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Physicochemical and Rheological Properties of Stirred Yoghurt during Storage Induced from High-Intensity Thermosonicated Goat and Cow Milk

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Abstract: The effect of high-intensity thermosonication (HIT) pretreatment (20 kHz frequency, output power 4000 W and 25% amplitude for 5, 10 and 15 min) on the physicochemical and rheological properties of stirred yoghurt made from goat milk was studied. Various parameters of the milk were evaluated, such as the particle size, pH and soluble calcium and phosphorus, while other parameters of the stirred yoghurt were evaluated during storage (up to 18 days), such as the rheological measurements, syneresis, pH values, titratable acidity, color, and sensory properties. The microstructure had more interconnected chains than the stirred yoghurt made from homogenized milk on the first day of the storage period. Moreover, the HIT process reduced the diameter of the fat globules in the goat milk, making them smaller than those of homogenized milk. This pretreatment could be used successfully in the production of stirred yoghurt to improve major quality parameters such as delayed syneresis, increased viscosity and enhanced sensory properties during storage.

Keywords: high-intensity thermosonication; goat milk; fermented milk; stirred yoghurt; rheological properties; microstructure

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1. Introduction

Yoghurt is a fermented dairy product that is widely manufactured and consumed. It is prepared by the fermentation of milk with lactic starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*). Additionally, the yoghurt becomes more nutritious than the milk used to make it [1]. Goat milk is a healthy alternative to cow milk, with benefits such as improved digestion, decreased allergic qualities and medicinal capabilities [2]. These benefits may be further enhanced by using goat milk in the stirred yoghurt industry as a vehicle for promoting human nutrition and health [3]. However, goat milk yoghurt has a lower consistency and apparent viscosity and a higher tendency for syneresis than cow milk yoghurt [4,5]. This is because goat milk casein micelles are smaller, have a lower proportion of αs1-casein, and have a different arrangement of the phosphate groups [6,7].

The homogenization of fat globules is a standard process commonly applied in the dairy industry to improve the yoghurt consistency, prevent whey release and reduce the unsolicited phenomenon of phase separation (creaming) in the final product [8]. Although ultrasound (US) is considered a non-thermal technique in the ultrasonic application, heat is generated unless the temperature is carefully controlled during the ultrasonic treatment [9,10]. Thermosonication is the combined impact of thermal processing and radiation treatment using US power, which is more successful than thermal treatment alone for the homogenization of fat, denaturation of whey proteins and inactivation of bacteria and enzymes. Meanwhile, in dairy processing, a combination of sonication and heat has been used to alter the characteristics and functionality of dairy proteins [11].

The application of thermosonication prior to inoculating milk with the starting culture improved the microstructure and rheological properties of yoghurt and prevented the whey separation [12,13]. A specific study on Turkey's fermented product (ayran) reported that thermosonication enhanced the rheology and sensory properties with a long shelf life during storage [14]. Ultrasonicating skim milk at a frequency of 22.5 kHz and output power of 50 W for up to 30 min, with or without temperature control, resulted in the generation of considerable heat, which induced denaturation of the whey proteins, modified the particle size of the casein and promoted the acid-induced gel reaction [5]. Furthermore, ultrasound homogenization of milk (frequency 20 kHz, amplitude 150–750 W) reduced the size of milk fat globules and caused the denaturation of whey proteins and the formation of protein aggregates [15]. Previous studies reported that thermosonication of skim milk before acidification by starter culture to produce gels/yoghurt enhanced the rheological, microstructure and sensorial properties compared to those produced from conventionally heated skim milk [12,16]. The ultrasound treatment of the casein solution before acidification (24 and 130 kHz ultrasound for 0, 60 and 120 min) had a significant impact on the gelation characteristics and increased the firmness of the acid gel [17]. In addition, the US increased the firmness and caused a homogenization of the fat globules of the acid gels prepared from ultrasonicated whole milk samples [10,12,15]. Furthermore, many studies found that thermosonication treatment improved the rheology and sensory properties, and prevented whey separation, giving a long shelf life during storage of the fermented milk [12,14,16]. Furthermore, thermosonication treatment is used to inactivate bacteria, homogenize fat globules and improve goat milk quality [18].

