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Can ultrasonication improve coagulation properties of indigenous and exotic cow milk for dairy product processing?

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ABSTRACT

This study investigates the effects of ultrasonication on milk coagulation properties, focusing on milk from indigenous Thamankaduwa White (TW) and exotic Holstein Friesian (HF) cattle. Milk samples were subjected to ultrasound treatment (20 kHz with a 2 cm probe depth and 60 % amplitude) at three energy densities (504, 612, and 720 J mL^{-1}). Acid-induced gels were formed using a lactic acid starter culture, while rennet-induced gels were produced through enzymatic coagulation. The results demonstrate that ultrasonication significantly enhances the water-holding capacity (WHC) in both gel types, particularly in HF milk treated at 720 J mL^{-1}. Ultrasonication also altered gel hardness, cohesiveness, and microstructure, with TW milk showing more pronounced changes. These include larger and more abundant void spaces in rennet gels and a coarser protein network in acid-induced gels. Ultrasonication offers a practical and efficient method to improve dairy product quality by enhancing WHC, hardness, and cohesiveness while reducing syneresis in milk gels. This technology also promotes the utilization of milk from underutilized indigenous cattle breeds, such as TW, in the dairy industry, contributing to sustainability and addressing industry challenges.

1. Introduction

The coagulation properties of cow milk vary depending on the breed, which can influence the technological characteristics during dairy product processing. Variations in milk composition among cattle breeds stem from their genetic differences, leading to unique compositional profiles (Poulsen et al., 2012). Previous studies have shown that indigenous cattle tend to produce milk with superior composition and nutritional properties compared to exotic cattle. Sharma et al. (2018) reported that Sahiwal milk had higher fat and total solid content compared to Holstein-Friesian (HF) milk. Similarly, Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva and Vidanarachchi (2022) reported better milk coagulation properties and higher κ-casein content in the milk of Thamankaduwa White (TW) cattle compared to Friesian and Jersey cattle breeds.

Indigenous cattle types such as TW and common local breeds ("*Batu*") play a substantial role in both milk production and dairy manufacturing in Sri Lanka (Abeykoon et al., 2016). Although they produce lower milk yields compared to generic breeds, these cattle are highly adaptable to harsh environmental conditions and can subsist on low-quality feed. They also display greater disease resistance than generic breeds. As a result, indigenous cattle are crucial to traditional rural production systems, thriving without expensive inputs (Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva and Vidanarachchi, 2022). They are essential for the livelihoods of rural communities, offering a variety of products and services (Silva et al., 2008).

Processed dairy products such as set-yoghurt and traditional fermented milk gels (*Meekiri* and *Deekiri*) made from indigenous buffalo or cattle milk are in high demand due to their firm curd structure, distinct flavour, and therapeutic properties (Priyashantha et al., 2021). According to traditional beliefs, milk from indigenous cattle in Sri Lanka is considered free from allergens responsible for milk allergies in humans (Silva et al., 2019). This characteristic can attract consumers in markets that prioritize allergy-friendly products. Thamankaduwa White, a key indigenous cattle breed predominantly found in Eastern and North Central provinces of Sri Lanka, is also known as "White cattle". These

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cattle are well adapted to the arid zone climate and resistant to most parasitic diseases (Shanjayan & Lokugalappatti, 2015). Additionally, TW milk demonstrates exceptional milk coagulation properties (Abeykoon et al., 2016). A study comparing Milk Clotting Profile (MCP) among various cattle breeds in Sri Lanka, including improved (Friesian and Jersey) and indigenous (TW and Lankan cattle) cattle types, discovered that TW milk exhibited the shortest rennet coagulation time, highest coagulum yield, and superior meltability values compared to the other breeds (Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva, Vidanarachchi, & Johansso, 2022).

The low milk production of indigenous cattle is the primary barrier preventing indigenous dairy farming from becoming a significant contributor to Sri Lanka's dairy sector. Many indigenous cattle types are under threat in various dairy farming systems due to their low productivity compared to generic breeds with high milk production capacities through genetic improvement programs for high and specialized productivity (Priyashantha & Vidanarachchi, 2024). Employing innovative technologies such as ultrasound may enhance the properties of Indigenous cow milk and provide valuable insights into improving nutritional quality, processing efficiency and market potential to empower the farming and preservation of Indigenous cattle. This approach will promote the conservation of valuable Indigenous cattle gene pools, preventing their extinction through enhanced utilization and protecting the agricultural system that sustains rural livelihoods.

Currently, dairy product manufacturers are increasingly adopting novel processing technologies to replace traditional methods. These advancements may enhance efficiency, food safety, shelf life, and the functional properties of dairy products (Ashokkumar et al., 2010). In this context, ultrasound technology provides a promising alternative to heat treatments and offers benefits like microbial reduction, milk fat homogenization, and improved milk functionality (Chandrapala et al., 2012). As a non-thermal method, ultrasonication can preserve the quality and nutritional value of fresh milk (Wang et al., 2022). Ultrasound, characterized by high-frequency sound waves, induces compressions and rarefactions in the liquid medium, forming cavitation bubbles. These bubbles implode, leading to physical and chemical changes like microstreaming, agitation, and turbulence, altering the milk's properties significantly (Carrillo-Lopez et al., 2021).

Ultrasound has shown significant benefits in various dairy processing methods. For example, ultrasound reduced the size of the fat globules to sub-micron levels, improving homogenization and emulsion ability. This enhances the texture and mouth feel of products like ice cream, beverages and chocolate milk. In chocolate milk, ultrasound treatment reduced particle sizes, resulting in improved fat distribution, enhanced antioxidant activity, and better nutrient retention. In Ice cream production, Ultrasound reduces ice crystal sizes, enhances freezing rates and improves the creaminess of ice cream making it more appealing to consumers. Ultrasound reduces the churning times and improves the viscosity and hardness of cream and butter. Ultrasound effectively reduces the microbial load in dairy beverages without compromising their sensory quality (Carrillo-Lopez et al., 2021).

Previous studies have shown that ultrasonication could improve the renneting behavior of milk which will have a huge potential in the cheese manufacturing industry (Liu et al., 2014). Moreover, it has been shown that ultrasound treatment could improve the strength of acid-induced gels, thus it can be used in the yoghurt manufacturing industry (Gregersen et al., 2019). The current study seeks to investigate the effects of ultrasound treatment on both rennet–induced and acid-induced coagulation of Thamankaduwa White (TW) milk, comparing it to milk from Holstein Friesian (HF) cattle. With limited research on the application of ultrasound to milk from indigenous cattle breeds in Sri Lanka, this study aims to fill this gap by examining the impact of ultrasound on milk from Indigenous breeds and comparing these results with those from exotic breeds, focusing on niche dairy products derived from Indigenous cattle.

2. Materials and methods

2.1. Experimental design

Fresh milk samples from three individual cows of HF were collected and pooled. These HF cows were raised in an intensive system with stall feeding using a Total Mixed Ration (TMR). Fresh milk samples from three individual TW cows were obtained and pooled. TW cows were reared in an extensive management system, exclusively practising free grazing. All sampled cows shared similar physiological conditions, specifically being in the second parity and mid-lactation stages. Following sample collection, the milk was promptly cooled to 4 °C and stored at that temperature until further analyses. All analyses were conducted within 2–3 days of milk sample collection.

