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Thermoultrasonication as an emerging technology for raw cow's milk processing: A review of its impacts on food safety and physicochemical quality

La termoultrasonicación como tecnología emergente para la leche de vaca: Una revisión de su impacto sobre la inocuidad alimentaria y la calidad fisicoquímica

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Abstract

Ultrasonication combined with mild heating (TUS) has emerged as a promising alternative to conventional heat treatment (CHT) for the processing of raw cow's milk (RCM). Recent studies report significant microbial inactivation—typically around 2.5 log reductions—while preserving key nutritional and physicochemical properties. The majority of findings indicate minimal degradation of proteins and lipids, improved viscosity, enhanced stability of bioactive compounds, and a marked decrease in aflatoxin M1 (AFM1) levels. Although processing parameters such as amplitude, frequency, and duration vary across studies, there is a consistent emphasis on the synergistic effect of acoustic cavitation and moderate thermal input. This synergy not only contributes to microbial safety but also offers potential reductions in energy consumption compared to traditional thermal processing. Nevertheless, further efforts are required to scale up the technology and standardize operating protocols to facilitate regulatory acceptance. These findings collectively underscore the potential of TUS as an innovative and efficient approach for enhancing the safety and quality of dairy products.
Keywords: milk, thermoultrasonication, microbial inactivation, physicochemical properties, dairy processing.

Resumen

La ultrasonicación combinada con temperatura suave (TUS) ha surgido como una alternativa prometedora al tratamiento térmico convencional (CHT) para el procesamiento de leche cruda de vaca (RCM). Estudios recientes reportan una inactivación microbiana significativa —típicamente alrededor de 2.5 reducciones log—, mientras se preservan las propiedades nutritivas y fisicoquímicas clave. La mayoría de los hallazgos indican una degradación mínima de las proteínas y los lípidos, una mejor viscosidad, una mayor estabilidad de los compuestos bioactivos y una marcada disminución de los niveles de aflatoxina M1 (AFM1). Aunque los parámetros de procesamiento como la amplitud, la frecuencia y la duración varían entre los estudios, hay un énfasis constante en el efecto sinérgico de la cavitación acústica y la entrada térmica moderada. Esta sinergia no sólo contribuye a la seguridad microbiana, sino que también ofrece reducciones potenciales en el consumo de energía en comparación con el tratamiento térmico tradicional. Sin embargo, se requieren más esfuerzos para ampliar la tecnología y estandarizar los protocolos de funcionamiento a fin de facilitar la aceptación reglamentaria. Estos resultados subrayan colectivamente el potencial del TUS como un enfoque innovador y eficiente para mejorar la seguridad y calidad de los productos lácteos.
Palabras clave: leche, termoultrasonicación, inactivación microbiana, propiedades fisicoquímicas, procesamiento de leche.

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1 Introduction

Conventional heat treatments (CHT), such as pasteurization and ultra-high-temperature processing, are widely employed in the food industry to eliminate pathogenic microorganisms and extend shelf life. However, these methods often degrade heat-sensitive nutrients and alter the physical and sensory characteristics of food products (Albenzio *et al*. 2012; Cadwallader & Singh, 2009). In response to these drawbacks, alternative technologies have been proposed to ensure microbial safety while minimizing physical, chemical, and nutritional losses. Emerging food processing technologies such as pulsed electric fields, high hydrostatic pressure, microfiltration, ultrasonication (US), and thermoultrasonication (TUS) offer promising alternatives to reduce thermal damage in liquid matrices and potentially complement conventional methods in the dairy industry (Cregenzán *et al*. 2014; Hernandez-Falcón *et al*. 2018; Sharma *et al*. 2014; Stratakos *et al*. 2019).

Among these technologies, US has been reported to be used at low (<1 W/cm^2) or high (>1 W/cm^2) intensities, with frequencies ranging from 20 to 500 kHz (Karlović *et al*. 2014). The US has demonstrated considerable utility in food processing, improving meat texture and aging, modifying physicochemical properties, and regulating enzymatic activity in bovine muscle (Castillo-Andrade *et al*., 2025). Building on this, Loan *et al*. (2024) showed that US-assisted enzyme extraction of purple rice bran uses acoustic cavitation to accelerate mass transfer and alter plant cell structures, resulting in higher polyphenol recovery and faster reaction rates. Similarly, Obando-Galicia *et al*. (2024) reported that US or high-shear extraction with a food-grade soybean oil/lecithin system improves polyphenol recovery from the red pear shell of the cactus; the extracts were also tailored to match the rheology and microstructure of the oleo-gel. Additionally, Ramos-Villacob *et al*. demonstrated that complexing lauric acid with amylose in cassava starch promotes V-type inclusion complexes by enhancing diffusion and inducing transient disorder, enabling quicker kinetics and more precise control of functionality. Under specific conditions, however, this technology may degrade nutrients and produce undesirable flavor compounds due to the formation of peroxides and lipid oxidation products (Chandrapala *et al*. 2012; Marchesini *et al*. 2015). To minimize these effects, US has been combined with other treatments, such as pressure (manosonication), heat and pressure (manothermosonication), or heat alone (TUS). Although the temperature in TUS can be controlled, potential effects on thermolabile components such as proteins and vitamins cannot be ruled out (Mahmoud *et al*., 2022).

Compared to CHT, TUS has been shown to cause minimal changes in the nutritional properties, physical characteristics, antioxidant capacity, and enzymatic activity of fruits and vegetables and/or fermented beverages derived from blackberry, apple, strawberry, mango, carrot, beet, almond, cactus fruit, aguamiel, and milk (Cervantes-Elizarrarás *et al*., 2017; Cruz-Cansino *et al*., 2015; Hernández-Falcón *et al*., 2018; Parreiras *et al*., 2020; Zafra-Rojas *et al*., 2023). The consumption of liquid cow's milk remains an integral part of the human diet due to its content of high-biological-value proteins, calcium, essential fatty acids, amino acids, fats, water-soluble vitamins, and various bioactive compounds that play crucial roles in multiple biochemical and physiological processes (Albenzio *et al*., 2016).

In recent years, several studies have investigated TUS as a promising technique to improve the microbiological quality, rheological properties, and bioavailability of nutritional compounds in raw cow's milk (RCM), positioning it as a potential alternative for the dairy industry. This study aimed to evaluate the changes induced by TUS in raw milk intended for human consumption. Although some studies have reported temperatures exceeding the thermal threshold (>50 °C) during the US processing of RCM, none have surpassed the temperatures typically associated with CHT.