Based on previous studies, high-intensity thermosonication is a promising tool for milk homogenization, microbial inactivation and protein function promotion, which could result in a superior-quality stirred yoghurt. Despite the positive effects of sonication treatment on dairy processing, there are few publications available about the effects of high-intensity thermosonication application on stirred yoghurt made from goat milk. Therefore, by controlling the heating and ultrasound treatment for goat milk, it was possible to markedly modify the rheological properties, syneresis, sensory properties and microstructure of the stirred yoghurt.

2. Materials and Methods

2.1. Samples

Goat milk and cow milk were obtained from a goat farm (Hebei, China) and the Sanyuan Lvhe dairy farm (Beijing, China), respectively. The chemical compositions of the goat milk (pH 6.50) and cow milk (pH 6.79), as determined by the Beijing Dairy Cattle Center using Milko-Scan FT1 analyzer (Foss Electric, Hillerød, Denmark), were 3.27% protein, 3.69% fat, 5.00% lactose and 13.50% total solids for goat milk and 3.14% protein, 3.33% fat, 5.19% lactose and 12.40% total solids for cow milk. The samples were kept at 4 °C overnight.

2.2. Homogenization and High-Intensity Thermosonication Process

The control milk samples (goat and cow milk) were heated to 65 °C and homogenized at a pressure of 20 MPa (AH100D, ATS industrial systems limited company, Shanghai, China). For the high-intensity thermosonication process, a 20 kHz horn-transducer ultrasound processing unit (Beijing, China) was used. The processor was fitted with an ultrasonic probe with a titanium flat tip (14 mm diameter). Ultrasound was performed at a frequency of 20 kHz and output power of 4000 W with a 25% amplitude for 5, 10 and 15 min in 3 s/2 s work/rest cycles. The probe's temperature was adjusted to 60 °C during the sonication process, and the samples' temperature was kept at 60 °C by circulating hot water around the container. Throughout the process, the temperature of the sample was constantly monitored (Figure 1). All milk samples were pasteurized at 95 °C for 10 min in a water bath and then cooled rapidly to 42 °C. All sample measurements were performed following pasteurization.

