values are mean ± SD (n=3). Different letters within each column indicate significant differences ($p < 0.05$).

The results reveal clear differences in phenolic content, antioxidant activity, enzymatic activities, HMF levels, and turbidity across the four milk types and their various treatments. Among the raw milks, sheep milk exhibited the highest total phenolic content and antioxidant activity, followed closely by buffalo milk, while goat and cow milks showed comparatively lower levels (Table 2). The variation can be attributed to the differences in the natural composition of each milk type, as sheep and buffalo milk generally have higher fat and protein contents, which can bind and stabilize phenolic compounds, enhancing their extractability and antioxidant effects (Rasheed et al., 2016). Additionally, genetic factors, diet, and lactation stage influence the concentration of bioactive compounds, and sheep and buffalo may naturally synthesize or accumulate more phenolics or antioxidant peptides in their milk (Stobiecka et al., 2022). The higher antioxidant activity corresponds to the greater phenolic content (Yildiz, 2022), suggesting that these compounds contribute significantly to the milk's ability to neutralize free radicals and protect against oxidative stress.

Thermal pasteurization at 90 °C for 1 minute caused a significant reduction in phenolic content and antioxidant activity across all milk types, which is expected due to heat-induced degradation of sensitive compounds. The application of heat accelerates oxidation and breakdown of phenolics and other antioxidants, causing them to lose their bioactivity (Yildiz, 2021). Furthermore, thermal processing can cause structural changes in milk proteins, which may result in the release or binding of phenolics, affecting their availability and measurement (Khongphakdee et al., 2025). Pasteurized milks also showed increased HMF levels compared to raw samples, indicating some degree of sugar breakdown and Maillard reaction product formation as a result of heating. HMF is a known marker of heat damage formed by the dehydration of hexose sugars, and its accumulation during pasteurization confirms the exposure to high temperatures (Choudhary et al., 2020). The enzymatic activities of polyphenol oxidase (PPO) and pectin methyl esterase (PME) were effectively reduced by pasteurization, confirming the efficiency of heat treatment for enzyme inactivation. Heat denatures these enzymes, preventing them from catalyzing reactions that degrade milk quality or cause browning during storage. However, the loss of enzymes may also diminish some functional benefits related to natural enzymatic activity.

Sonication treatments, applied at ambient temperature for varying durations, demonstrated a beneficial effect in preserving phenolic compounds and antioxidant capacity compared to pasteurized samples, though still lower than raw milks. Ultrasound treatment

induces cavitation (the formation and collapse of microbubbles)that can physically disrupt cells or particles, releasing bound phenolics and improving extraction efficiency without significant heat generation. This mild physical disruption can increase the bioavailability of antioxidants while avoiding thermal degradation. For example, buffalo and sheep milks treated with longer sonication times showed improved retention of phenolics and antioxidant activity, suggesting that ultrasound processing can be a gentler alternative that better maintains nutritional quality while reducing enzymatic activity. However, prolonged sonication can also generate free radicals and localized heating that might partially degrade sensitive compounds, which explains why the antioxidant levels do not fully match those of raw milks. Turbidity values after sonication were slightly increased compared to raw milks, likely due to physical changes in milk structure caused by ultrasonic cavitation, which can cause protein denaturation, fat globule size reduction, or particle aggregation, leading to higher light scattering (Yıldız, 2018).

Thermosonication treatments, which combine mild heat (40–60 °C) with ultrasound for different time intervals, effectively reduced enzymatic activities while moderately preserving bioactive compounds. The application of moderate heat along with sonication enhances enzyme inactivation more efficiently than sonication alone by denaturing enzyme proteins and disrupting their active sites, while the ultrasound cavitation improves heat transfer and exposes enzymes to denaturing conditions. Phenolic content and antioxidant activity were generally higher than in pasteurized milk but slightly lower than in raw milk, striking a balance between microbial safety and nutritional quality. The milder temperature regimes limit heat-induced degradation reactions, while the physical effects of ultrasound promote the release of phenolics. HMF levels remained lower than those observed in pasteurized milks, indicating that the lower temperatures used in thermosonication prevent excessive sugar degradation and Maillard reaction progress. Turbidity changes were variable but generally showed acceptable stability, suggesting minimal negative impact on milk appearance, as moderate heat can reduce protein aggregation, and ultrasound prevents the formation of large particles that cause cloudiness.

3.2. Inactivation of Escherichia coli ATCC 25922 in Milk Samples

The microbial inactivation efficiency of different processing methods applied to milk samples was assessed by evaluating the log reduction of Escherichia coli ATCC 25922. Conventional thermal pasteurization at 90 °C for 1 min effectively achieved ≥5-log reductions across all milk types (Figure 1), aligning with widely accepted safety standards and confirming its reliability in inactivating heat-sensitive pathogens (Koutsoumanis et al., 2022). This level of inactivation is attributed to the denaturation of vital enzymes and proteins, as well as the disruption of membrane integrity caused by high temperatures, leading to irreversible microbial damage (Pegu & Arya, 2023).