This review synthesizes current evidence from studies examining the application of TUS to RCM intended for direct human consumption. Only investigations that applied TUS directly to RCM were included, thereby ensuring that the analysis remains focused on the specific challenges and outcomes associated with unheated dairy matrices (Annandarajah *et al*., 2018; Bermúdez-Aguirre *et al*., 2008, 2009a, 2009b, 2011; Deshpande & Walsh, 2021; Dhiny *et al*., 2023; Herceg *et al*., 2012; Hernández-Falcón *et al*., 2018; Vijayakumar *et al*., 2015; Wang *et al*., 2022; Zhou *et al*., 2020). Focusing on RCM enhances comparability and industrial relevance for three main reasons: (1) cow's milk is the most widely consumed and processed worldwide, supported by standardized supply chains; (2) native microbial and enzymatic profiles differ among species, potentially affecting the comparability of microbial log reductions and product stability; and (3) from both regulatory and technological perspectives, parameters validated in bovine milk are directly applicable to the dominant dairy sector (Cimmino *et al*., 2023; Marangoni *et al*., 2019). Overall, this approach enables a more rigorous assessment of how TUS influences microbial safety and physicochemical quality in the most commercially significant dairy matrix. The objective of this review is to consolidate existing knowledge on the sensorial, functional, and microbiological implications of TUS, to evaluate its advantages relative to CHT, and to identify current research gaps and future directions in dairy processing.

2 Conventional heat treatment of raw cow's milk

CHT, such as pasteurization and ultra-high-temperature (UHT) processing, are commonly used to eliminate pathogenic microorganisms and preserve food, although they often lead to nutrient losses (Cadwallader & Singh, 2009). Pasteurization, typically referred to as high-temperature short-time (HTST), raises the temperature of RCM to 72 °C for 15 seconds, effectively inactivating pathogens such as *Coxiella burnetii*, *Salmonella typhi, Streptococcus pyogenes*, and *Mycobacterium tuberculosis* (Bastam *et al*. 2021). In contrast, UHT treatments raise the temperature to 135 °C for 2–4 seconds, producing commercially sterile milk and extending shelf life for several months at ambient temperature (Bai *et al*. 2023). Although these thermal processes are effective for microbial inactivation, they often cause thermal damage to heat-sensitive components, thereby altering nutritional quality and affecting the sensory attributes of the final product (Lucey *et al*. 2022).

3 Principles and mechanisms of thermoultrasonication

TUS, also referred to as thermosonication, thermoultrasound, or thermosound, typically involves a control panel, an ultrasonic probe, and a temperature-controlled recirculating bath (Figure 1). This technology stands out as a sustainable alternative due to its energy efficiency, operational simplicity, and low environmental impact. Additionally, it reduces processing time while enhancing food quality and shelf life. Automated systems can further optimize labor and production costs (Akdeniz & Akalin, 2022; Arvanitoyannis *et al*. 2017).

TUS occurs when US is applied in combination with moderate temperatures (often <55 °C) (Bariya *et al*. 2023). The underlying principle of this technique is acoustic cavitation, in which sound waves oscillating at frequencies typically between 20 and 40 kHz generate microscopic vapor bubbles in the liquid medium (Asaithambi *et al*. 2022). These bubbles expand and grow during wave oscillations until they reach a resonant size, at which point they collapse due to alternating pressure cycles, releasing localized energy and generating shock waves (Crudo *et al*. 2014). This intense mechanism can disrupt cell membranes, facilitate the release of nutritionally

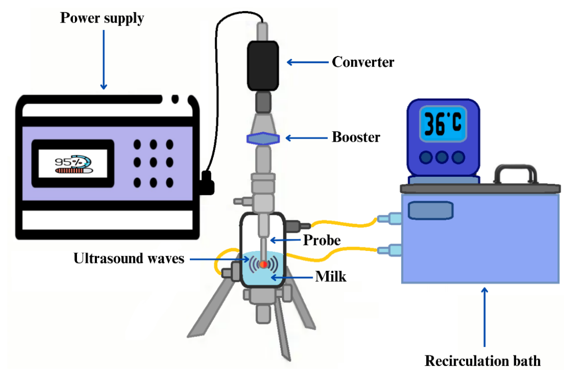

Figure 1. Equipment for thermoultrasonication.

relevant compounds, and promote the formation of stable emulsions (Shen *et al*. 2017).

The US mechanism relies on piezoelectric transducers that convert electrical energy into high-frequency sound waves. When applied to a liquid such as milk, these waves generate alternating compression and rarefaction cycles, leading to acoustic cavitation. With increasing sound intensity, microbubbles are formed and undergo oscillation, expansion, and subsequent collapse. The implosion of these bubbles produces highly localized extreme conditions, including transient temperatures approaching 4,700 °C, pressures exceeding 100 MPa, and intense shear forces (Zhang *et al*., 2018).

When US is combined with heat, as in TUS, the efficiency of acoustic cavitation is markedly enhanced. Elevated temperatures reduce the viscosity and surface tension of the liquid, thereby facilitating microbubble formation, expansion, and collapse. This thermal contribution amplifies cavitation intensity, resulting in greater molecular disruption, improved emulsification, and accelerated microbial inactivation. The synergistic interaction between thermal energy and US thus enables more effective modifications of food structure and functional properties, ultimately leading to optimized processing outcomes.

Other effects of TUS include enzyme inactivation, microbial reduction, and nutrient preservation due to lower processing temperatures (Villamiel & De Jong, 2000). These outcomes contribute to enhanced nutritional properties, minimized loss of heat-sensitive components, reduced development of cooked flavors, and minimal adverse effects on texture or appearance (Binti-Maklin *et al*. 2025). Additional studies on goat milk have shown increased solubility of calcium and phosphorus, while in camel milk cream, a reduction in fat globule size has been observed, positively affecting product stability, viscosity, adhesiveness, and hardness (Kashaninejad & Razai, 2020; Ragab *et al*. 2019).

Despite these advantages, TUS may present some limitations, such as the potential generation of free radicals and modifications in the structure and texture of foods, which are matrix-dependent (Pérez-Andrés *et al*. 2018).