2.2. Analysis of milk composition

The milk fat, protein, lactose, and solids-not-fat (SNF) content were analyzed using an ultrasonic milk analyzer (Lactoscan SP, SLP60, Milkotronic Ltd, Nova Zagora, Bulgaria).

2.3. Ultrasonication

Milk was subjected to ultrasonication in 50 mL aliquots in glass beakers with an Ultrasonic Mixer (Model TF-650 N, Tefic Biotech Co., Limited, China). The mixer consisted of a 6 mm diameter ultrasonic probe operating at a frequency of 20 kHz, with a probe depth of 2 cm and an amplitude set to 60 %. During ultrasonication, the beakers containing the samples were kept in an ice bath to control the temperature below 40 °C. During ultrasonication, the temperature of the core of the samples was continuously measured by the temperature probe of the ultrasonicator. The Energy Density (ED) applied to the samples was calculated according to equation (1) as mentioned by Nguyen & Anema (2017).

$$ED\left(\frac{J}{mL}\right) = \frac{Nominal\ power\ (W)\ X\ Process\ time\ (s)}{Sample\ Volume\ (mL)} \quad (1)$$

Ultrasonication was performed at three different power levels, 42 W, 51 W, and 60 W for a duration of 10 min, resulting in ultrasound energy densities of 504, 612, and 720 J mL^{-1}, respectively.

2.4. Milk coagulation

2.4.1. Preparation of rennet-induced coagulation of milk

Both ultrasound-treated and untreated milk samples were kept in a water bath at 37 °C for 10 min. One mL of 1 % (w/v) rennet solution prepared from commercial calf rennet with an enzyme activity of 600 IMCU (International Milk Clotting Units)/mL (Maxiren, DSM Food Specialties, The Netherlands) was added to 50 mL of milk and incubated at 37 °C for 40 min.

2.4.2. Preparation of acid-induced coagulation of milk

TW and HF milk acid-induced gels were prepared according to the procedure described previously by Abesinghe et al. (2020). Treated and untreated milk samples were batch pasteurized at 85 °C for 30 min followed by cooling to 40 °C. Then the milk samples were inoculated with 0.04 g/L freeze-dried direct vat set thermophilic yoghurt starter culture (STI Chr. Hansen, Denmark) consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. After the inoculation samples were incubated at 42 ± 2 °C for approximately 4 h, until the coagulum pH reached 4.6. Once the pH reached 4.6, the coagulum was stored at 4 °C for 15 h before measuring its coagulation properties.

2.5. Analysis of milk coagulation properties

2.5.1. Texture measurements

To assess the texture of rennet-coagulated milk, preheated milk at 37 °C was maintained for 10 min in plastic cups, after which rennet solution was added. Following the addition of rennet, the samples were incubated for 1 h at 37 °C using an incubator (Velp Scientifica FOC 215I, Velp Scientifica Srl, Italy). Acid-induced gel samples were prepared in a cylindrical form, with a diameter of 4 cm and a height of 4 texture profiles of prepared coagulated milk samples were analyzed using a texture analyzer (Brookfield CT3, Brookfield Engineering Laboratories, USA). For rennet coagulated milk samples, a TA-25/1000 cylindrical flat probe was used at a speed of 2 mm s^{-1} and a depth of 10 mm into the curd. For acid-induced gel samples, a cylindrical probe (25 mm diameter and 36 mm high) was used at a speed of 1 mm s^{-1} and a depth of 25 mm into the curd. Curves of force versus time were analyzed by in-built software TexturePro CT V1.8 Build 31.

2.5.2. Water holding capacity

WHC of rennet gels was determined by the centrifugation method. Gels were centrifuged at 4000 × *g* for 10 min (Sorvall ST 40R, Thermo Fisher Scientific, Germany). The supernatant was drained, and the remaining gel pellets were weighed to calculate the water-holding capacity (WHC). WHC of acid-induced gels was measured by the method described by Riener et al. (2009) with slight modifications. Twenty-five grams of coagulum samples from each treatment were transferred into 50 mL centrifuge tubes and centrifuged at 3000 × *g* for 10 min at 4 °C. The whey was drained from pellets and the weights of the pellets were obtained. The WHC of both rennet gels and acid-induced gels was calculated using the following equation (Riener et al., 2009).

$$\boldsymbol{WHC} = \frac{\textbf{Weight of remaining gel pellets}}{\textbf{Weight of initial milk sample}}\boldsymbol{X}100 \quad (2)$$

2.5.3. Syneresis

Syneresis of rennet gels was determined by the centrifugation method described by Daviau et al. (2000). In brief, 30 mL milk samples were placed in 50 mL centrifuge tubes and preheated in a water bath at 37 °C for 10 min. Then the samples were renneted and incubated in a water bath at 37 °C for another 40 min. The prepared rennet gels were then centrifuged at 1000 × *g* for 15 min (Sorvall ST 40R, Thermo Fisher Scientific, Germany). The separated whey was weighed and expressed as grams of whey per 100 g of milk. Syneresis of acid-induced gels was determined using the method described by Hassan et al. (2015). About 25 mL of coagulum from each treatment were transferred to 50 mL centrifuge tubes. The samples were then centrifuged at 1000 × *g* in a centrifuge (Sorvall, ST 40R, Thermo Fisher Scientific, Germany) for 20 min. The quantity of whey separated at the top of the coagulum inside centrifuge tubes was recorded in millilitres. The supernatant liquid's weight fraction was used as an index of whey syneresis (mL/100 g coagulum).

2.5.4. Microstructure

The coagulum samples were dehydrated using an air dryer. The specimens were mounted on aluminium SEM (Scanning Electron Microscope) stubs with carbon stickers and coated with a layer of gold–palladium by a Mini-Sputter coater/ Glow discharge system (SC 7620, Quorum Technologies Ltd, East Sussex, UK). Specimens were viewed on a scanning electron microscope (Zeiss EVO LS 15, Carl Zeiss Microscopy, 07,745 Jena, Germany), operated at 3.00 kV accelerating voltage at different magnifications.

2.6. Statistical analysis

The experiment followed a two-factor factorial completely randomized design (CRD) model. All samples were analyzed in triplicates, and the data were processed using the SPSS program (IBM – SPSS, V29.0), with a significance level set at $p < 0.05$. Tukey's HSD test was applied to identify significant differences, with results reported as means and standard deviations. To assess interaction effects, a two-factor factorial ANOVA was conducted, with a significance level of $p < 0.05$. The factors considered were ultrasound energy density, which had four levels (0 J mL^{-1}, 504 J mL^{-1}, 612 J mL^{-1}, 720 J mL^{-1}), and breed/cattle type, with two levels (TW and HF).

3. Results and discussion

3.1. Chemical composition of HF and TW milk

The chemical composition of HF and TW milk is shown in Fig. 1. The composition of bovine milk is significantly influenced by factors such as cattle breed or genotype, environmental conditions, stage of lactation, parity, and diet. Variations in the composition of milk can result in advantageous technological and functional characteristics in dairy products (Weerasingha et al., 2021). Based on the analysis of milk composition, it is apparent that cattle breed or type has a clear impact on the content of milk fat, protein, lactose, and solids-not-fat (SNF) contents. The fat, protein, lactose and SNF contents of Thamankaduwa White (TW) milk were significantly higher ($p < 0.05$) compared to Holstein Friesian (HF) milk, with average values of 4.80 %, 3.26 %, 4.09 % and 8.08 % respectively, for TW milk, versus 4.28 %, 2.79 %, 3.51 %, 6.92 % for HF milk.

