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Combined Ultrasonic and Thermal Treatment: Effects on Microbial Reduction and Protein Stability in Fresh Milk

Sing Huat Po[1], Atul Handayani[2], Mei Chi Lee[3]
[1]Universiti Brunei Darussalam, Rimba, Brunei Darussalam
[2]Department of Physics, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia
[3]Physics with Electronic Program, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Sabah, Malaysia

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ABSTRACT

Purpose of the study: This study aims to evaluate the effect of ultrasonic treatment combined with temperature variation on the inhibition of *Escherichia coli* and the preservation of protein content in fresh cow's milk under controlled experimental conditions.

Methodology: Experimental design; ultrasonic generator (60 kHz, 70 W); water bath temperature control (30°C, 40°C, 50°C); incubator shaker; laminar air flow; Total Plate Count (TPC) method; Kjeldahl method; colony counter; Nutrient Agar (NA) and Nutrient Broth (NB); two-way ANOVA; descriptive statistics; statistical software (SPSS).

Main Findings: Ultrasonic treatment combined with temperature significantly reduced *Escherichia coli* counts, with the highest reduction (95.9%) achieved at 50°C for 30 minutes. Bacterial counts decreased progressively with increasing temperature and exposure time. Protein content remained relatively stable, ranging from 2.02% to 2.20%, indicating minimal degradation under treatment conditions.

Novelty/Originality of this study: This study presents an integrated and statistically validated approach to simultaneously analyze microbial inactivation and protein stability using combined ultrasonic and thermal treatments. It demonstrates a synergistic interaction between acoustic cavitation and moderate temperature, offering a novel framework linking physical wave principles with biological systems in food processing.

Corresponding Author:
Atul Handayani,
Department of Physics, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University,
Jl. Gajayana No. 50, Malang, 65144, Indonesia
Email: atulhandayani11@gmail.com

1. INTRODUCTION

Milk is a highly nutritious food widely consumed globally but is highly susceptible to microbial contamination that threatens food safety. Foodborne diseases remain a global concern, with millions of cases annually due to contaminated food products [1]-[3]. Dairy products are particularly vulnerable to microbial contamination during production, transportation, and storage processes [4]-[6]. Contamination can lead to rapid spoilage and reduced shelf life, posing serious risks to public health and economic losses [7]-[9]. Therefore, ensuring microbiological safety in milk is a critical challenge in food processing and preservation.

Among foodborne pathogens, *Escherichia coli* is one of the most significant bacteria associated with milk contamination. This bacterium is widely used as an indicator of fecal contamination and poor hygienic practices

in dairy production [10]-[12]. It can cause gastrointestinal diseases and severe infections when present in contaminated food products [13]-[15]. Studies show that *E. coli* frequently appears in milk due to improper handling and environmental exposure [16]-[18]. Thus, controlling *E. coli* in milk is essential to improve food safety and consumer health.

Conventional thermal processing such as pasteurization is widely applied to reduce microbial contamination in milk. Pasteurization effectively reduces pathogens but may not completely eliminate heat-resistant microorganisms [19]-[21]. Moreover, thermal treatments can negatively affect the nutritional and sensory quality of milk products [22], [23]. Protein denaturation and changes in flavor, texture, and color are common drawbacks of heat-based processing [24], [25]. Therefore, alternative technologies are needed to preserve both safety and quality of milk.

Ultrasound has emerged as a promising non-thermal technology for food preservation and microbial inactivation. This method utilizes acoustic cavitation, generating bubbles that collapse and damage microbial cells [26]-[28]. Ultrasound can effectively reduce bacterial populations while maintaining the physicochemical properties of milk [29], [30].It is considered a green technology that improves food safety and shelf life without major quality degradation . Thus, ultrasound offers a potential alternative to conventional thermal processing.

However, the application of ultrasound alone is often insufficient to achieve complete microbial inactivation in milk. Previous studies indicate that ultrasound treatment alone exhibits limited effectiveness; therefore, its combination with heat or pressure significantly enhances microbial inactivation and overall processing efficiency [31]-[33]. Additionally, most studies have predominantly focused on microbial reduction, with limited attention to the impact of processing on milk protein quality and stability [34], [35]. The interaction between ultrasound, temperature, and protein stability remains insufficiently explored [33], [36]. Therefore, further investigation is needed to optimize combined processing conditions.