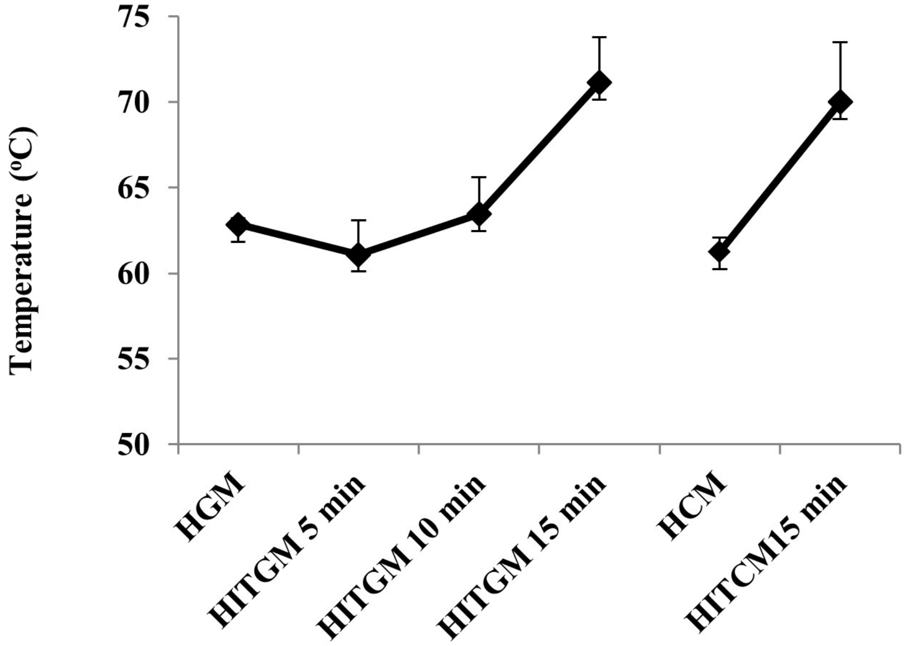

Figure 1. Changes in the temperature of goat and cow milk samples during the high-intensity thermosonication treatment. Values presented as means of triplicate ± standard deviation. HGM, homogenized goat milk; HITGM 5 min, goat milk, high-intensity thermosonicated for 5 min; HITGM 10 min, goat milk, high-intensity thermosonicated for 10 min; HITGM 15 min, goat milk, high-intensity thermosonicated for 15 min; HCM, homogenized cow milk; HITCM 15 min, cow milk, high-intensity thermosonicated for 15 min.

2.3. Particle Size Measurements

The average particle size of milk samples was evaluated using a Malvern Zetasizer Nano-ZS (Malvern Instruments Ltd., Worcestershire, UK) [18]. Appropriate dilutions (1:10, v/v) of the samples in distilled water were used to determine the particle size of the proteins (at refractive index 1.46 for casein micelle); each sample was diluted (1:1, v/v) with EDTA solution (10 g L^{-1} adjusted to pH 7.6 with 1 M of sodium hydroxide) to observe the size distribution of the fat globules at refractive index 1.45.

2.4. The pH, Soluble Calcium and Phosphorus Analyses

All milk samples were determined at room temperature using direct immersion with a glass electrode of a pH meter (Shanghai INESA Scientific Instrument CO., Ltd., Shanghai, China). Before the measurement, the pH electrode was calibrated using standard buffers at pH 4.0, 7.0 and 10.0. Furthermore, the concentrations of soluble Ca and P in the milk samples were determined using an inductively coupled plasma mass spectrophotometer (ICP-MS, Agilent, Santa Clara, CA, USA). The milk samples were prepared as assessed by Ragab et al. [18]. Using an ultracentrifuge, ten milliliters of milk were ultracentrifuged at 100,000× g for one hour at 4 °C (Beckman Coulter Inc., Brea, CA T4-TI-70 rotor, Ultracentrifuge Hitachi, Brea, CA, USA). The supernatant was carefully obtained for the determination of soluble calcium and phosphorus contents in the serum phase using ICP-MS. All the samples were measured in triplicate.

2.5. Stirred Yoghurt Preparation

Processed milk (2 L) was pasteurized at 95 °C for 10 min, with added 6% (*w*/*v*) commercial sugar, equilibrated at 45 °C, inoculated with a yoghurt starter culture (R-704-3332792, CHR-HANSEN, Guangzhou, China), and mixed well. The mixture was stirred for 2 min and then incubated for 4 h at 42 °C. The fermentation was stopped when pH 4.6 was reached and the samples cooled to 4 °C. The stirred yoghurt was produced by breaking the set yoghurt manually in the beaker with a spatula (4.7 cm × 5.5 cm) in a circular motion for 1 min. This stirring time had been determined in preliminary tests to give a visually smooth reference stirred yogurt [19].

2.6. Rheological Properties

The rheological parameters of stirred yoghurt samples were determined according to Gursoy et al. [20] at 5 °C using the controlled-strain rheometer (Physica MCR 301, Anton Paar Company, Graz, Austria) with a cup and probe type CC27SN13078. Measurements were taken at shear rates between 30 and 120 rpm. Analyses were performed at 10 s intervals and repeated 3 times.

2.7. Syneresis

For the syneresis that occurred during storage, stirred yoghurts were measured and spread evenly; 30 mL of the samples were taken on a Whatman No. 1 filter paper (Whatman Ltd., Maidstone, UK) in a funnel, placed on top of a 50 mL graduated cylinder at 4 °C for 2 h, and the collected liquid volume recorded in triplicate.