4 Thermoultrasonication of raw cow's milk

The studies published between 2008 and 2024 included in this review were conducted in various geographical regions: Canada (Wang *et al*., 2022), China (Zhou *et al*., 2021), Croatia (Herceg *et al*., 2012), Indonesia (Dhiny *et al*., 2023), Mexico (Hernández-Falcón *et al*., 2018), and the USA (Annandarajah *et al*., 2018; Bermúdez-Aguirre *et al*., 2008, 2009a, 2009b, 2011; Deshpande & Walsh, 2021; Vijayakumar *et al*., 2015). All studies applied high-intensity US simultaneously with heat, either in batch or continuous systems, using amplitudes between 30% and 100%, temperatures ranging from 15 °C to 72 °C, and treatment durations between 0.25 and 30 minutes. Most protocols adhered to the moderate temperature regime typically associated with TUS; increases beyond this range were only reported in two studies, which documented temperatures between 29–55 °C and 20–71 °C, respectively (Hernández-Falcón *et al*., 2018; Wang *et al*., 2022). The operating parameters are summarized in Table 1, which provides a comparative overview of US intensity, frequency, treatment duration, and thermal strategies, thereby illustrating the variability in TUS implementations and their potential effects on processing outcomes. While most studies included comparisons with CHT, Wang *et al*. (2022) and Herceg *et al*. (2012) did not report any direct comparative analyses. Table 2 summarizes the principal variables assessed across microbiological, physicochemical, and proximate composition domains, together with the changes associated with TUS application. Under certain conditions, TUS consistently reduced microbial counts, altered physicochemical properties, and modified techno-functional attributes (Table 3).

Table 1. TUS[1] conditions in the included studies.

Temperature (°C)			Time (min)	Amplitude (%)	kHz[2]	Watts	Reference
Water bath	Initial	Final					
72	15 – 75	15 – 75	1	100	20	140	Annandarajah *et al*. (2018)
65	63	63	10 – 30	100	24	400	Bermúdez-Aguirre *et al*. (2008)
65	63	63	30	100	24	400	Bermúdez-Aguirre *et al*. (2009a)
65	63	63	30	30 – 100	24	400	Bermúdez-Aguirre *et al*. (2009b)
57	63	63	10 – 30	100	24	400	Bermúdez-Aguirre *et al*. (2011)
72	20 – 25	67 – 72	0.11 – 0.19	90	–	168 – 180	Deshpande & Walsh, (2021)
55	–	60	5 – 20	–	20 – 22	365	Dhiny *et al*. (2023)
–	40 – 60	40 – 60	6 – 12	50 – 100	20	600	Herceg *et al*. (2012)
45	29 – 31	48 – 55	10 – 15	95	20	1500	Hernández-Falcón *et al*. (2018)
72	65	–	1 – 3	100	20	115	Vijayakumar *et al*. (2015)
90	63	–	1 – 9	–	25	400	Wang *et al*. (2022)
–	55 – 63	-	315 – 30	–	20	200 – 400	Zhou *et al*. (2021)

[1]TUS: termoultrasonication, [2]kHz: kilohertz.

Table 2. CHT1 conditions in the included studies.

Temperature (°C)	Time (min)	Reference
72	0.25	Annandarajah *et al*. (2018)
65	30	Bermúdez-Aguirre *et al*. (2008)
65	30	Bermúdez-Aguirre *et al*. (2009a)
63	30	Bermúdez-Aguirre *et al*. (2009b)
65	30	Bermúdez-Aguirre *et al*. (2011)
72	0.11 – 0.19	Deshpande & Walsh (2021)
65	30	Dhiny *et al*. (2023)
NI[2]	NI[2]	Herceg *et al*. (2012)
85	0.25	Hernández-Falcón *et al*. (2018)
72	1 – 3	Vijayakumar *et al*. (2015)
NI[2]	NI[2]	Wang *et al*. (2022)
63/121	30/0.25	Zhou *et al*. (2021)

[1]CHT: conventional heat treatment, [2]NI: not indicated.

Table 3. Summary of variables examined in the included studies and their observed changes.

Variables				
Microbiology	Physicochemical	Proximate analysis	Other	Reference
–	–	–	Plasmin (↓)	Annandarajah *et al.* (2018)
–	Color (↑), fat globule size (↓)	Fat (↑)	–	Bermúdez-Aguirre *et al.* (2008)
Mesophilic aerobic (↓)	pH (↓), color (↑), density (↓), acidity (↑),	Fat (↑), protein (↓), NFS[1] (↓)	–	Bermúdez-Aguirre *et al.* (2009a)
L. innocua (↓), mesophilic bacteria (↓)	Color (↑), pH (↓), acidity (↑)	Fat (↑), protein (↓)	–	Bermúdez-Aguirre *et al.* (2009b)
L. innocua (↓)	–	–	–	Bermúdez-Aguirre *et al.* (2011)
G. stearothermophilus (↓)	pH (↑)	–	FFAs[4] (↓)	Deshpande & Walsh, (2021)
E. coli (↓)	pH (↓), viscosity (↓)	Fat (↑), Protein (↓)	–	Dhiny *et al.* (2023)
E. coli (↓), *S. aureus* (↓)				Herceg *et al.* (2012)
Mesophilic aerobic (↓), enterobacteria (↓)	pH (↓), color (↑), density (↓), stability (↑), acidity (↑)	NFS (↑), TS (↑)	AFM1[3] (↓), antioxidants (↑)	Hernández-Falcón *et al.* (2018)
Total aerobic (↓), coliform count (↓)	Color (↑), fat globule size (↓), viscosity (↑)	–	Sensory evaluation, plasmin (↑),	Vijayakumar *et al.* (2015)
–	–	Protein (↑)	Enzymatic digestion (↑)	Wang *et al.* (2022)
S. aureus (↓)	pH (↓), color (↑), particle size (↓)	Protein (↓)		Zhou *et al.* (2021)

[1]NFS: nonfat solid. [2]TS: total solid. [3]AFM1: aflatoxin M1. [4]FFAs: free fatty acids. ↑: increase of the variable. ↓: decrease of the variable.

5 Impact of thermoultrasonication on milk microstructure and component functionality

TUS alters casein micelles and their spatial organization, two critical factors in firm gel formation and structural integrity of dairy matrices. It also enhances micellar interactions and promotes cross-linking, resulting in more stable and consistent gels that improve the texture of products such as yogurt and cheese (Ragab *et al.*, 2019; Song *et al.*, 2021). Silva, Zisu & Chandrapala (2018) refined this view by showing that the effect of US on micelles depends on the casein-to-whey protein ratio: in casein-dominant systems, sonication reduces particle size by promoting aggregate reorganization through exposure of hydrophobic regions, whereas in formulations with a higher serum fraction, primary aggregates are formed via disulfide bonds involving κ-casein and whey proteins, thereby altering micellar architecture and functional interactions.