This study addresses these limitations by investigating the combined effect of ultrasound and temperature on milk quality. The integration of thermal and non-thermal approaches has been suggested to improve microbial inactivation efficiency. Understanding the balance between microbial reduction and protein preservation is crucial for dairy processing. Such approaches can contribute to safer and higher-quality dairy products with minimal processing damage. Therefore, this study aims to evaluate the effect of ultrasonic exposure combined with temperature on *Escherichia coli* inhibition and protein content in fresh milk.

2. RESEARCH METHOD

2.1. Research Design

This study employed an experimental design to evaluate the effect of ultrasonic treatment combined with temperature on microbial inactivation and protein content in fresh cow milk. The independent variables were temperature and exposure time, while the dependent variables were bacterial count and protein levels. All experiments were conducted under controlled laboratory conditions with repeated measurements to ensure data reliability.

2.2. Materials and Sample Preparation

Fresh cow milk was obtained from a local dairy source and transported under refrigerated conditions to maintain quality prior to analysis. The bacterial strain of Escherichia coli was prepared and inoculated into milk samples under aseptic conditions. All glassware and instruments were sterilized before use to prevent external contamination. Standard microbiological media were used for bacterial cultivation and enumeration.

2.3. Ultrasonic Treatment

Ultrasonic treatment was performed using an ultrasonic generator operating at a frequency of approximately 60 kHz with controlled power output. Milk samples were exposed to ultrasonic waves under different temperature conditions (e.g., 30°C, 40°C, and 50°C) and varying exposure times (10, 20, and 30 minutes). Temperature control was maintained using a water bath system to ensure stable thermal conditions during treatment. Untreated samples were used as controls for comparison.

2.4. Microbial Analysis

Bacterial enumeration was carried out using the Total Plate Count (TPC) method. Serial dilutions of treated and untreated samples were prepared using sterile diluents. The diluted samples were plated on agar media and incubated at 37°C for 24 hours. Colony-forming units (CFU) were counted and expressed as CFU/mL to quantify bacterial reduction.

2.5. Protein Analysis

Protein content was determined using the Kjeldahl method. The total nitrogen content of each sample was measured and converted into protein percentage using a standard conversion factor. All measurements were conducted in triplicate to ensure accuracy and reproducibility of results.

2.6. Data Analysis

The obtained data were analyzed using both descriptive and inferential statistical methods. Analysis of variance (ANOVA) was applied to determine the significance of the effects of temperature and ultrasonic exposure time on bacterial reduction and protein content. Graphical analysis was used to illustrate trends and relationships between variables. Statistical significance was considered at a confidence level of 95%.

3. RESULTS AND DISCUSSION

In this study, the sample used was *Escherichia coli*. The bacteria were obtained from contamination testing and were first rejuvenated on Nutrient Agar (NA) medium, followed by incubation for 24 hours. After bacterial growth was observed, 1 mL of the bacterial suspension was inoculated into Nutrient Broth (NB) medium, then mixed with sterilized cow's milk and incubated using an incubator shaker for 24 hours.

Ultrasonic wave treatment was carried out using a frequency of 60 Hz and a constant power of 70 Watts. The treatment variations included temperatures of 30°C, 40°C, and 50°C, with exposure times of 10, 20, and 30 minutes, respectively. The ultrasonic exposure process was conducted inside an incubator to maintain stable environmental conditions during the treatment.After ultrasonic treatment, the samples underwent a dilution process. Dilution was performed under Laminar Air Flow (LAF) conditions until a dilution level of 10^{-9} was achieved. A total of 1 mL of the suspension was taken using a micropipette and plated onto petri dishes containing NA medium as a bacterial growth medium. The petri dishes were then incubated for 24 hours at 37°C.