2.8. Titratable Acidity and pH Values

During the storage period, the titratable acidity as lactic acid (%) and pH of stirred yoghurt samples were determined using 0.1 M NaOH with phenolphthalein indicator, and a pH meter (Shanghai INESA Scientific Instrument CO., Ltd., Shanghai, China), respectively.

2.9. Confocal Scanning Laser Microscopy (CSLM)

The visualization of the microstructure of the stirred yoghurt samples was observed by CLSM according to Andoyo et al. [21]. The samples were stained by rhodamine B isothiocyanate (RITC) solution (85 g L^{-1} RITC in dimethyl sulfoxide, Sigma Aldrich, St Quentin Fallavier, France) at 0.2 g kg^{-1}. Then the samples were examined using a Leica TCS SP8 confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany) and observed through helium–neon laser (excitation at 543 nm and emission at 625 nm). The images were digitalized in grey level as 512 × 512 pixels matrix.

2.10. Color Measurements

According to Nassar et al. [22], color values of stirred yoghurt samples at 1 and 18 days of storage were determined on the CIE L^* a^* b^* color space, where L^* refers to lightness, a^* to redness/greenness and b^* to yellowness or blueness, using a Hunter Lab Color Quest XE Spectrophotometer (Birstall, Leicestershire, UK). The samples were calibrated for the original value with standard plates. Color measurements were taken using the reflectance specular included with the D65 illuminant, a 10° observer angle, and an 8 mm aperture. Each yoghurt sample (20 mL) was placed in an optical glass cell provided by the manufacturer of the colorimeter (diameter of the cell, 34 mm) and 10 measurements were taken at 3-s intervals at approximately 5 °C. Measurements were averaged for each sample.

2.11. Sensory Properties

Sensory evaluation was conducted according to Erkaya et al. [14]. The stirred yoghurt samples were organoleptically analyzed by 15 well-trained (5 males and 10 females, average

age 27) members of the Dairy Processing Department (Food Science and Technology Institute, Beijing, China) who were familiar with stirred yoghurt and had previous test panel experience of storage periods of 1, 8 and 18 days. Stirred yoghurt products were packaged in 3-digit-coded white plastic pots (40 g/pot) and stored at 4 °C. The order of presentation of yoghurt samples was randomized. Panelists evaluated each yoghurt in duplicate. The samples were at approximately 10 °C when they were tested (left at room temperature for 10–15 min). The sessions were also carried out in an air-conditioned room at 20 °C under white lighting in individual booths. During the evaluation process, the assessors rinsed their mouths between samples with warm water to ensure the accuracy of the experimental data according to the method developed by the International Dairy Federation [6]. Each yoghurt sample was evaluated for appearance (milky white level), consistency (smooth mouth feel for texture), odor (typical yoghurt fragrant), taste (good for sweet and sour), acidity and total acceptability. Each sensory attribute was rated on a hedonic scale (a 9-point scale), where the highest score indicates the highest quality, and the lowest score represents the lowest quality.

2.12. Electronic Nose

The electronic nose (PEN3.5, AIRSENSE, Schwerin, Germany) with 18 metal oxide sensors and an automatic headspace sampling system was used to detect the changes in the aroma [23]. Three replicates were prepared for analysis, and the data was evaluated using the mean of the replicates.

2.13. Statistical Analysis

Analysis of variance (ANOVA) was performed using SAS software (version 9.3 for SAS Institute Inc., Cary, NC, USA), with a probability level of 5% used to indicate significance. The LSD test was used to determine the significance of the results. The data of the electronic nose was analyzed by principal components analysis (PCA) which is commonly used for complex data analysis to summarize variation.

3. Results and Discussion

3.1. Particle Size Measurements

As shown in Table 1, the particle size of the high-intensity thermosonicated goat milk samples (HITGM) decreased steadily when compared to the homogenized goat milk sample (HGM). As found independently in our previous study [18], sonication could induce changes in the fat globules' size that reduced them. Even HITGM for 15 min showed a slight increase but not significantly ($p > 0.05$) compared to the other samples, which could be attributed to increased heating, which induces the whey protein denaturation associated with the casein micelles. Nevertheless, all the high-intensity thermosonicated samples (HIT) had smaller particle sizes than the homogenized samples. Similar findings showed that the ultrasound treatment of goat milk at 20 kHz and 800 W for 15 min reduced particle size due to the disruption of the casein micelles, causing the release of protein from the micellar to the serum phase [24].