TUS enhances interactions between casein micelles primarily by inducing conformational changes and rearrangements within the protein matrix, which promote greater connectivity and cohesion of the gelling network. Acoustic cavitation exposes hydrophobic regions and free sulfhydryl groups, favoring hydrophobic interactions and the formation of disulfide bonds between caseins and between caseins and whey proteins. These molecular events densify the network and increase gel firmness (Shanmugam *et al.*, 2012; Shen *et al.*, 2017; Meng *et al.*, 2021; Pérez-Andrés *et al.*, 2018). Such effects translate into functional improvements, including increased resistance to syneresis, shorter acid gelation times, and improved elasticity of fermented milk gels, attributable to a more interconnected and stable micellar network (Reiter *et al.*, 2023; Liu *et al.*, 2014). Hemar *et al.* (2020) further demonstrated that high-power US could moderately reduce the apparent size of reconstituted micelles without disrupting their internal structure, suggesting surface compaction that enhances structural density. In mixed protein systems, US facilitates complementary protein–protein interactions that further strengthen the network, as observed in combinations of casein with other proteins (Nascimento *et al.*, 2023; Yuno-Ohta *et al.*, 2020).

6 Microbiological effects of thermoultrasonication on raw cow's milk

In RCM, it is important to recognize that pathogenic microorganisms such as *Brucella abortus* and *Mycobacterium* sp may be present within the dairy chain and pose significant public health concerns

(Ninković *et al*., 2024; Ullah *et al*., 2024). Tavsanli *et al*. (2022) evaluated the effect of low-frequency US on the microbiota of raw goat milk inoculated with *Brucella melitensis*, reporting reductions exceeding 2 log CFU after 10 min of treatment at 20 kHz and 400 W. Similarly, Al Bsoul *et al*. (2010) investigated the effectiveness of US against *Mycobacterium* sp 6PY1, achieving >3 log reductions in cell density in aqueous suspension after 15 min of sonication at 20 kHz and 0.5 W/mL.

Under controlled conditions, TUS can achieve microbial reductions approaching sterilization. At US powers of 200–250 W, damage to the cell wall and membrane is intensified, leading to intracellular leakage. At 300 W, severe rupture occurs, with large cavities and fragmentation of the cell wall (Jiang *et al*., 2024). The extent of membrane disruption correlates positively with ultrasonic power density. Cavitation increases membrane permeability, allowing the entry of macromolecules and resulting in leakage of intracellular contents and damage to organelles, enzymes, and nucleic acids (Ahmad *et al*., 2023). In dairy matrices, multiple studies have demonstrated multilogarithmic microbial reductions consistent with these mechanisms. Under optimized conditions, no detectable growth has been observed following treatment and during refrigerated storage (Bermúdez-Aguirre *et al*., 2011; Hernández-Falcón *et al*., 2018; Dhiny *et al*., 2023). Nevertheless, sterilization efficacy is influenced by initial microbial load, reactor geometry, energy density, and matrix composition. Thus, pilot- and industrial-scale validations, along with regulatory frameworks defining process criteria and microbiological verification, are necessary.

Research summarized in Table 4 presents the application of TUS for microbial reduction, comparing pre- and post-treatment levels in RCM. For context, conventional HTST achieves >5 log reductions in indicator pathogens such as *Escherichia coli* and *Listeria* spp., while UHT treatment achieves commercial sterility levels (>6 log reductions) across a wide range of microorganisms (Cadwallader & Singh, 2009; Bai *et al*., 2023).

TUS treatments demonstrated effectiveness equal to or greater than that of traditional HTST in inactivating fecal contamination indicators and other pathogenic bacteria, including *E. coli*, *Listeria innocua*, *Staphylococcus aureus*, and Enterobacteriaceae (Bermúdez-Aguirre *et al*., 2008, 2009a, 2009b; Deshpande & Walsh *et al*., 2021, Dhiny *et al*., 2023; Herceg *et al*., 2012; Hernández-Falcón *et al*., 2018; Vijayakumar *et al*., 2015; Zhou *et al*., 2021).

Escherichia coli exhibited marked sensitivity to TUS, with reductions of up to 3.07 log CFU/mL, accompanied by pronounced structural damage to the cell envelope, including multiple cracks, fractures, and outward bulging, as well as alterations in milk viscosity and fat composition (Dhiny *et al*., 2023; Herceg *et al*., 2012). Mechanistic studies further demonstrate that cavitation induces severe membrane disruption, characterized by extensive cracking, fracturing, and outward deformation of the cell membrane, thereby confirming the destructive effects of acoustic cavitation on *E. coli* integrity (Lin *et al*., 2019). *Listeria innocua* was reduced by more than 5 log CFU/mL after 10 minutes of TUS at 63 °C, while CHT achieved only a 3 log CFU/mL reduction under similar conditions (Bermúdez-Aguirre *et al*. 2009b, 2001). *Staphylococcus aureus* was completely inactivated in milk subjected to TUS for 7.5 minutes at 63 °C highlighting the microbicidal potential of the combined treatment (Bastam *et al*. 2021; Arvanitoyannis *et al*., 2017). Additionally, mesophilic bacteria and the native microbiota experienced significant reductions, and in some cases, no microbial growth was detected after 15 days of refrigerated storage post- treatment (Hernández-Falcón *et al*., 2018, Bermúdez-Aguirre *et al*., 2009a, 2009b).

In a continuous-flow system, such as the one studied by Deshpande & Walsh (2020), the antimicrobial effect of TUS was consistent, showing reductions in both native microbiota and thermophilic bacteria. Although this study did not report detailed measurements of the initial pathogen loads, the findings suggest that, under optimized conditions, TUS can be as effective as CHT in microbial inactivation.

The effectiveness of TUS is strongly influenced by the structural characteristics of bacterial cell envelopes. In Gram-negative bacteria, the lipopolysaccharide-rich outer membrane combined with a thin peptidoglycan layer in the periplasmic space renders them particularly susceptible to shear forces, pressure pulses generated by cavitation, and oxidative stress caused by reactive oxygen species such as hydrogen peroxide produced during bubble collapse (Herceg *et al*., 2012; Li *et al*., 2019). By contrast, Gram-positive bacteria possess a thick (20–80 nm) peptidoglycan cell wall reinforced with peptide cross-bridges, which increases structural rigidity and provides greater resistance even against reactive species (Vadillo-Rodríguez & Dutcher, 2011; Zupanc *et al*., 2019). Under typical thermal conditions of TUS, increasing vapor pressure together with decreasing surface tension and viscosity of the medium enhances cavity formation and collapse, thereby intensifying microbial inactivation at temperatures approaching those of CHT, with Gram-negative bacteria displaying notably higher susceptibility (Ugarte-Romero *et al*., 2007).