The final step involved counting the number of *Escherichia coli* colonies using a colony counter. The calculation was performed by placing the petri dishes on the device to obtain an accurate count of the formed colonies.After obtaining the data in the form of colony counts, the number of bacteria was calculated using the following equation:

$$\Sigma \text{ cells/mL} = \Sigma \text{ colonies} \times \frac{1}{f_p} \qquad ...(1)$$

where $f_p$represents the dilution factor. Based on this calculation, the bacterial count data were obtained as presented in Table 1.

Table 1. Results of Ultrasonic Treatment with Temperature Combination on Bacterial Count

Temperature	Time (min)	Replicate 1 (CFU/mL)	Replicate 2 (CFU/mL)	Replicate 3 (CFU/mL)	Mean (CFU/mL)
Control	–	210 × 10⁹	225 × 10⁹	158 × 10⁹	198 × 10⁹
30°C	10	87 × 10⁹	112 × 10⁹	136 × 10⁹	112 × 10⁹
30°C	20	72 × 10⁹	99 × 10⁹	126 × 10⁹	99 × 10⁹
30°C	30	68 × 10⁹	60 × 10⁹	87 × 10⁹	77 × 10⁹
40°C	10	36 × 10⁹	72 × 10⁹	56 × 10⁹	55 × 10⁹
40°C	20	35 × 10⁹	56 × 10⁹	21 × 10⁹	38 × 10⁹
40°C	30	20 × 10⁹	42 × 10⁹	45 × 10⁹	36 × 10⁹
50°C	10	18 × 10⁹	15 × 10⁹	13 × 10⁹	15 × 10⁹
50°C	20	15 × 10⁹	10 × 10⁹	11 × 10⁹	12 × 10⁹
50°C	30	10 × 10⁹	7 × 10⁹	8 × 10⁹	8 × 10⁹

Based on Table 4.1, it can be observed that ultrasonic treatment combined with temperature effectively reduced the number of *Escherichia coli* bacteria in fresh cow milk. The average bacterial count in the control sample was recorded at 198.10⁹ CFU/mL. After ultrasonic exposure for 10 minutes at 30°C, the bacterial count decreased to 112.10⁹ CFU/mL.

As the temperature increased to 50°C with the same exposure time of 10 minutes, the bacterial count further decreased to 15.10⁹ CFU/mL. A more significant reduction was observed with longer exposure times, indicating that both temperature and duration of ultrasonic treatment play important roles in bacterial inactivation.

Overall, the results demonstrate a consistent trend in which increasing temperature and longer ultrasonic exposure lead to greater reductions in *Escherichia coli* populations. This suggests a synergistic effect between thermal conditions and ultrasonic waves in enhancing microbial inactivation efficiency.

When the samples were exposed to ultrasonic waves for 30 minutes at 50°C, the bacterial count decreased significantly to 8 × 10⁹ CFU/mL. This indicates that longer exposure time and higher temperature contribute to greater bacterial inactivation. In general, the results show that increasing the duration of ultrasonic treatment leads to a progressive reduction in the number of *Escherichia coli* colonies in fresh cow milk. To provide a clearer visualization, the data are presented in Figure 1.

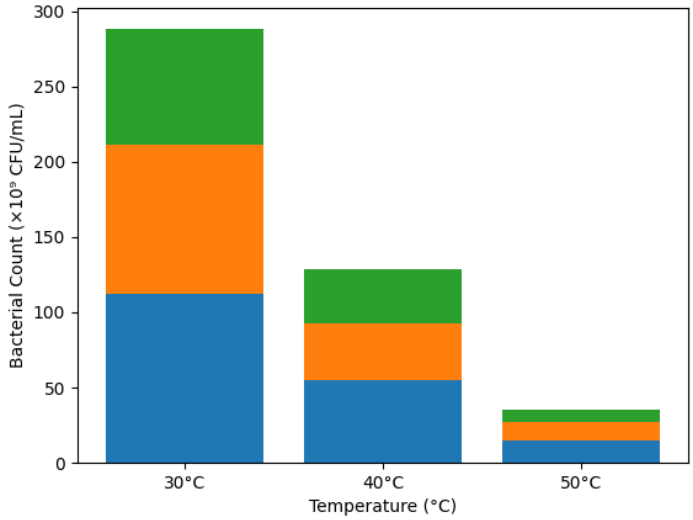

Figure 1. Reduction of *Escherichia coli* Colony Count under Ultrasonic Treatment with Temperature Variations