The results of this study indicate that breed or type differences significantly affect milk fat content. Similarly, the higher milk fat content in TW compared to HF milk aligns with findings from previous studies. (Abeykoon et al., 2016; Weerasingha et al., 2021). The SNF content in TW milk was higher than that in HF milk. These findings align with those reported by Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva, Vidanarachchi, & Johansso (2022), who studied the same breed and cattle type, showing significantly higher SNF content in TW milk at 7.89 % compared to 7.29 % in HF milk.

However, in the current study, a higher protein and lactose content was observed in TW milk compared to HF milk in contrast to the results obtained for the same cattle breed and type by Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva, Vidanarachchi, & Johansso (2022). In another study, a notable increase in milk protein content was documented in pure indigenous Ethiopian cattle type in comparison to HF, where animals were subjected to the same feeding and management conditions (Kebede, 2018). Accordingly, the present study suggests that indigenous cattle breeds may exhibit superior milk compositional traits, specifically higher levels of fat, protein, lactose, and solids-not-fat (SNF) compared to exotic breeds. These traits should be considered in the planning of breed improvement programs.

3.2. Coagulation properties of rennet gels and acid-induced gels prepared from ultrasound-treated and untreated TW and HF milk

3.2.1. Effect of ultrasound on WHC and syneresis of milk gels prepared from TW and HF milk, within milk types

The application of ultrasound energy has been shown to influence various functional properties of milk gels. The variation of WHC and syneresis of rennet gels and acid-induced gels with applied ultrasound energy densities of TW and HF cow milk is shown in Table 1. The rennet gels prepared from ultrasound-treated samples had a higher ($p < 0.05$) WHC compared to rennet gels prepared from untreated samples in both TW and HF cow milk. Furthermore, with the increase of the applied ultrasound energy density, an increase in the WHC could be observed in both TW and HF milk gels. TW milk samples ultrasonicated at 720 J mL^{-1} had a higher ($p < 0.05$) WHC compared to samples ultrasonicated at 504 and 612 J mL^{-1}. It could be explained that ultrasound treatment can increase the binding of water inside the three-dimensional network of the gel coagulum. The application of ultrasound increases the

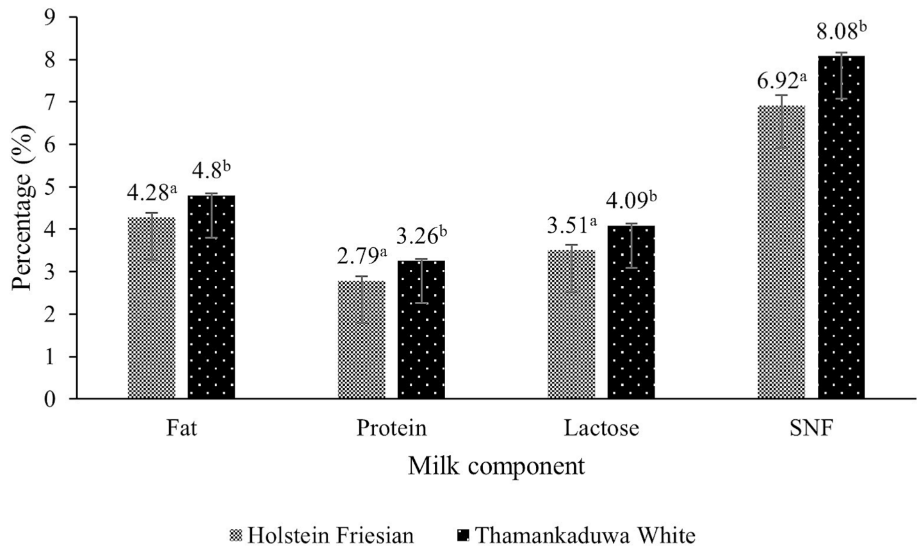

Fig. 1. Milk composition analysis of HF and TW milk The values followed by different superscripts for respective milk component are significantly different ($p < 0.05$).

Table 1
WHC and syneresis of rennet gels and lactic acid gels of ultrasound-treated and untreated TW and HF milk.

US ED (JmL^{-1})	WHC %		Syneresis (mL/100 g gel)	
	TW	HF	TW	HF
	Rennet Gels			
0	35.57 ± 2.17^{Aa}	28.54 ± 6.98^{Aa}	32.80 ± 1.60^{Aa}	39.62 ± 1.86^{Ab}
504	59.83 ± 8.26^{Ba}	40.92 ± 6.21^{ABb}	20.53 ± 2.09^{Ba}	30.07 ± 4.36^{ABb}
612	63.44 ± 5.92^{BCa}	47.97 ± 5.52^{Bb}	13.32 ± 3.90^{Ca}	28.83 ± 1.40^{Bb}
720	78.74 ± 5.88^{Ca}	55.76 ± 6.03^{Bb}	12.84 ± 8.70^{Ca}	13.78 ± 6.19^{Ca}
	Lactic Acid Gels			
0	81.43 ± 1.49^{Aa}	57.20 ± 10.13^{Ab}	26.83 ± 0.76^{Aa}	53.58 ± 1.14^{Ab}
504	78.07 ± 2.41^{ABa}	74.70 ± 0.63^{Ba}	28.51 ± 1.20^{Aa}	42.23 ± 11.90^{Aa}
612	75.73 ± 1.27^{Ba}	79.12 ± 5.82^{Ba}	29.84 ± 4.48^{Aa}	36.47 ± 8.44^{Aa}
720	75.00 ± 1.31^{Ba}	80.76 ± 2.50^{Bb}	29.99 ± 3.26^{Aa}	40.62 ± 1.51^{Ab}

The values (Mean ± SD) followed by different uppercase superscripts within the column for respective gel type and lowercase superscripts within the row for WHC and syneresis are significantly different ($p < 0.05$).
WHC: Water Holding Capacity, US ED: Ultrasound Energy Density.

denaturation of whey proteins in milk (Almanza-Rubio et al., 2015). The binding of whey proteins to casein micelles enhances the water-holding capacity (WHC) of the rennet gel, which may explain the higher WHC observed in the ultrasound-treated samples. Ultrasound partially denatures whey proteins, leading to the exposure of hydrophobic and sulfhydryl groups, which increases the surface hydrophobicity and free thiol (SH) groups. This modification enhances intramolecular hydrophobic interaction, promoting the formation of whey-whey aggregates (Shokri et al., 2022) According to Shanmugam et al., (2012), ultrasonication also causes the formation of whey-whey and whey- casein aggregates, which interact with casein micelles to form micellar casein aggregates. This crosslinking of proteins enhances water binding within the three-dimensional gel matrix, improving water-holding capacity (WHC) (Nguyen & Anema, 2010; Zaho et al., 2014; Abesinghe et al., 2020). The larger protein aggregates physically trap water molecules, leading to improved WHC in the gel.