For mesophilic aerobic microorganisms, higher ultrasonic amplitudes result in greater CFU reductions. Cavitation imposes a combination of mechanical, thermal, and chemical stresses. The implosion of microbubbles generates shear forces that disrupt cell membranes, alter permeability, and may lead to partial or complete lysis.

Table 4. Reducing microbial count (CFU/mL[1]) in RCM[2] after TUS[3] application.

Microorganism	CHT[4]	TUS[2]	Reference
Aerobics totals	1.86	1.86	Vijayakumar *et al.* (2015)
Aerobic mesophilic	~3.00	~0.30 - 2.20	Hernández-Falcón *et al.* (2018)
Coliforms	>3.60	>3.60	Vijayakumar *et al.* (2015)
Enterobacteriaceae	~4.3	~ 1.2 - 3.32	Hernández-Falcón *et al.* (2018)
Mesophilic bacteria	3.00 1.89- 3.75	3.00 0.53 – 4.25	Bermúdez-Aguirre *et al.* (2008) Bermúdez-Aguirre *et al.* (2009b)
E. coli	1.54 – 2.44 2.90	1.92 – 3.07 ~4.68	Herceg *et al.* (2012) Dhiny *et al.* (2023)
L. innocua	0.50 – 5.0 5.30	5.00 – 6.00 ~5.50	Bermúdez-Aguirre *et al.* (2011) Bermúdez-Aguirre *et al.* (2009b)
S. aereus	0.09 – 0.94 3.25	0.39 – 1.49 4.19	Herceg *et al.* (2012) Zhou *et al.* (2021)
Geobacillus stearothermophilus	0.05 – 0.37	0.26 – 0.54	Deshpande & Walsh (2021)

[1]CFU/mL: $\log_{10}$ colony-forming unit per milliliter. [2]RCM: raw cow´s milk. [3]TUS: thermoultrasonication [4]CHT: conventional heat treatment.

Simultaneously, the dissociation of water molecules produces hydrogen and hydroxyl radicals, while additional reactive oxygen and nitrogen species—including hydrogen peroxide, nitric oxide, and nitrous acid—are formed. These species induce oxidative stress, contributing to protein denaturation and the degradation of intracellular components (Furuta *et al*., 2004; Tsukamoto *et al*., 2004; Zhang *et al*., 2018). Moreover, TUS modifies surface electrostatic properties and destabilizes extracellular polymeric substances, thereby reducing adhesion and biofilm formation and diminishing microbial protection (Kentish & Feng, 2014; Ojha *et al*., 2017). These effects are further amplified by elevated temperatures, which accelerate bubble formation and pore development in membranes, ultimately causing rupture and leakage or accumulation of cytoplasmic material (Bermúdez-Aguirre *et al*., 2011).

7 Effects on physicochemical characteristics

Most authors reported a moderate pH decrease after treatments at 60–65 °C, without compromising colloidal stability or sensory properties; final pH values stayed within acceptable ranges for fluid milk products (Bermúdez-Aguirre *et al*. 2008, 2009b; Deshpande & Walsh, 2021; Hernández-Falcón *et al*. 2018; Zhou *et al*. 2021). Cavitation during TUS often raises the local temperature, likely contributing observed acidification. Additional pH changes during storage may come from acid metabolites produced surviving microorganisms, potentially linked to lipolysis (Bermúdez-Aguirre *et al*. 2009b) or from formation of carboxyl groups after TUS-induced molecular changes (Li *et al*. 2019). These processes can cause variations in the proximate composition reported in some studies (Bermúdez-Aguirre *et al*. 2008, 2009a).

Regarding color, multiple studies reported an increase in whiteness (L*) and a reduction in particle size (Bermúdez-Aguirre *et al*. 2008, 2009a, Hernández-Falcón *et al*. 2018; Zhou *et al*. 2021 The color increase specifically refers to higher luminosity (L*), not a change in hue (*a**) or yellowish/bluish (b*). Color is a key factor in consumer acceptance of milk and is related to the dispersion of fat globules and casein micelles (Owen *et al*. 2001). Light reflection is enhanced by reduced fat globule size and protein denaturation caused by TUS-induced homogenization, making milk appear whiter (Gaucher *et al*. 2008; Li *et al*. 2019).

Protein denaturation under TUS predominantly affects membrane-associated proteins, particularly xanthine oxidase and butyrophilin, thereby weakening the integrity of the milk fat globule membrane (MFGM) (Zhao *et al*., 2024). Gregersen *et al*. (2019) reported that high-intensity US combined with moderate temperatures (50–70 °C) and intermediate power levels (30–50 W) can denature up to 40% of the major whey proteins β-lactoglobulin and α-lactalbumin, demonstrating that both acoustic energy and heat contribute significantly to protein destabilization. Cavitation also disrupts the fat globule membrane, promoting the adhesion of casein micelles and the formation of a granular interfacial network, which enhances emulsion stability and improves product texture (Gaucher *et al*., 2008; Li *et al*., 2019; Villamiel & De Jong, 2000).

TUS reorganizes protein–lipid interactions, thereby strengthening emulsion stability. Cavitation reduces the size of fat globules, increasing surface area and facilitating efficient adsorption of casein micelles and whey proteins. These proteins anchor to exposed lipid surfaces through hydrophobic and electrostatic interactions; polar residues such as lysine and glutamic acid interact with phospholipid head groups, reinforcing the interfacial layer. The resulting network enhances colloidal stability, reduces creaming, and improves viscosity and uniformity—parameters critical for producing high-quality dairy products (Bermúdez-Aguirre *et al*., 2008; Ragab *et al*., 2019).

Conversely, studies assessing lipid fractions have not detected significant changes in free fatty acid concentration or total fat content following TUS treatment, confirming lipid stability under the evaluated conditions (Deshpande & Walsh, 2021; Hernández-Falcón *et al*., 2018). Nevertheless, disruption of the MFGM during TUS exposes the triglyceride core, enabling casein micelle adhesion and the development of a granular interfacial structure that contributes to improved emulsion stability (Villamiel & De Jong, 2000).

An increase in viscosity was observed in studies using higher amplitudes (100%) or extended treatment times (>10 min), attributed to interactions between protein and lipid fractions induced by localized shear forces (Dhiny *et al*. 2023). No signs of phase separation, gel formation, or sedimentation were reported, even after 15 days of storage (Annandarajah *et al*. 2018; Deshpande & Walsh, 2021; Hernández-Falcón *et al*. 2018; Vijayakumar *et al*. 2015). TUS also improved emulsion stability by reducing particle size through cavitation (Bermúdez-Aguirre *et al*. 2008; Hernández-Falcón *et al*. 2018). These results indicate that TUS enhances milk functionality without compromising its essential properties.