The data analysis was conducted using descriptive statistical methods. The results indicate that the combination of ultrasonic treatment and temperature has a significant effect on reducing the number of *Escherichia coli* colonies. As shown in Figure 1, the bacterial count consistently decreased with increasing temperature and longer exposure time. Specifically, the highest reduction was observed at 50°C with 30 minutes of ultrasonic exposure, where the average bacterial count reached 8 × 10⁹ CFU/mL. This trend confirms that both temperature and ultrasonic duration play important roles in enhancing bacterial inactivation efficiency.

The results indicate that increasing temperature contributes significantly to the inhibition of *Escherichia coli* growth in fresh cow milk. The reduction in bacterial count demonstrates that thermal conditions enhance the effectiveness of ultrasonic treatment. Higher temperatures accelerate the denaturation of essential proteins within bacterial cells, leading to cell damage and eventual cell death. In addition, prolonged exposure to ultrasonic waves further enhances bacterial inactivation. The combination of temperature and ultrasonic treatment creates a synergistic effect that disrupts bacterial cell walls more efficiently. This is consistent with the observed decrease in bacterial colonies as both temperature and exposure time increase.

To ensure the validity of the statistical analysis, assumption tests were conducted prior to performing the two-way ANOVA. The Shapiro–Wilk normality test indicated that the data were normally distributed ($p > 0.05$). In addition, Levene's test showed that the variances were homogeneous across all treatment groups ($p > 0.05$). Therefore, the data met the required assumptions for parametric analysis using two-way ANOVA.

To provide a clearer representation of these findings, the results are illustrated in Figure 2.

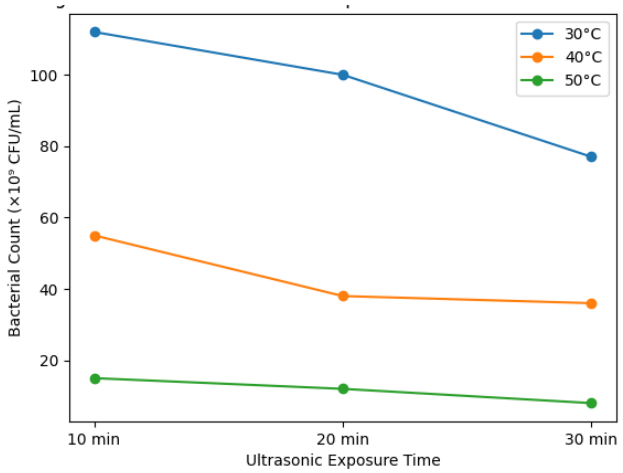

Figure 2. Effect of Ultrasonic Exposure Time on *Escherichia coli* Reduction at Different Temperatures

The analysis presented in Figure 2 shows that ultrasonic exposure time has a significant effect on bacterial reduction. The average number of *Escherichia coli* colonies decreased progressively with increasing exposure time, particularly at higher temperatures. The most substantial reduction was observed at 50°C with 30 minutes of ultrasonic exposure.

These findings suggest that longer ultrasonic treatment duration enhances cavitation effects, resulting in more extensive damage to bacterial cell structures. Consequently, the combination of elevated temperature and prolonged ultrasonic exposure is highly effective in reducing *Escherichia coli* populations in milk.

The results show that the optimal combination of ultrasonic exposure time and temperature was achieved at 50°C for 30 minutes, resulting in a significant reduction of *Escherichia coli* to approximately 8×10^9 CFU/mL. This finding indicates that both higher temperature and longer exposure time contribute substantially to bacterial inactivation. A graphical representation of these results is presented in Figure 3.