In acid-induced gels, an increase ($p < 0.05$) of WHC due to ultrasonication was observed only in the HF samples. Whereas, TW samples showed an opposite trend where ultrasound-treated samples had lower WHC ($p < 0.05$) compared to untreated samples. A similar trend in water holding capacity (WHC) was observed in HF samples in previous studies. Shanmugam et al. (2012) found that ultrasonication causes denaturation of whey proteins and as a result, whey-whey and whey-casein aggregates are formed, which react with casein micelles and form micellar casein aggregates. Crosslinking may enhance water binding within the three-dimensional network of the gel matrix, thereby improving water holding capacity (WHC). (Nguyen & Anema, 2010; Zhao et al., 2014; Abesinghe et al., 2020). A likely explanation for the trend observed in TW samples is the higher κ-casein content in TW milk, which leads to smaller casein micelles (Abeykoon et al., 2016). Additionally, a previous study reported a slight reduction in casein micelle size following ultrasonication of skim milk (Shanmugam et al., 2012). In Zaho et al.'s (2024) study, excessive Ultrasound pretreatment resulted in decreased gel firmness, coagulum strength, texture stability index (TSI), cohesiveness and water-holding capacity (WHC). This negative impact on gel properties is attributed to the generation of smaller particle sizes, which hinder effective particle aggregation and gel formation. Therefore, the additional reduction in casein particles in TW milk due to ultrasonication may explain the decrease in water holding capacity (WHC) observed in its milk gels. (Nguyen & Anema, 2010; Zhao et al., 2014). An increase in water holding capacity (WHC) is desirable in acid-induced gels to restrict the free movement of water within the gel matrix, while the opposite is true for rennet gels. Thus, applied ultrasound energy density can be utilized to customize different milk types for these two types of gels.

Syneresis (contraction/ shrinkage of milk gels) is a phenomenon of liquid separation from coagulated milk products, resulting in undesirable effects. This is a significant visible defect that can occur during the storage of certain processed dairy products (Zhao et al., 2014; Joon et al., 2017). When ultrasound treatments were applied to milk, the syneresis was reduced ($p < 0.05$) in rennet gels prepared from both TW and HF milk. The decrease in syneresis upon ultrasound treatment was possibly associated with the sonochemical effects on milk fat globules (MFG) and milk proteins, which reduced the particle size while increasing the surface area. In addition, ultrasound may change the structure of whey proteins, causing partial denaturation and exposing more hydrophilic regions to the surrounding water molecules

(Abesinghe et al., 2019). Ultrasound treatment did not affect the syneresis of the acid-induced gels prepared from both TW and HF milk ($p > 0.05$).

The primary factor influencing consumer acceptance of fermented milk products, such as yoghurt and cheese, is their textural characteristics. Texture profiles comprising hardness, cohesiveness, adhesiveness, and springiness of rennet gels and acid-induced gels are shown in Table 2. Hardness (resistance of a sample to de-formation until an external force is applied) is the most commonly assessed physical parameter for the evaluation of both rennet and acid-induced gel texture. When considering the rennet gels, there was a reduction ($p < 0.05$) in the hardness of gels in ultrasound-treated samples in HF milk compared to untreated samples. However, there was no difference ($p > 0.05$) in the hardness of rennet gels of ultrasound-treated and untreated samples of TW milk. Studies conducted by Zhao et al. (2014) on goat milk, using an ultrasound power level of 800 W for up to 20 min, demonstrated a significant increase in the hardness of rennet gels. However, in the current study conducted on cow milk, no such increase in hardness was observed. A likely explanation for this observation is the insufficient energy inputs used in the current study to achieve the desired hardness in the milk coagulation process. Ultrasound at low power may not significantly enhance interactions between casein and whey proteins or improve their cross-linking ability, and might not sufficiently alter the milk's microstructure (e.g., fat globule dispersion or casein micelle structure), resulting in weaker gel matrices. However, the findings of the current study regarding the hardness of rennet gels produced from HF milk align with those of Hammam et al. (2021), which reported a decrease in hardness in ultrasound-treated samples compared to untreated ones. Further, the same authors stated that within the ultrasound-treated samples, there was a slight increase in the hardness with the increase in the ultrasound energy density. However, this trend was not observed in the present study, as shown in Table 2. The hardness of acid-induced gels from both TW and HF milk increased significantly ($p < 0.05$) due to ultrasonication, with a notable effect at an energy density of 720 J/ml. Similar findings have been documented in prior research.

Abesinghe et al. (2020) found that ultrasonication of buffalo milk at 66 W for 15 min (Energy Density: 1188 J mL^{-1}) increased the gel hardness by 98 % compared to the untreated milk gels. According to Gregersen et al. (2019), the increase in hardness of acid-induced full-fat milk was due to ultrasonication is probably because of the whey protein denaturation and aggregation of whey-whey and whey-casein aggregates. The same author noted that increased gel strength may be linked to the strong association between caseins and the milk fat globule membrane (MFGM) resulting from ultrasound treatment. They suggest that this may be a result of caseins associated with the MFGM to compensate for the increased surface space without resulting loss of MFGM proteins. According to Zhao et al. (2014), the interaction of the gel network and fat globule size is crucial in determining the product's texture. Joon et al. (2017) emphasize that lipid content and fat globule size significantly influence gel firmness. Ultrasound treatment causes substantial homogenization of the fat globules to very small sizes. The smaller fat globules in ultrasonicated samples likely explain the increased hardness observed in milk gels. Ultrasound pretreatment enhances the interaction among milk components, particularly milk proteins (Zhao et al., 2014), and is an effective method for improving milk coagulation properties in both rennet and acid-induced gels.

Cohesiveness is tensile strength and internal bond strength within samples (Zhao et al. 2014). There was no difference ($p > 0.05$) in the cohesiveness of rennet gels between the ultrasound-treated and untreated samples of TW cow milk. However, in HF cow milk, there was an increase ($p < 0.05$) in the cohesiveness of ultrasound-treated samples compared to untreated samples. The reason for the observed higher cohesiveness values may be due to the compaction of the structure of the gel due to the reduction in the particle sizes. In acid-induced gels, ultrasonication did not ($p > 0.05$) affect the cohesiveness of either TW or HF samples. Abesinghe et al. (2020) observed an increase in the cohesiveness of milk gels in ultrasound-treated buffalo milk (1188 J mL^{-1}), which may be attributed to the denaturation of whey proteins caused by ultrasonication. However, the current study did not observe such a trend.

Adhesiveness refers to the force required to detach material adhering to teeth during eating and is considered a negative force. It has a positive effect on the thickness of the gel samples and is an important factor governing the stability of the products (Delikanli and Ozcan, 2017). A significant increase ($p < 0.05$) was noted in the adhesiveness of rennet gels derived from ultrasound-treated TW milk compared to untreated TW samples. However, this trend was not observed for rennet gels from HF milk. In acid-induced gels, ultrasound did not ($p > 0.05$). Show a significant effect on the adhesiveness of gels derived from either TW or HF milk.

According to the results of the texture profile in the present study, ultrasound had no effect ($p > 0.05$) on the springiness of rennet gels or acid-induced gels from both types of milk. Springiness is the rate and extent to which a deformed material returns to its original condition after the force is removed. It depends on many factors such as heat treatment, interaction of proteins, degree of the unfolding of proteins and flexibility (Delikanli and Ozcan 2017). Abesinghe et al. (2020) noted enhanced springiness in buffalo milk gels following ultrasound treatment (1188 J mL^{-1}). However, this trend was not observed in the present study.

3.2.2. *Variability of the effect of ultrasound across different milk types*

As shown in Table 1, the WHC of rennet gels from untreated TW and HF milk was similar, with no significant difference ($p > 0.05$) between the two. The use of ultrasound in milk significantly increased the WHC

Table 2
Textural Parameters (Hardness, Cohesiveness, Adhesiveness and Springiness) of rennet gels and lactic acid gels of ultrasound-treated and untreated TW and HF milk.