8 Proximate analysis after thermoultrasonication

Several studies assessed the proximate composition of RCM following TUS by measuring fat, total protein, non-fat solids (NFS), and total solids (TS) (Table 5) (Bermúdez-Aguirre *et al*. 2008, 2009a; Dhiny *et al*. 2023; Hernández-Falcón *et al*. 2018; Wang *et al*. 2022; Zhou *et al*. 2021). In most studies, protein content was not significantly affected, indicating that protein denaturation was not detectable under the evaluated conditions (Bermúdez-Aguirre *et al*., 2009a, 2009b; Zhou *et al*., 2021). For instance, total protein quantification by nitrogen determination (Kjeldahl or Dumas methods) is insensitive to conformational changes, and thus partial denaturation induced by cavitation may occur without affecting total protein values. The variations observed in some measurements likely reflect changes in solubility, recovery efficiency, or artefacts associated with colorimetric assays, rather than a true loss of protein mass.

Table 5. Effect of TUS[1] on the physicochemical and proximate analysis of RCM[2].

Parameter	CHT[3]	TUS[1]	Reference
Physicochemical properties			
pH	6.74± 0.06	6.67 ± 0.06 – 6.71 ± 0.06	Hernández-Falcón *et al*. (2018)
	6.74± 0.06	6.61 ± 0.01 – 6.66 ± 0.05	Bermúdez-Aguirre *et al*. (2009a)
	6.74± 0.06	6.61 ± 0.01 – 6.66 ± 0.05	Bermúdez-Aguirre *et al*. (2009b)
	6.82± 0.02	6.80 – 6.85	Deshpande & Walsh (2021)
	6.71± 0.04	6.66 ± 0.02	Zhou *et al*. (2021)
Acidity (%)	0.20± 0.05	0.19 ± 0.04 – 0.20 ± 0.05	Hernández-Falcón *et al*. (2018)
	0.126± 0.008	0.136 ± 0.012 – 0.14 ± 0.00	Bermúdez-Aguirre *et al*. (2009a)
	0.126± 0.008	0.136 ± 0.012 – 0.146 ± 0.00	Bermúdez-Aguirre *et al*. (2009b)
Density (g/mL)	1.032 ± 0.00	1.032 ± 0.00	Hernández-Falcón *et al*. (2018)
	1.0317 ± 0.0010	1.026 ± 0.001 – 1.026 ± 0.00	Bermúdez-Aguirre *et al*. (2009a)
Viscosity (mPa/s)	1.41 ± 0.14 – 1.42 ± 0.14	1.39 ± 0.00 – 1.60 ± 0.05	Vijayakumar *et al*. (2015)
Stability (%)	~ 96.2	~ 96.25–97.19	Hernández-Falcón *et al*. (2018)
Proximate analysis properties			
Fat (%)	4.22 ± 0.02	4.24 ± 0.02	Bermúdez-Aguirre *et al*. (2008)
	4.22 ± 0.02	4.21 ± 0.04 – 4.29 ± 0.05	Bermúdez-Aguirre *et al*. (2009a)
	4.22 ± 0.02	4.21 ± 0.04 – 4.29 ± 0.05	Bermúdez-Aguirre *et al*. (2009b)
Protein (%)	3.55 ± 0.01	3.00 ± 0.01 – 3.04 ± 0.03	Bermúdez-Aguirre *et al*. (2008, 2009a)
	2.53 ± 0.38 – 3.02 ± 0.16	3.22 ± 0.16	Zhou *et al*. (2021)
NFS[3] (%)	8.60 ± 0.40	8.63 ± 0.41 – 8.67 ± 0.54	Hernández-Falcón *et al*. (2018)
	9.50 ± 0.03	8.03 ± 0.04 – 8.36 ± 0.43	Bermúdez-Aguirre *et al*. (2009a)
TS[4] (%)	11.59 ± 0.40	11.62 ± 0.40 – 11.67 ± 0.54	Hernández-Falcón *et al*. (2018)

[1]TUS: thermoultrasonication. [2]RCM: raw cow´s milk. [3]CHT: conventional heat treatment. [3]NFS: nonfat solids. [4]TS: total solid.

Fat content, by contrast, was reported to increase by approximately 5%. In one study, TUS applied at 400 W, 24 kHz, and 100% amplitude for 30 min reduced the mean fat globule diameter to approximately 1 μm. Mechanistically, cavitation disrupts fat globule aggregates into triacylglycerol microdroplets, yielding a finer and more homogeneous lipid dispersion and thereby increasing the fraction of dispersed and analytically recoverable lipids (Bermúdez-Aguirre *et al*., 2008). Consequently, the altered size distribution of fat droplets results in a modestly higher measured fat content (~5%), even though the total lipid mass in the system remains unchanged (Bermúdez-Aguirre *et al*., 2008, 2009a, 2009b; Dhiny *et al*., 2023). TS and NFS values remained stable, showing no significant differences compared to CHT, indicating that TUS does not compromise the chemical integrity of RCM (Hernández-Falcón *et al*. 2018).

9 Functional and technological properties

TUS has also been shown to enhance the hydrolysis of soluble proteins when treated with pepsin and pancreatin (Wang *et al*., 2022). This effect is driven by significant conformational changes in milk proteins, including partial unfolding of tertiary structures, increased exposure of hydrophobic residues and peptide bonds, and disruption of intramolecular hydrogen and disulfide bonds. These structural modifications improve the accessibility of digestive enzymes to cleavage sites, thereby accelerating enzymatic hydrolysis. Fourier transform infrared (FTIR) spectroscopy revealed a decrease in α-helix content accompanied by an increase in β-sheet and random coil structures, consistent with enhanced enzymatic binding and fragmentation efficiency (Wang *et al*., 2022).

US -assisted enzymatic hydrolysis benefits from several mechanistic improvements, as reviewed by Qian *et al*. (2023). Cavitation enhances mass transfer by disrupting boundary layers, reducing particle size, and increasing surface area. US may also induce subtle conformational changes in enzyme molecules, improving flexibility and catalytic efficiency without compromising the integrity of active sites. Optimal US parameters (20–40 kHz, 100–500 W) have been reported to reduce hydrolysis times by up to 30%, increase the degree of hydrolysis by 10–20%, and generate bioactive peptides with enhanced functional properties (Yang *et al*., 2018; Balthazar *et al*., 2019; Qin *et al*., 2018; Magalhães *et al*., 2022). These findings suggest that integrating US into enzymatic protocols can significantly improve protein degradation and produce peptides with targeted bioactivities relevant to dairy applications.