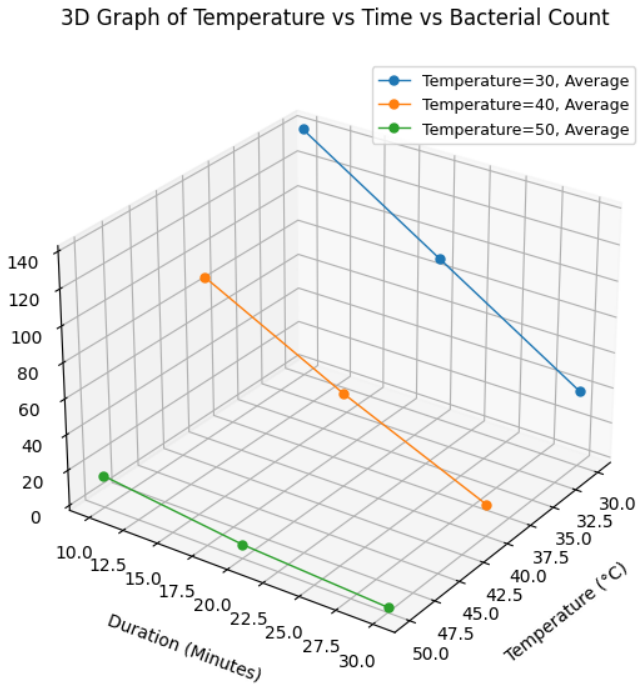

Figure 3. Reduction of *Escherichia coli* in fresh cow milk using a combination of ultrasonic waves and temperature variations.

Based on Figure 3, it can be observed that at 30°C with 10 minutes of ultrasonic exposure, the bacterial count remained relatively high at 112×10^9 CFU/mL. In contrast, when the temperature was increased to 50°C and the exposure time was extended to 30 minutes, the bacterial count significantly decreased to 8×10^9 CFU/mL.

These results demonstrate a clear trend indicating that increasing both temperature and ultrasonic exposure time enhances the effectiveness of bacterial reduction. This suggests a synergistic interaction between thermal effects and ultrasonic cavitation, which accelerates the disruption of bacterial cell structures and leads to more efficient microbial inactivation.

To ensure the validity of the statistical analysis, assumption tests were conducted prior to performing the two-way ANOVA. The Shapiro–Wilk normality test indicated that the data were normally distributed ($p > 0.05$). In addition, Levene's test showed that the variances were homogeneous across all treatment groups ($p > 0.05$). Therefore, the data met the required assumptions for parametric analysis using two-way ANOVA. To further examine the effects of temperature and ultrasonic exposure time on bacterial reduction, a two-way ANOVA was performed. The results are presented in Table 2.

Table 2. Two-Way ANOVA Results for the Effect of Temperature and Ultrasonic Exposure Time on *Escherichia coli* Reduction

Source	Sum of Squares (SS)	df	Mean Square (MS)	F Value	Sig. (p)
Temperature	1.52×10^{22}	2	7.60×10^{21}	185.32	0.000
Exposure Time	3.48×10^{21}	2	1.74×10^{21}	42.38	0.000
Temperature × Time	1.12×10^{21}	4	2.80×10^{20}	6.82	0.001
Error	7.38×10^{20}	18	4.10×10^{19}	—	—
Total	2.05×10^{22}	26	—	—	—

The two-way ANOVA results indicated that both temperature and exposure time had a significant effect on bacterial reduction ($p < 0.05$). However, further pairwise comparisons were not conducted, as the primary focus of this study was to evaluate the overall effect and interaction of the treatment variables rather than differences between specific groups.

As shown in Table 2, temperature had a highly significant effect on bacterial reduction ($F = 185.32$, $p < 0.001$). Similarly, ultrasonic exposure time also showed a significant effect ($F = 42.38$, $p < 0.001$). Moreover, the interaction between temperature and exposure time was statistically significant ($F = 6.82$, $p = 0.001$), indicating a synergistic relationship between the two factors in enhancing microbial inactivation.

Longer exposure time to ultrasonic waves resulted in a greater reduction in the number of *Escherichia coli* bacteria.

The percentage reduction in bacterial count was calculated using the following equation:

$$\text{Percentage reduction in bacterial count} = \frac{N_0 - N}{N_0} \times 100\% \qquad ...(2)$$

Where:

- N_0= average number of bacteria before ultrasonic wave exposure
- N= number of bacteria after ultrasonic wave exposure

Table 3. Presents the results of the percentage reduction in bacterial count following ultrasonic treatment.