US ED (JmL^{-1})	Hardness (N)		Cohesiveness		Adhesiveness (mJ)		Springiness (mm)	
	TW	HF	TW	HF	TW	HF	TW	HF
	Rennet Gels							
0	483 ± 69.86^{Aa}	453 ± 15.04^{Aa}	0.39 ± 0.09^{Aa}	0.39 ± 0.04^{Aa}	0.70 ± 0.36^{Aa}	0.57 ± 0.21^{Aa}	9.88 ± 0.31^{Aa}	9.69 ± 0.26^{Aa}
504	566 ± 104.80^{Aa}	244 ± 17.62^{Bb}	0.36 ± 0.03^{Aa}	0.47 ± 0.01^{Bb}	0.80 ± 0.44^{Aa}	1.03 ± 0.15^{Aa}	10.43 ± 0.82^{Aa}	15.93 ± 11.01^{Aa}
612	456 ± 27.97^{Aa}	223 ± 7.55^{Bb}	0.36 ± 0.02^{Aa}	0.49 ± 0.01^{Bb}	1.67 ± 0.20^{Ba}	1.17 ± 0.45^{Aa}	10.27 ± 0.84^{Aa}	11.31 ± 2.90^{Aa}
720	540 ± 41.22^{Aa}	240 ± 17.01^{Bb}	0.36 ± 0.01^{Aa}	0.47 ± 0.02^{Bb}	1.63 ± 0.58^{Ba}	0.47 ± 0.47^{Aa}	10.36 ± 0.33^{Aa}	22.25 ± 10.80^{Aa}
	Lactic Acid Gels							
0	155 ± 7.94^{Aa}	115 ± 5.03^{Ab}	0.33 ± 0.02^{Aa}	0.31 ± 0.02^{Aa}	3.93 ± 0.15^{Aa}	2.57 ± 1.19^{Aa}	23.27 ± 0.34^{Aa}	22.12 ± 0.48^{Ab}
504	177 ± 10^{ABa}	128 ± 7.57^{Ab}	0.31 ± 0.03^{Aa}	0.32 ± 0.01^{Aa}	5.33 ± 1.01^{Aa}	2.70 ± 0.36^{Ab}	23.20 ± 0.39^{Aa}	23.21 ± 0.45^{Aa}
612	200 ± 14.42^{Ba}	143 ± 11.06^{ABb}	0.34 ± 0.01^{Aa}	0.35 ± 0.01^{Aa}	5.30 ± 1.21^{Aa}	2.67 ± 0.42^{Ab}	22.73 ± 0.90^{Aa}	23.48 ± 0.21^{Aa}
720	190 ± 1.53^{Ba}	158 ± 16.37^{Bb}	0.33 ± 0.02^{Aa}	0.33 ± 0.02^{Aa}	4.97 ± 0.53^{Aa}	3.03 ± 0.35^{Ab}	22.40 ± 1.60^{Aa}	23.66 ± 0.97^{Aa}

The values (Mean ± SD) followed by different uppercase superscripts within the column for respective gel type and lowercase superscripts within the row for respective textural properties are significantly different ($p < 0.05$).

of rennet gels from TW milk ($p > 0.05$) compared to rennet gels from HF milk treated with the same ultrasound energy densities.

WHC of acid-induced gels of untreated TW milk was higher ($p < 0.05$) compared to the acid-induced gels from untreated HF milk. Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva, Vidanarachchi, & Johansso (2022) found that TW milk had higher fat content, casein content, κ-casein content, and lower somatic cell count (SCC) compared to HF milk. Furthermore, Abeykoon et al. (2016) stated that gels from TW milk had higher curd yield compared to that of HF milk. These properties can be attributed to the higher WHC in the TW milk gel compared to the HF milk gel, which is in line with the results of the present study. The water-holding capacity of acid-induced gels prepared from ultrasound-treated TW and HF milk showed similarity ($p > 0.05$) at energy densities of 504 and 612 J mL^{-1}. However, at 720 J mL^{-1}, the WHC of HF gels was higher ($p < 0.05$) than that of TW milk gels.

The syneresis of rennet gels prepared from untreated samples was lower in TW cow milk compared to rennet gels prepared from untreated samples of HF cow milk. The syneresis of TW milk rennet gels treated at ultrasound energy densities 504 and 612 J mL^{-1} were lower ($p < 0.05$) compared to HF milk samples treated at the same ultrasound energy densities. In acid-induced gels, the syneresis of gels from untreated TW milk was lower ($p < 0.05$) compared to the gels from untreated HF milk. The higher fat content in TW milk leads to a greater number of bonds being occupied by fat globules. Perhaps, whey drainage from the coagulum would have been more difficult in TW milk gels due to the more tortuous path the fluid must navigate, resulting in lower syneresis. According to the studies conducted by Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva, Vidanarachchi, & Johansso (2022), TW cow milk possesses higher κ-casein content and calcium content compared to HF cow milk. Both the κ-casein and calcium contents are important in milk coagulation which may also be a reason for the observed lower syneresis which represents higher coagulation ability in TW milk gels. Higher coagulation ability reflects improved aggregation of casein micelles, enabling them to trap water and other components more effectively within the gel matrix. A strong and uniform gel matrix can lower syneresis by minimizing whey expulsion, thereby enhancing the product's appearance. Conversely, excessive syneresis results in a watery and unappealing product. In acid gels, syneresis of ultrasound-treated TW samples was lower ($p < 0.05$) than in HF gels only in the 720 J mL^{-1} treatment (Table 1).

The texture profile, including hardness, cohesiveness, adhesiveness, and springiness, of rennet gels and acid-induced gels of TW and HF milk is provided in Table 2. When comparing the hardness of rennet gels derived from TW and HF milk, the untreated samples from both milk types exhibited similar hardness ($p > 0.05$). However, ultrasound-treated samples from TW milk displayed higher hardness ($p < 0.05$) than gels from HF milk subjected to the same energy densities. Whereas acid-induced gels from untreated TW milk exhibited significantly higher hardness ($p < 0.05$) compared to acid-induced gels from untreated HF milk. Similarly, acid-induced gels from ultrasound-treated TW milk showed greater hardness ($p < 0.05$) than those from HF milk treated with the same energy densities. This observation is probably due to the high amounts of total solids in TW milk. Amatayakul et al. (2006) previously documented a positive association between the overall solid content of milk and the firmness of the gel. Similar results for acid-induced gels from TW milk were reported previously by Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva and Vidanarachchi (2022). The higher casein concentration in TW milk compared to HF milk accelerates gel formation. Consequently, the heightened cross-linking within the gel network leads to a faster aggregation rate, resulting in a more solid curd (Dimassi et al., 2005).

The cohesiveness of rennet gels from untreated TW milk was similar ($p > 0.05$) to the rennet gels from untreated HF milk. However, rennet gels prepared from ultrasound-treated HF milk exhibited greater ($p < 0.05$) cohesiveness compared to those prepared from TW milk treated with the same energy densities. In acid-induced gels, untreated TW samples exhibited cohesiveness comparable ($p > 0.05$) to untreated HF samples). There were no significant differences ($p > 0.05$) observed in acid-induced gels between TW and HF samples treated with the same ultrasound energy densities.