The microstructural changes induced by TUS also result in techno-functional improvements, including increased protein solubility, higher emulsifying capacity, and improved gel stability in fermented products, with minimal differences in flavor, aroma, or mouthfeel compared with CHT (Abesinghe *et al*., 2019; Zlatev *et al*., 2018; Vijayakumar *et al*., 2015). In a sensory evaluation involving a trained panel (n = 8), participants used a triangle test to characterize odor attributes and quantify intensities on a 15-cm scale. Panelists generally rated TUS-treated milk as acceptable, with no significant sensory drawbacks (Vijayakumar *et al*., 2015). In addition, Vijayakumar *et al*. (2015) and Annadarajah *et al*. (2018) reported that TUS inactivated over 90% of plasmin, maintaining low activity through 49 days of storage. This inactivation is relevant for commercial shelf life, particularly in UHT milk, where plasmin degradation of caseins produces hydrophobic peptides associated with bitterness, viscosity changes, and protein destabilization. Controlling plasmin activity thus contributes to flavor and texture preservation during storage (7–14 days for HTST milk) (Bui *et al*., 2021). However, in cheese manufacture, plasmin plays a key role in proteolysis during ripening; therefore, its inactivation should be adjusted according to cheese type and ripening objectives (Vélez *et al*., 2016; France *et al*., 2021; Wang *et al*., 2024).

Beyond microbiological and physicochemical changes, TUS also affects sensory attributes and product stability through interconnected structural and molecular processes. Treated milk generally exhibits increased whiteness (L^*), smoother texture, and higher viscosity, primarily due to reduced fat globule size and enhanced protein–lipid interfacial stability (Zhou *et al*., 2021; Gou *et al*., 2023). Under moderate conditions, flavor remains comparable to HTST; however, high-intensity treatments (e.g., 200 W for 2 min) may produce subtle off-flavors described as gummy, burnt, or foreign, likely associated with whey protein unfolding and mild lipid oxidation (Pérez-Andrés *et al*., 2018). Overall, sensory panels have consistently rated TUS-treated milk as acceptable when processing parameters are properly optimized (Vijayakumar *et al*., 2015).

9.1 Shelf-life and stability under refrigeration

TUS extends the refrigerated shelf life of RCM by inhibiting microbial regrowth and maintaining physicochemical stability. Hernández-Falcón *et al*. (2018) observed stable microbial counts for up to 15 days at 4 °C without phase separation or sedimentation. Other studies reported that TUS-treated milk preserved its pH and fat quality over similar periods (Deshpande

& Walsh, 2021; Hernández-Falcón *et al*., 2018). At the molecular level, cavitation exposes hydrophobic amino acid residues and free sulfhydryl groups in proteins such as β-lactoglobulin and generates low levels of reactive species, including lipid hydroperoxides. These modifications enhance interfacial behavior and structural functionality; however, if TUS parameters are not properly controlled, mild oxidative reactions may occur, leading to subtle sensory changes (Astráin-Redín *et al*., 2023; Zhang *et al*., 2018). Nevertheless, oxidative stability is generally maintained under optimized conditions, with no significant increases in FFAs or off-flavor development (Shokri *et al*., 2022).

FTIR (post-treatment TUS) analysis of the Amide I band confirmed TUS-induced alterations in secondary protein structure, characterized by increased α-helix and random coil content at the expense of β-sheets and β-turns. While these spectra reflect the overall protein matrix rather than specific proteins, whey proteins such as β-lactoglobulin are particularly susceptible to denaturation. Such modifications may reduce immunoglobulin E binding and allergenicity, while also enhancing the digestibility of casein fractions (Ehn *et al*., 2004; Wang *et al*., 2022).

TUS has also been reported to reduce aflatoxin M1 (AFM1) content, potentially through structural modifications in the lactone or furan rings (Mortazavi *et al*. 2015). This reduction was associated with an increase in phenolic content and antioxidant activity (Hernández-Falcón *et al*. 2018). One study noted that the increase in antioxidant activity may result from weakened Van der Waals forces, hydrogen bonds, and hydrophobic interactions, leading to the release of phenolic compounds into the medium (Hassam *et al*. 2013).

10 Methodological limitations and experimental constraints

Although the overall evidence highlights the potential of TUS, the comparability and generalizability of findings are constrained by methodological heterogeneity among the reviewed studies. Key challenges for rigorous synthesis include variations in amplitude (30–100%), frequency (20–40 kHz), temperature (15–72 °C), sample volume, probe design, and treatment duration (0.25–30 min), as well as differences between batch and continuous applications, thermal control strategies, measurement of initial microbial loads, and inadequate characterization of the milk matrix. Several studies also lack sufficient replication or appropriate thermal controls, making it difficult to distinguish the specific contributions of TUS from temperature-related effects. Furthermore, few investigations report complete energy profiles, acoustic transfer efficiency, or scaling parameters, which hampers the assessment of industrial relevance. In addition, the absence of systematic analyses of oxidative by-products and markers of protein damage limits the capacity to balance potential risks against technological benefits. Most studies were conducted on a laboratory or pilot scale, with limited reproduction in industrial environments (Table 2). The survey by Deshpande & Walsh (2021) is a notable exception, as it assessed a continuous flow system on a small scale.

Further validation is necessary to translate these promising findings into industrial practice. Regulatory approval will also require reproducible data on microbial inactivation, enzyme activity reduction, and possible formation of toxic by-products. Additionally, new research into energy consumption and cost-effectiveness will help determine whether TUS offers economic advantages over traditional CHT.

11 Toward practical adoption in the dairy sector

The application of TUS to RCM aligns with current trends in the dairy industry, which seek to ensure microbial safety while preserving nutritional and sensory quality. The synergy between ultrasonic cavitation and moderate temperatures presents a promising option for processing raw milk and developing specialized dairy products that benefit from partial homogenization, extended shelf life, or reduced contaminants such as AFM1. However, practical adoption requires thorough pilot-scale experimentation, robust engineering for high-throughput systems, and full regulatory compliance. Integration into existing production lines, potentially before or after cream separation, must be carefully designed to avoid unwanted homogenization that could lead to rancidity if lipase activity remains high. Despite these challenges, the potential of TUS as an energy-efficient, environmentally friendly technology capable of improving final product quality highlights its promise in future dairy operations.