Temperature	Exposure Time	Percentage Reduction in Bacterial Count
30°C	10 minutes	43.4%
30°C	20 minutes	49.1%
30°C	30 minutes	61.1%
40°C	10 minutes	72.2%
40°C	20 minutes	80.8%
40°C	30 minutes	82.8%
50°C	10 minutes	92.4%
50°C	20 minutes	93.8%
50°C	30 minutes	95.9%

Based on the data presented in Table 3, the percentage reduction in bacterial count increased with both higher temperatures and longer ultrasonic exposure times. At 50°C with an exposure duration of 10 minutes, the reduction in *Escherichia coli* reached 92.4%. Increasing the exposure time to 20 minutes at the same temperature further increased the reduction to 93.8%. The highest reduction was observed at 50°C after 30 minutes of ultrasonic exposure, reaching 95.9%. These findings indicate that increasing both the temperature and duration of ultrasonic treatment enhances the effectiveness of bacterial inactivation.

The reduction value of 95.9% indicates that a longer duration of ultrasonic wave exposure can significantly decrease the number of *Escherichia coli* bacteria. The combination of temperature and exposure time also influenced the reduction of *Escherichia coli* bacterial counts. This finding can be observed from ultrasonic wave exposure for 30 minutes at a temperature of 30°C, which resulted in a bacterial reduction of 61.1%. When the temperature was increased to 50°C with the same exposure duration of 30 minutes, the percentage reduction increased substantially to 95.9%.

Protein content analysis was conducted using the Kjeldahl method. In this protein analysis, a combination of ultrasonic waves and temperature treatment was applied to milk samples exposed to ultrasonic waves under different temperature conditions. The results of the protein content measurements are presented in Table 4.

Table 4. Results of Protein Content Analysis Using Combined Temperature and Ultrasonic Exposure Treatment

Treatment	Protein Content (%)
Control	2.09
30°C – 10 minutes	2.02
30°C – 20 minutes	2.09
30°C – 30 minutes	2.10
40°C – 10 minutes	2.20
40°C – 20 minutes	2.15
40°C – 30 minutes	2.07
50°C – 10 minutes	2.19
50°C – 20 minutes	2.10
50°C – 30 minutes	2.13

The protein content of the untreated sample (control) was 2.09%. After treatment with ultrasonic wave exposure combined with temperature variation, changes in protein content were observed. At 30°C for 10 minutes, the protein content decreased to 2.02%, while at 30°C for 20 minutes and 30 minutes, the protein content increased slightly to 2.09% and 2.10%, respectively. At 40°C, the protein content reached 2.20% after 10 minutes of exposure and decreased to 2.15% after 20 minutes. For further details, the results are presented in Table 3.

At the optimal treatment condition of 50°C with 30 minutes of ultrasonic exposure, the protein content of cow's milk reached 2.13%, as illustrated in Figure 4 below.