In contrast, ultrasound (US) treatments performed at ambient temperature for up to 30 minutes resulted in only partial microbial reduction, with log reductions ranging between 3.0 and 4.4, depending on the milk type (Figure 1). This moderate efficacy is consistent with earlier studies suggesting that ultrasound alone primarily causes sublethal injury through mechanical effects such as cavitation, shear stress, and microstreaming, but may not be sufficient to destroy all cells unless combined with additional lethal factors (Peterson & Pitt, 2000; Huang et al., 2017; Luo et al., 2023). The observed variation in log reduction among different milk types may be influenced by compositional differences such as fat and protein content, which can act as protective

barriers against acoustic cavitation, thereby limiting the ultrasound's effectiveness.

Thermosonication (TS), which combines moderate heat (50–60 °C) with ultrasound, significantly enhanced microbial inactivation across all milk samples, achieving consistent log reductions above the 5-log safety threshold (Figure 1). This synergistic effect likely results from the combined action of thermal stress and acoustic cavitation. While heat increases microbial membrane fluidity and weakens cellular defenses, ultrasound-induced cavitation generates intense local shear forces, microjets, and transient high temperatures, collectively leading to enhanced cell lysis (Yıldız, 2022). Moreover, the increased diffusivity of dissolved gases and localized pressure changes during TS may contribute to oxidative stress and further compromise microbial viability (Zupanc et al., 2019).

Interestingly, goat and buffalo milk treated with TS exhibited slightly higher log reductions compared to cow milk. This may be explained by the smaller fat globule size and higher medium-chain fatty acid content in goat milk, which may facilitate more effective acoustic energy transmission and enhance cavitation intensity (Yildiz & Feng, 2019). In buffalo milk, the higher total solids and protein concentration may increase the viscosity and thermal conductivity, resulting in more uniform energy dispersion and prolonged retention of thermal and mechanical energy during processing (Mejares et al., 2022). These compositional attributes could potentially enhance the mechanical disruption of microbial cells under TS conditions.

Overall, these findings confirm thermosonication as a promising non-thermal or minimally thermal alternative to conventional pasteurization. It not only ensures microbial safety but may also help retain the nutritional and sensory quality of milk by minimizing exposure to high temperatures.

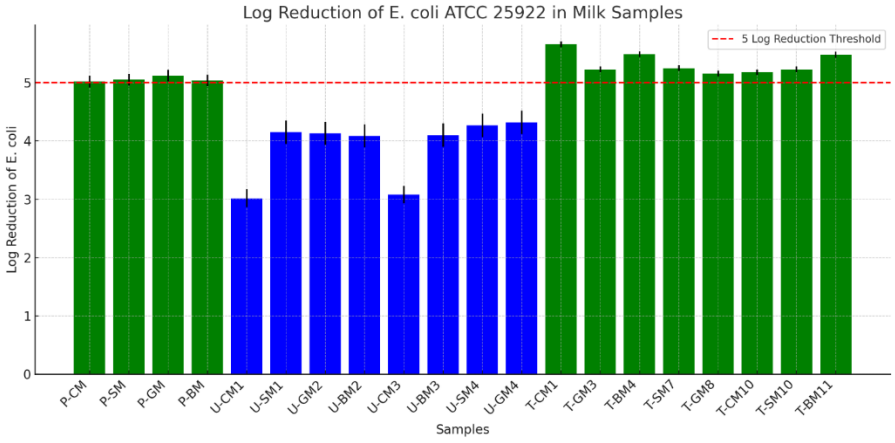

Figure 1. Inactivation of E. coli ATCC 25922 in Milk Samples

4. CONCLUSIONS

The results of this study demonstrate that sonication, and more notably thermosonication, offer effective alternatives to conventional thermal pasteurization in milk processing. These innovative techniques not only ensure enhanced inactivation of *E. coli* ATCC 25922 but also preserved the bioactive compounds that are often degraded during heat-based treatments. Thermosonication, which combines moderate heat with ultrasonic waves, was particularly effective in achieving both microbial safety and functional quality, suggesting its potential as a dual-purpose intervention.

Compared to traditional pasteurization, which can compromise nutritional and sensory attributes, these non-thermal and hybrid technologies provide a more sustainable and consumer-friendly approach. Their ability to operate at lower temperatures with shorter

processing times contributes to energy savings, reduced thermal damage, and potentially extended shelf life—features which are highly desirable in contemporary dairy processing systems.

However, despite these advantages, the industrial-scale implementation of sonication and thermosonication still faces several challenges. Future research should focus on optimizing operational parameters such as amplitude, frequency, and temperature-time combinations for different milk types. In addition, it is important to evaluate the long-term effects on nutritional, sensory, and rheological properties, ensure broad-spectrum microbial and enzymatic stability under varied processing conditions, and conduct comprehensive techno-economic and life cycle assessments to determine commercial feasibility and environmental impact.

Overall, the integration of sonication-based techniques holds significant promise for transforming traditional dairy processing practices by aligning with sustainability goals, advancing consumer health, and promoting innovation within the food industry.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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