Although this review focuses on studies applying TUS directly to RCM, related research provides complementary insights. For example, Gautam *et al*. (2025) examined the effects of TUS on the plasmin system in buffalo milk, observing structural changes in proteolytic pathways that may also apply to cow's milk, albeit with species-specific matrix differences. Furthermore, recent work on US energy density and modulation modes (continuous versus pulsed) in fresh cow's milk without combined heating demonstrated significant effects on microbial, physicochemical, and techno-functional properties. While these findings fall outside the strict definition of TUS, they underscore the importance of standardizing energy input and wave characteristics to improve reproducibility. Overall, structural and functional modifications induced by TUS enhance microbial safety and improve techno-functional properties of milk (emulsification, gelation, texture), positioning it as a promising tool for next-generation dairy products.

Building on the mechanisms described above, the advantages and limitations of TUS relative to CHT can be outlined. Comparative studies show that TUS achieves microbial reductions equal to or greater than those of HTST or UHT, while better preserving thermolabile compounds such as vitamins, bioactive peptides, and native enzymes, and maintaining the colloidal integrity of milk—attributes that support clean-label formulations (Wang *et al*., 2022; Shen *et al*., 2017). These features give TUS a clear advantage in final product quality without compromising safety. Nevertheless, several operational challenges persist precise control of parameters is required to avoid variability in cavitation distribution; prolonged treatments may increase energy consumption; and high intensities can generate undesirable flavors. To strengthen competitiveness against CHT, systematic comparisons under diverse raw milk conditions, harmonized regulatory criteria, and assessments of potential sub-lethal injury and microbial recovery are essential.

None of the included studies evaluated the effectiveness of TUS against *Brucella* sp. or *Mycobacterium* sp. in milk, highlighting a critical gap and an opportunity for future research to validate its capacity to inactivate these pathogens.

11.1 Scale-up and engineering considerations

Scaling TUS from laboratory to industrial systems presents several engineering challenges. Uniform cavitation distribution in larger volumes is hindered by acoustic attenuation, reflections, and dead zones; thus, reactor design must optimize transducer geometry, operating frequency, and sonication pattern (batch versus continuous flow) to ensure consistent energy densities. Thermal management must be integrated with US delivery to prevent hot spots and maintain synergy between acoustic and thermal effects. Real-time monitoring of acoustic energy density, effective temperature, and local intensity requires calibrated sensors or indirect methods (e.g., calorimetry, dissolution profiles), as the absence of robust metrics limits reproducibility and validation.

Continuous-flow systems offer advantages in scalability and uniformity compared to batch processes, but require balancing residence time, amplitude, and flow rate to ensure effective microbial inactivation while preserving product quality. Assessments of acoustic transfer efficiency and energy dissipation are essential at larger scales to optimize power usage and minimize energy losses from reflections or poor coupling. Integration into existing production lines also demands compatibility with upstream and downstream processes (e.g., cream separation, homogenization), while practical considerations such as maintenance, clean-in-place procedures, and noise-resistant materials must be addressed.

11.2 Regulatory and safety validation

For widespread adoption, TUS must undergo rigorous regulatory review to confirm its safety and consistency. Establishing critical control parameters and acceptance criteria for microbial inactivation, potential toxic by-products (e.g., aflatoxins), and preservation of functional compounds is essential. Validation should include inter-laboratory studies and benchmarking against HTST, with assessments of pathogen reduction, sub-lethal injury, and microbial recovery. Comprehensive analyses of acoustic energy transfer, thermal profiles, and oxidation products are also needed to identify hidden risks and balance potential drawbacks against benefits. Documentation should adhere to international standards (e.g., Codex Alimentarius, FDA, EFSA) to facilitate global market acceptance.

11.3 Economics and adoption roadmap

Adoption of TUS in dairy plants depends on both its technological benefits and economic feasibility. Initial cost–benefit analyses should compare energy use per liter with HTST/UHT, accounting for potential savings in refrigeration from extended shelf life and reduced product loss. Investments in specialized equipment, online monitoring, and operator training must be weighed against gains in waste reduction, quality retention, and product differentiation (e.g., clean-label marketing, longer shelf life). A phased implementation is recommended: beginning with pilot studies integrated into existing lines, followed by development of performance indicators that combine technical and economic metrics, and eventually scale-up to optimized continuous systems. Validation groups comprising academia, industry, and regulators should be established to share data, mitigate risks, and accelerate standardization.

*An adoption roadmap may include:

1. **Standardization and multi-centre validation** – development of consensus protocols for TUS parameters (acoustic energy density, temperature profiles, residence time) and inter-laboratory studies to ensure reproducibility.

2. **Industrial-scale pilot studies** – combining engineering optimization (reactor geometry, continuous-flow designs, real-time monitoring)

with techno-economic assessments of energy use, product quality retention, and process robustness.

3. **Safety and quality frameworks** – validation schemes covering microbial inactivation kinetics (including sub-lethal effects), oxidative and structural changes, and sensory stability under real storage conditions, aligned with international guidelines.

4. **Application-specific optimization** – tailoring TUS regimes for products such as yogurt or cheese, with endpoints including gel strength, emulsion stability, and flavor retention.

5. **Stakeholder integration** – creation of consortia involving academia, industry, and regulators to share data, develop certification standards, and establish labelling strategies*.

Conclusions

The evidence indicates that TUS is a viable technology for treating RCM, achieving microbial reductions comparable to CHT. In contrast to heat-based methods, TUS preserves heat-sensitive nutrients, bioactive compounds, and colloidal integrity, thereby supporting desirable techno-functional properties. By coupling temperature control with acoustic cavitation, TUS provides safety benefits with reduced thermal damage, positioning it as an innovation for "quality preservation" rather than a mere alternative to HTST. However, significant hurdles remain in methodological standardization, regulatory validation, and market integration. Addressing these challenges is essential to transition TUS from promising laboratory findings to widespread industrial adoption.

Although the antimicrobial potential of TUS has been consistently demonstrated under laboratory conditions, several critical limitations restrict its broader application. The variability in experimental setups, including reactor geometry, transducer configuration, and amplitude calibration, introduces substantial heterogeneity in reported microbial inactivation levels, complicating cross-study comparisons. Furthermore, most investigations have been performed at bench scale using model systems or artificially inoculated milk, which may not accurately replicate the microbial diversity and load present in commercial dairy chains. The lack of standardized reporting of acoustic parameters, energy densities, and thermal profiles further hampers reproducibility and limits the development of predictive models. Consequently, while TUS shows promise as a non-thermal or minimally thermal alternative to CHT, systematic pilot-scale evaluations, harmonized methodologies, and regulatory validation are essential before reliable industrial adoption can be achieved.

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