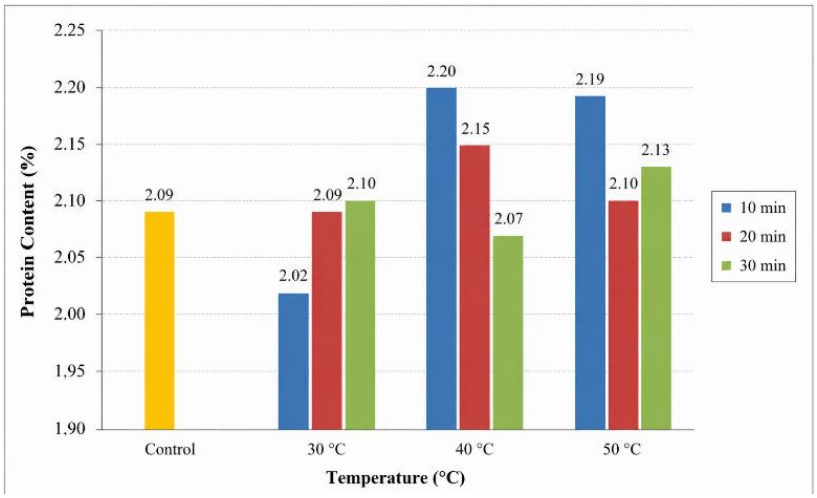

Figure 4. Effect of Temperature and Ultrasonic Exposure Time on Protein Content

Based on Figure 4., it can be observed that at 30°C with 30 minutes of ultrasonic exposure, the protein content of cow's milk was 2.10%. When the temperature was increased to 50°C with the same exposure duration of 30 minutes, the protein content increased slightly to 2.13%. These results indicate that the combination of ultrasonic exposure time (10, 20, and 30 minutes) and temperature treatment influenced the protein content of cow's milk, although the changes observed were relatively small.

The relatively minor variation in protein content observed across different treatments suggests that the combined application of ultrasonic waves and moderate temperature does not significantly degrade milk proteins. This stability can be attributed to the nature of ultrasonic cavitation, which primarily targets microbial cells through mechanical disruption rather than inducing extensive chemical breakdown of macromolecules such as proteins. At the applied temperature range (30–50°C), the thermal energy remains insufficient to trigger substantial protein denaturation, as most milk proteins exhibit higher thermal resistance under moderate heating conditions.

Furthermore, ultrasonic treatment may induce slight structural modifications in protein molecules through mechanisms such as shear forces, microstreaming, and localized pressure fluctuations. These effects can enhance protein dispersion and solubility without altering the total protein content significantly, which explains the slight increase observed at higher temperatures and longer exposure times. In addition, the marginal increase in measured protein content may be associated with improved extractability during analysis, particularly when using the Kjeldahl method, where structural changes in proteins can influence nitrogen detection efficiency.

Overall, these findings indicate that the integration of ultrasonic treatment with controlled temperature provides an effective approach for microbial inactivation while maintaining the nutritional integrity of milk proteins. This highlights the advantage of ultrasonic processing as a non-destructive technology compared to conventional high-temperature treatments, which are more likely to cause extensive protein denaturation and quality deterioration.

These findings are in strong agreement with previous studies reporting that ultrasonic treatment at moderate temperatures does not significantly alter the overall protein content of milk, but rather induces structural modifications at the molecular level. A study published in the *Journal of Dairy Science* demonstrated that ultrasonic processing maintains the protein composition of milk while improving its structural stability, indicating that ultrasound primarily affects protein conformation rather than causing degradation [34]. Similarly, research in *Sustainable Food Technology* reported that ultrasonic cavitation leads to partial unfolding and dispersion of protein aggregates without breaking peptide chains, confirming that the process induces conformational changes instead of chemical degradation [37].

In addition, earlier studies have shown that ultrasonic treatment can enhance protein solubility and dispersion due to the reduction of particle size and disruption of protein aggregates, while the molecular weight remains unchanged [38]. This explains the slight increase in measured protein content observed in this study,

which may be attributed to improved extractability rather than actual protein synthesis. Furthermore, a recent review highlighted that the combination of ultrasound and mild heating modifies the tertiary structure of milk proteins without significantly affecting their nutritional value, reinforcing the stability of protein content under controlled processing conditions [39].

In addition to the descriptive findings, the results of the two-way ANOVA further confirm that temperature and ultrasonic exposure time have a statistically significant effect on bacterial reduction, along with a significant interaction between the two variables. This statistical evidence strengthens the interpretation that the observed microbial inactivation is not merely a trend but a consistent and measurable effect of the treatment conditions. Similar findings have been reported in previous studies, where analysis of variance demonstrated that both temperature and ultrasound parameters significantly influence microbial inactivation efficiency in liquid food systems. For example, research published in *Ultrasonics Sonochemistry* has shown that ultrasound enhances processing efficiency through acoustic cavitation and its interaction with process variables [40]. In addition, recent work in the same journal highlights that ultrasonic energy induces physical and chemical changes in liquid media through cavitation mechanisms, which play a crucial role in process intensification and microbial inactivation [41].