The fat size changes of the HIT and homogenized milk samples are presented in Table 1. Fat provides a perception of creaminess and improves the mouthfeel and texture of the product. The fat size of the HIT goat milk samples decreased to 356, 272 and 292 nm after 5, 10 and 15 min, respectively, while the HGM was 503 nm. A similar result was reported by Ragab et al. [19], who observed a decrease in the size of the fat globules in raw goat milk treated by thermosonication (20 kHz at a power variance of 150 W, 200 W, 300 W and 400 W for 10 min). Further significant decreases ($p < 0.05$) in size were observed in the HITCM 15 min compared to the HCM samples, of 289 nm and 915 nm.

Table 1. Changes in soluble calcium and phosphorus concentrations (mg/100 g) in the water-soluble phase, pH values and fat size (nm) following the high-intensity thermosonication treatments compared to homogenized goat milk and homogenized cow milk.

Samples	Particle Size (nm) *	Soluble Calcium (mg/100 g)	Soluble Phosphorus (mg/100 g)	pH Values	Fat Size (nm)
HGM	302.9 ± 3.63 ^{c}	298.25 ± 6.21 ^{cb}	692.31 ± 14.89 ^{cb}	6.45 ± 0.004 ^{b}	503 ± 4.01 ^{b}
HITGM 5 min	294.9 ± 1.10 ^{cd}	289.40 ± 0.47 ^{cb}	695.83 ± 0.86 ^{cb}	6.45 ± 0.001 ^{b}	356 ± 6.48 ^{c}
HITGM 10 min	283.3 ± 0.20 ^{d}	279.31 ± 3.44 ^{c}	676.89 ± 6.32 ^{c}	6.41 ± 0.012 ^{c}	272 ± 0.77 ^{d}
HITGM 15 min	295.6 ± 0.65 ^{cd}	310.62 ± 2.16 ^{b}	713.44 ± 0.76 ^{b}	6.45 ± 0.004 ^{b}	292 ± 1.43 ^{d}
HCM	426.2 ± 1.39 ^{a}	367.56 ± 31.85 ^{a}	944.28 ± 17.84 ^{a}	6.79 ± 0.004 ^{a}	915 ± 43.45 ^{a}
HITCM 15 min	354.0 ± 3.18 ^{b}	378.21 ± 2.05 ^{a}	954.28 ± 17.84 ^{a}	6.79 ± 0.009 ^{a}	289 ± 0.60 ^{d}

HGM, homogenized goat milk; HITGM 5 min, goat milk, high-intensity thermosonicated for 5 min; HITGM 10 min, goat milk, high-intensity thermosonicated for 10 min; HITGM 15 min, goat milk, high-intensity thermosonicated for 15 min; HCM, homogenized cow milk; HITCM 15 min, cow milk, high-intensity thermosonicated for 15 min. Different letters indicate statistical significance within the same column ($p < 0.05$). * The particle size refers to milk protein.

In the HIT samples, the fat globule diameter was reduced to a smaller size. These results indicated that the HIT treatment had greater efficiency than the homogenization effects over a prolonged time compared to homogenization. The HIT's effect on milk samples has been attributed to the cavitation phenomenon, and the resultant large shear forces break down the fat globules. This phenomenon is based on implosion bubbles produced by sonication in a liquid [25]. Other studies have found that ultrasound treatment reduces fat and increases the size of distributed particles [26,27].