The adhesiveness of untreated TW and HF samples was similar ($p > 0.05$) in both rennet and acid-induced gels. No significant differences ($p > 0.05$) were observed in the adhesiveness of rennet gels prepared from TW and HF milk treated with the same ultrasound energy densities. In contrast, in the acid-induced gels, the adhesiveness of ultrasound-treated TW samples was higher ($p < 0.05$) compared to those from HF milk treated with the same energy densities.

In rennet gels, the springiness of untreated samples of TW was similar to that of untreated HF samples. On the other hand, in acid-induced gels, untreated TW samples had higher ($p < 0.05$) springiness compared to the untreated HF samples. No significant changes ($p > 0.05$) were observed in the springiness of ultrasound-treated samples of TW and HF milk in both rennet and acid-induced gels.

3.2.3. *Interaction effect of ultrasound and milk composition (milk type) on the coagulation properties of rennet gels and acid-induced gels*

In rennet gels, the effect of interaction between the cattle breed/type (milk type) and ultrasound energy density was observed on syneresis, hardness, and cohesiveness ($p < 0.05$) which is graphically represented in Fig. 2. Such interaction effect was not observed ($p > 0.05$) in WHC, adhesiveness and springiness. Generally, ultrasound treatment decreases the syneresis of rennet gels. TW milk gels have lower syneresis than HF milk overall. A synergistic interaction can be observed in TW samples treated with ultrasound (Fig. 2 A). With increasing ultrasound energy density, TW milk gels exhibited a significant reduction in syneresis compared to HF milk gels. Excessive syneresis can make a product watery and visually unappealing, which is generally disliked by consumers. By reducing syneresis, whey separation from the gel matrix is minimized in the final product, thereby improving its texture and appearance, and ultimately enhancing consumer acceptance. This interaction effect can be attributed to the higher fat content in TW milk, which leads to a greater number of fat globules occupying binding sites. Ultrasound treatment has a homogenizing effect on fat globules, reducing their particle size and increasing surface area which allows for more water molecules to interact and bond with the fat globules. Additionally, ultrasound induces partial denaturation of whey proteins, exposing more hydrophilic regions that can interact with surrounding water molecules. The combination of these effects, fat globule modification and protein denaturation make whey drainage more difficult, thereby reducing syneresis. Consequently, the application of ultrasound to TW milk resulted in a noticeable reduction in syneresis, which may ultimately enhance consumer acceptance of these products. Ultrasound treatment did not increase ($p > 0.05$) the hardness of TW rennet gels, but it had a negative impact ($p < 0.05$) on the hardness of HF rennet gels (Fig. 2 B). While ultrasound does not affect the cohesiveness of TW gels ($p > 0.05$), it exhibited a synergistic effect ($p < 0.05$) on the cohesiveness of HF gels (Fig. 2 C). These findings suggest that the impact of ultrasound on the hardness and cohesiveness of rennet gels depends on the type of milk used.

In acid-induced gels, the effect of interaction between the cattle breed (milk type) and ultrasound energy density was observed only on WHC and syneresis ($p < 0.05$) which is graphically represented in Fig. 3. No such interaction was observed in the texture profile parameters ($p > 0.05$) of acid-induced gels. WHC of acid-induced gels increases with energy density for HF milk but decreases for TW milk, showing opposite trends and forming a crossover interaction pattern when plotted (Fig. 3A).

This indicates that the effect of ultrasound on WHC depends on the milk type. A probable reason for this could be, the higher casein content in TW milk which results in smaller casein micelles. Ultrasound treatment further decreases the micelle size, making it more difficult for these particles to aggregate and form a stable gel matrix. Consequently,

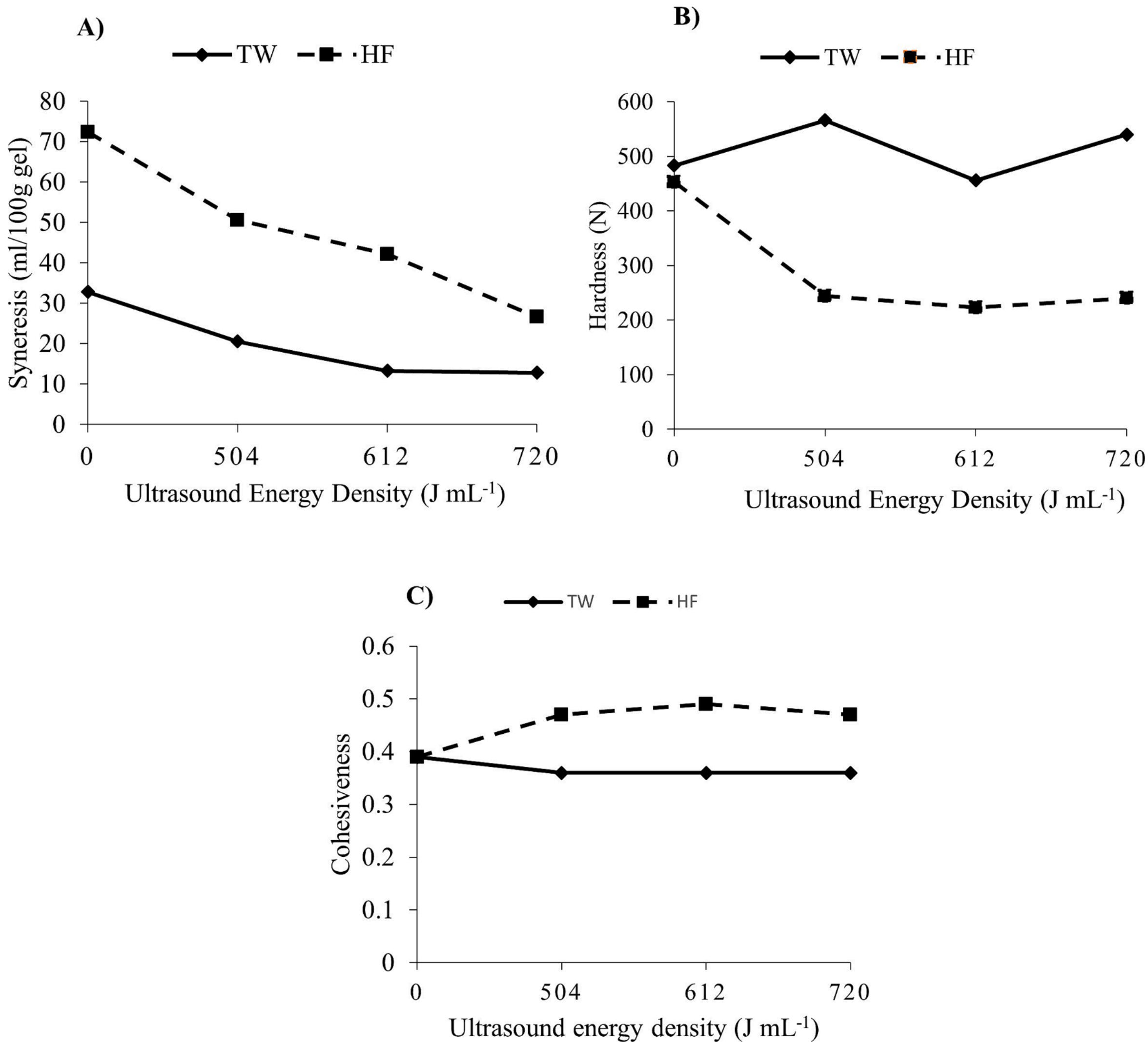

Fig. 2. A) Interaction effect of ultrasound and breed/milk type on the syneresis of rennet gels, B) Interaction effect of ultrasound and breed/milk type on the hardness of rennet gels C) Interaction effect of ultrasound and breed/milk type on the cohesiveness of rennet gels.