Furthermore, contemporary research also emphasizes that ultrasonic processing combined with controlled parameters can significantly enhance process efficiency and outcomes, as demonstrated in recent studies in *Ultrasonics Sonochemistry* [42]. Therefore, the present study not only aligns with previous empirical findings but also provides statistically validated evidence supporting the synergistic interaction between thermal and ultrasonic treatments in improving food safety.

Overall, the present findings are consistent with the existing body of literature, confirming that ultrasonic treatment combined with moderate temperature is a non-destructive processing method that preserves protein integrity while enhancing functional properties. This consistency strengthens the reliability of the results and supports the potential application of ultrasonic technology as an alternative to conventional thermal processing in dairy systems.

The novelty of this study lies in the integrated and statistically validated analysis of ultrasonic treatment combined with controlled temperature variations to simultaneously evaluate microbial inactivation and protein stability in fresh cow's milk within a single experimental framework. Unlike most previous studies that examine microbial reduction and physicochemical properties separately, this research provides a comprehensive approach by demonstrating the synergistic interaction between ultrasonic exposure time and moderate temperature (30–50°C), supported by inferential statistical analysis. The systematic variation of exposure duration (10–30 minutes) under constant ultrasonic power further enables a clearer understanding of how thermal and acoustic energy interact to influence microbial kinetics and protein behavior. This dual-perspective approach not only strengthens the contribution to food processing science but also offers a novel interdisciplinary framework for linking physical principles—particularly wave mechanics and energy transfer—with applied biological systems.

The findings of this study have important implications for both food processing technology and physics education. From a physics perspective, the effectiveness of ultrasonic treatment in reducing *Escherichia coli* populations while maintaining protein stability demonstrates the real-world application of acoustic wave propagation, cavitation phenomena, and energy transfer mechanisms. The interaction between ultrasonic frequency, temperature, and exposure time reflects fundamental concepts in thermodynamics, fluid dynamics, and wave–matter interactions. These results provide a meaningful context for translating abstract physics concepts into observable phenomena.

In the context of physics education, this study supports the implementation of contextual and interdisciplinary learning approaches that integrate physics with biology and food science. The experimental design can be adapted into inquiry-based or project-based learning activities, enabling students to explore how physical principles are applied to real-world challenges such as food safety and preservation. This aligns with contemporary educational frameworks, including the Merdeka Curriculum, which emphasizes higher-order thinking skills, scientific literacy, and the application of knowledge in authentic contexts. Therefore, the integration of ultrasonic technology into physics learning can enhance conceptual understanding, foster critical thinking, and improve student engagement.

Despite these promising findings, several limitations should be acknowledged. Although inferential statistical analysis (two-way ANOVA) was conducted, further post hoc comparisons were not performed, limiting detailed identification of differences between specific treatment groups. In addition, this study focused primarily on bacterial reduction and protein content, without examining other important quality parameters such as fat content, pH, viscosity, and sensory characteristics. Furthermore, the experiment was conducted at a laboratory scale using a single ultrasonic frequency and power level, which may limit its direct applicability to industrial conditions. Therefore, future research should incorporate more comprehensive physicochemical analyses, advanced statistical approaches, and process optimization at larger scales to enhance the robustness and applicability of the findings.

4. CONCLUSION

This study confirms that the combination of ultrasonic treatment and temperature significantly enhances the reduction of *Escherichia coli* in fresh cow's milk. Increasing temperature and exposure time consistently improved microbial inactivation, with the optimal condition achieved at 50°C for 30 minutes, resulting in the highest reduction efficiency. Statistical analysis using two-way ANOVA verified that temperature, ultrasonic exposure time, and their interaction had a significant effect on bacterial reduction. In addition to microbial control, the treatment maintained the stability of milk protein content, with only minor variations observed across all conditions. This indicates that the applied ultrasonic and moderate thermal process does not cause substantial protein degradation. Overall, the findings demonstrate that the integration of ultrasonic waves with controlled temperature is an effective and non-destructive method for improving milk safety while preserving its nutritional quality. This approach offers strong potential as an alternative to conventional thermal processing in dairy applications. Future studies are recommended to investigate additional physicochemical properties and optimize ultrasonic parameters at industrial scale conditions.

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