3.2. The pH, Soluble Calcium and Phosphorus Analyses

As shown in Table 1, the pH measurement showed no significant differences ($p > 0.05$) between the HIT milk samples and the homogenized samples. However, the pH values of the HIT milk samples were stable, with the HITGM for 10 min recording a significant ($p < 0.05$) decrease to 6.41 in comparison to the HGM (6.45) due to the enzymatic hydrolysis of phosphoric esters in the thermosonicated milk samples, according to Walstra et al. [28]. The calcium and phosphorus analyses were carried out to investigate the stability of the CCP relative to the casein micelle structure. Table 1 elucidates that there was no significant change in calcium and phosphorus concentrations in the serum phase between the HIT- and homogenized-milk samples. Similarly, the results have previously been reported [29].

3.3. Rheological Properties

During the 18-day storage period of the stirred yoghurt, the viscosity was measured (Figure 2a). In general, the viscosity of the stirred yoghurt samples made from cow milk had significantly higher values than that made from goat milk.

On the first day, the viscosity of the stirred yoghurt produced from HITGM increased from 188.5 mpas for HGM, to 220.5, 261.5 and 283.5 mpas for the HITGM for 5, 10 and 15 min, respectively. Furthermore, when the storage period was increased to 8 days, the viscosity of the stirred yoghurts produced from HITGM increased significantly ($p < 0.05$) to 233, 271.5 and 301 mpas compared to the stirred yoghurt produced from HGM (194.5 mpas). Furthermore, the viscosity of the stirred yoghurt produced from cow milk that was high-intensity thermosonicated (HITCM) for 15 min increased significantly ($p < 0.05$) from the 295.5 mpas of the homogenized cow milk (HCM) stirred yoghurt to 459 mpas on the first day of storage. However, the increase in viscosity of the stirred yoghurt produced by the HITCM for 15 min was the greatest.