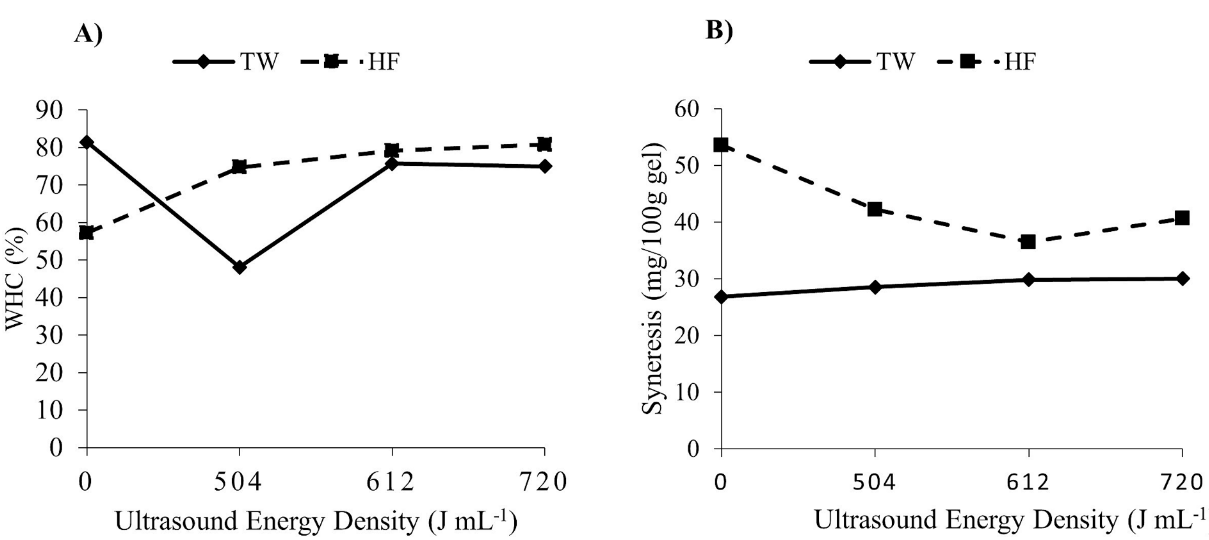

Fig. 3. A) Interaction effect of ultrasound and breed/milk type on the WHC of acid gels, B) Interaction effect of ultrasound and breed/milk type on the syneresis of acid gels.

this reduced aggregation capacity and negatively impact water retention, resulting in lower WHC in acid-induced gels from TW milk (Nguyen & Anema, 2010; Shanmugam et al., 2012; Zhao et al., 2014; Abeykoon et al., 2016).

TW acid-induced gels exhibited lower syneresis compared to HF acid gels overall. Ultrasound treatment does not lead to significant changes in the syneresis of acid-induced gels made from either TW or HF milk. However, a noticeable decrease in syneresis is observed in ultrasound-treated HF acid gels compared to the untreated HF acid-induced gels (Fig. 3B). These findings suggest that the impact of ultrasound on the syneresis of acid-induced gels is influenced by the type of milk used.

Further, the interaction effects suggest that the same ultrasound energy densities can differently affect the coagulation properties of rennet and acid-induced gels based on milk type. Therefore, in dairy processing, careful selection of ultrasound energy densities for each milk type is critical when applying ultrasound as a pre-treatment.

3.3. Microstructure

Microstructure acts as a key factor influencing texture and other physical properties of the final products resulting from rennet coagulation and acid-induced coagulation such as cheese and yoghurt, respectively. The gel comprises two main structures: the protein network and void spaces. The protein network includes casein micelles, crosslinks of casein, and fat globules embedded within the gel matrix. The void spaces are filled with lactose, water and other soluble components.

The scanning electron microscopic images of rennet gels of HF and TW cow milk are shown in Fig. 4. In untreated samples, the rennet gel from HF cow milk had a more uniform gel matrix with fewer void spaces compared to untreated samples of TW milk. In particular, TW rennet gel had more void spaces compared to HF milk rennet gels.

Ultrasound treatment affected the void spaces within the gel matrix in both TW and HF samples. The size and number of void spaces increased with an increase in the ultrasound energy density. Ultrasonic cavitation induces the denaturation of whey proteins. When β-lactoglobulin denatures, its free thiol groups are exposed, allowing covalent bonding with free thiol groups on κ-casein molecules located on the surface of casein micelles ((Steffl et al. 1999; Lucey, Munro and Singh 1999). As the ultrasonic energy density increases, whey protein denaturation also increases. Greater whey protein denaturation enhances protein–protein interactions within the gel network, resulting in a firmer structure with an increased number of void spaces. During processes such as ultrasonication, cavitation generates microbubbles that can become trapped within the gel matrix. These bubbles create void spaces, contributing to the gel's overall texture. Similarly, ultrasound can denature milk proteins (casein and whey), leading to enhanced aggregation. This creates a denser protein network that traps more water and air, resulting in both firmness and void spaces. However, it could be observed that this effect is more prominent in TW rennet gels compared to HF rennet gels. In the scanning electron microscopic images (Fig. 4) the void spaces may be filled with soluble components of the gel such as lactose.

The scanning electron microscopic images of acid-induced gels of HF and TW cow milk are shown in Fig. 5. The acid-induced gel from untreated HF milk had a uniform gel matrix with few void spaces whereas the acid-induced gel from untreated TW milk was not uniform and with more void spaces. These findings contradicted the results of a previous study by Weerasingha, Priyashantha, Ranadheera, Prasanna, Silva and Vidanarachchi (2022). In that study, set yoghurts made from TW milk exhibited a dense protein network with only a few small void spaces, while those made from HF milk displayed a porous structure with thinner protein strands.

When examining the ultrasonicated samples it appears that ultrasonication of raw Holstein Friesian milk had a minor effect on the final structure of the coagulum compared to that of TW milk. Ultrasonication significantly altered the microstructure of the coagulum in TW milk. Ultrasonication caused thinner gel networks in the coagulum from both milk types compared to untreated samples of the same milk type. The Coagulum of HF milk showed a thin and uniform protein network with small void spaces, whereas the acid-induced gel of TW milk resulted in a coarse protein network with large pores at treatments with 51 W and 60 W (energy densities of 612 J mL^{-1} and 720 J mL^{-1}, respectively). However, at a higher power level (*i.e.,* 720 J mL^{-1}) more porous structure of coagulum from both TW and HF milk was observed. In acid-induced gels from ultrasound-treated HF milk, a comparison of treatments at 612 J mL^{-1} and 720 J mL^{-1} revealed that the gel treated with 720 J mL^{-1} exhibited a greater number of small pores. Very large pores were observed in TW milk gels compared to HF milk gels at a higher level of power (*i.e.* 720 J mL^{-1}). These observations could be attributed to the larger pores created by ultrasonication which resulted in a decrease in the WHC of TW milk.