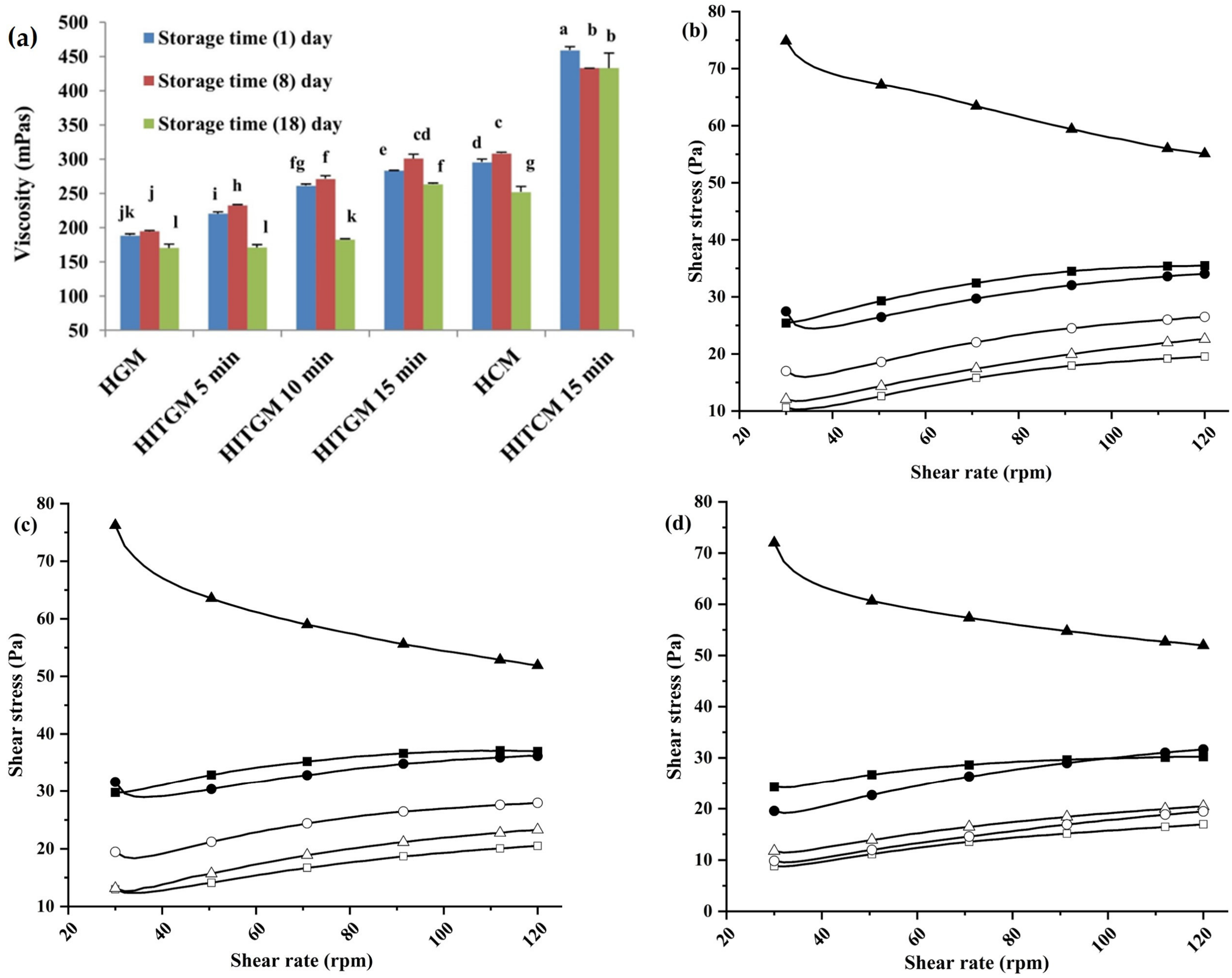

Figure 2. The changes in the viscosity during the storage (**a**) and shear stress of the stirred yoghurt samples during storage for 1 day (**b**), 8 days (**c**) and 18 days (**d**). The symbols represent for the stirred yoghurt from homogenized goat milk, from goat milk, high-intensity thermosonicated for 5 min, from goat milk, high-intensity thermosonicated for 10 min, from goat milk, high-intensity thermosonicated for 15 min, homogenized cow milk and from cow milk, high-intensity thermosonicated for 15 min. Data points not connected by the same letter are significantly different from each other ($p < 0.05$).

Regarding prolonged duration storage (8–18 days), the viscosity decreased to 433 mpas of the stirred yoghurt from HITCM, compared to that on the first day. During the 8-day storage period, the viscosity of both stirred yoghurts obtained from the HIT and homogenized milk samples increased. After 18 days, it had decreased significantly ($p < 0.05$) with the increased storage time.

It was concluded that HIT could significantly alter the viscosity of stirred yoghurt whether it is applied to goat or cow milk samples. The authors found that set type yoghurts manufactured from thermosonicated milk had increased gel firmness and viscosity [12]. Because ultrasound can increase the fraction of the available casein particles, the viscosity of yoghurt rises [8,30]. On the 30th day of storage, the highest viscosity of fermented milk was obtained with thermosonication at 70 °C for 5 min; however, heat treatment had a dramatically negative effect on the viscosity [14].

The correlation between the shear rate and shear stress is elucidated over the 18-day storage of the stirred yoghurt in Figure 2b–d. The trending behavior of these parameters means that all stirred yoghurt exhibited a non-Newtonian type of flow behavior. In contrast, shear stresses at which fracture occurs are shown as a function of applied stress duration [31].

3.4. Syneresis

Figure 3 shows the syneresis of the stirred yoghurt over the 18-days storage. The syneresis degree showed that HIT had a positive effect on the serum-release values for the stirred yoghurt compared to the homogenized. In other words, less syneresis occurred in the stirred yoghurt produced from HITGM samples than those obtained from the homogenized cow milk samples (Figure 3). This contributed to the higher level of protein and total solids content found in the goat milk compared to the cow milk composition.