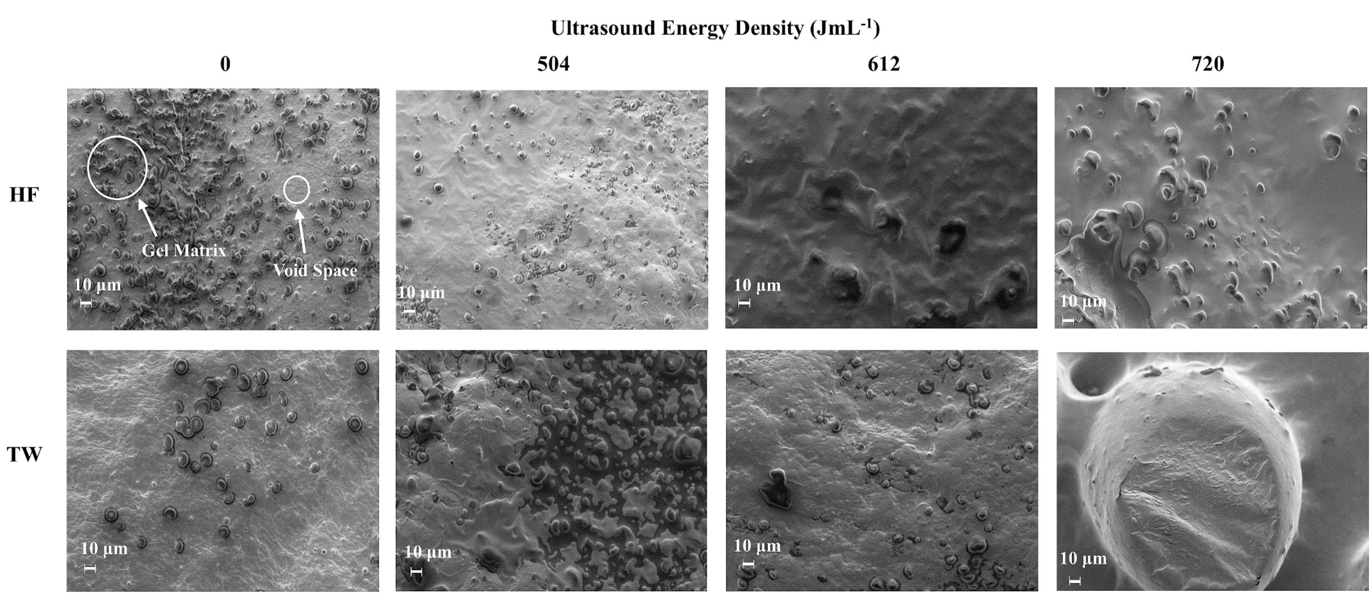

Fig. 4. Scanning electron microscopic images of rennet gels (Magnification. X 1000).

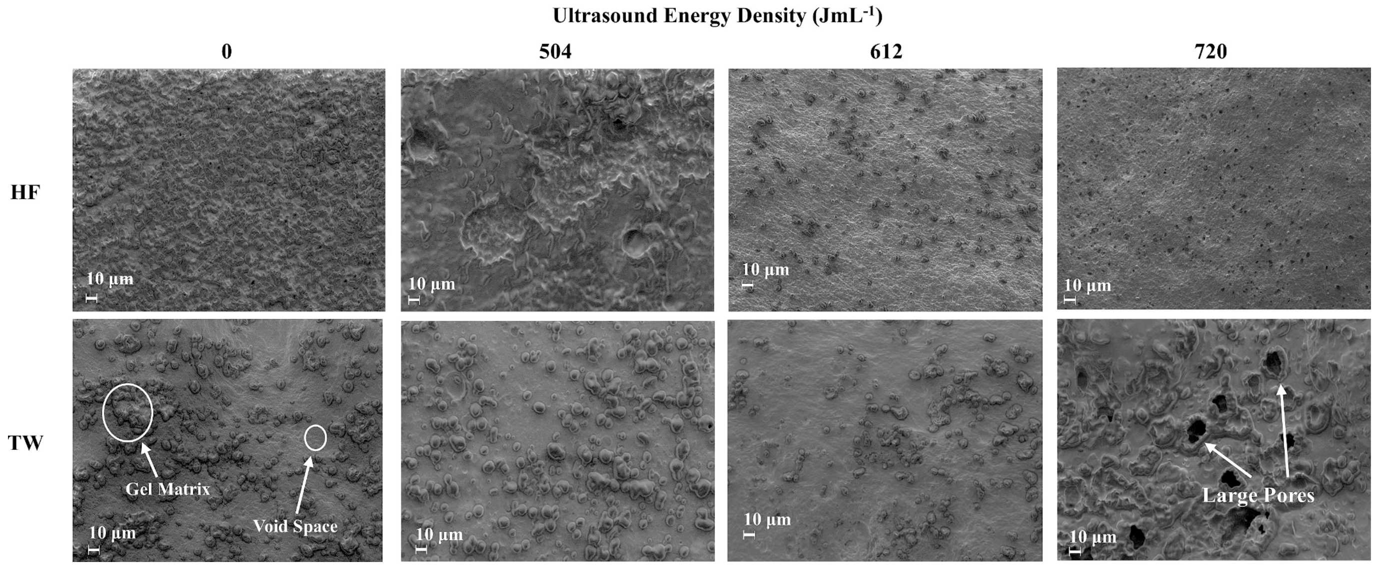

Fig. 5. Scanning electron microscopic images of acid gels (Magnification: X 1000).

4. Conclusion

Our study focused on assessing ultrasound's impact on TW and HF milk, revealing varying effects on gel properties. The present study revealed that the use of ultrasound as a pre-treatment can improve the rennet and acid-induced coagulation properties in both TW and HF cow milk. Furthermore, the study indicated that among the ultrasound treatments applied to the milk, an energy density of 720 J mL^{-1} was more effective in enhancing the rennet-induced coagulation properties. In the context of acid-induced coagulation, ultrasound pretreatment improves the hardness and WHC. However, milk from both TW and HF was differently affected by the same ultrasound power (energy density). The higher level of ultrasonication power (720 J/ml) has shown drastic changes in the overall coagulation properties of acid-induced milk gels from TW and HF cattle. Hence, ultrasound technology offers a viable solution for improving dairy product quality and preserving indigenous cattle types in Sri Lanka's dairy industry. The study found limited effects of ultrasound treatment on textural parameters, with significant changes observed only in WHC and syneresis for rennet gels and in WHC and hardness for acid gels, while other parameters remained unaffected. Further research and application of ultrasound hold the potential for addressing challenges and promoting sustainability in dairy farming.

Further, the interaction effects suggest that the same ultrasound energy densities can differently affect the coagulation properties of rennet and acid-induced gels. Therefore, in dairy processing, careful selection of ultrasound energy densities for each milk type is critical when applying ultrasound as a pre-treatment. This finding highlights the need for milk-type-specific optimization of ultrasound parameters to enhance gelation efficiency, improve product consistency, and potentially reduce production costs, which could benefit the development of tailored dairy products and innovative processing strategies. By incorporating the US into milk coagulation processes, milk processors can improve the texture, moisture retention, and overall quality of their dairy products, offering more appealing and high-value items to consumers. The effectiveness of ultrasound may vary depending on the milk type. Processors using indigenous cow-breed milk, such as TW may need to adjust treatment protocols to achieve the desired outcomes.

CRediT authorship contribution statement

L.B. Johnson: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **G. Diddeniya:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **J.K. Vidanarachchi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **P.H.P. Prasanna:** Validation, Supervision. **A.M.N.L. Abesinghe:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **H. Priyashantha:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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