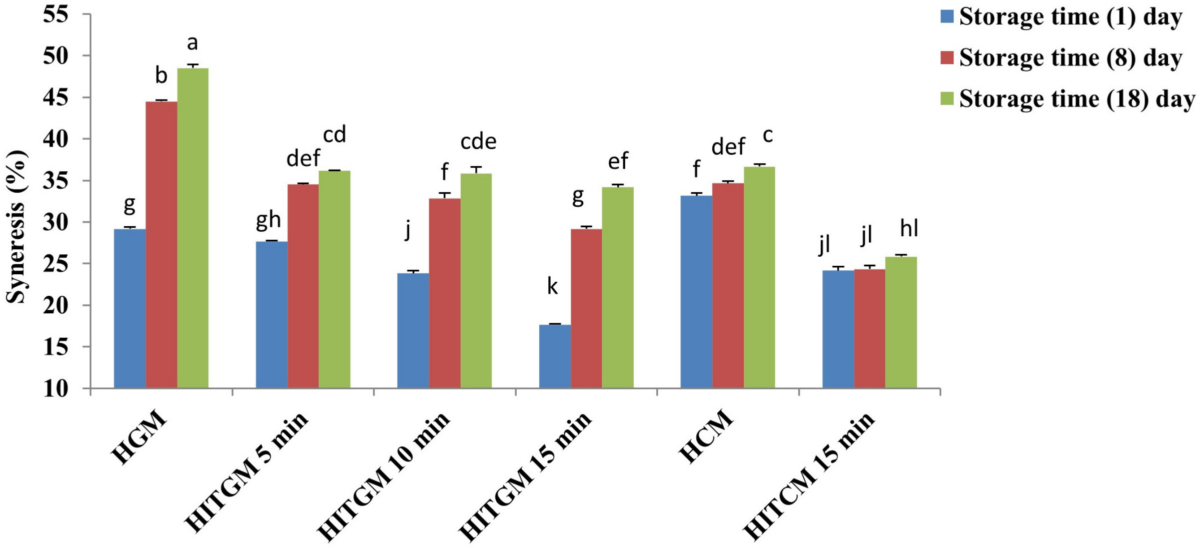

Figure 3. Syneresis of the stirred yoghurt samples during storage. HGM, homogenized goat milk; HITGM 5 min, goat milk, high-intensity thermosonicated for 5 min; HITGM 10 min, goat milk, high-intensity thermosonicated for 10 min; HITGM 15 min, goat milk, high-intensity thermosonicated for 15 min; HCM, homogenized cow milk; HITCM 15 min, cow milk, high-intensity thermosonicated for 15 min. Data points not connected by the same letter are significantly different from each other ($p < 0.05$).

On the first day, the syneresis of the stirred yoghurt made from HITGM decreased significantly ($p < 0.05$) from 29.2% (HGM) to 27.7, 23.8 and 17.7% of the yoghurt produced from HITGM for 5, 10 and 15 min, respectively. In the stirred yoghurt produced from the cow milk, the syneresis of the stirred yoghurt made from HCM reduced from 33.2 to 24.2% compared to the stirred yoghurt made from HITCM for 15 min. Even though the syneresis of all stirred yoghurt samples increased substantially after storage in a refrigerator, the HIT decreased the tendency towards syneresis for those in storage compared to the stirred yoghurt produced from homogenized milk.

Ultrasound cavitation induced a reduction in fat globule size. Hence, the total fat membrane surface area increased. The increased amount of membrane includes a lot of new-bonded casein, which would be hydrophilic [8,32]. Those authors also found that expanding the ultrasound amplitude level before inoculation of the homogenized milk improved the water-holding capacity, viscosity and reduced the syneresis of the induced yoghurt. The thermosonication of preheated milk (45 °C) showed superior texture and lower syneresis compared to those of the control yoghurts produced from conventionally heated milk (90 °C for 10 min) [16].

3.5. Titratable Acidity and pH Values

The pH levels of the stirred yoghurt samples during the storage periods (1, 8 and 18 days) are shown in Figure 4a. The highest decrease rate in pH was determined in the samples produced from the homogenized samples. The pH of the stirred yoghurt samples ranged from 4.43 to 4.58 on the first day and decreased during storage from 4.13 to 4.24 at the end of the storage time. While the HIT had a significant ($p < 0.05$) effect on the pH of the stirred yoghurt, it also had a significant ($p < 0.05$) effect on the acidity.

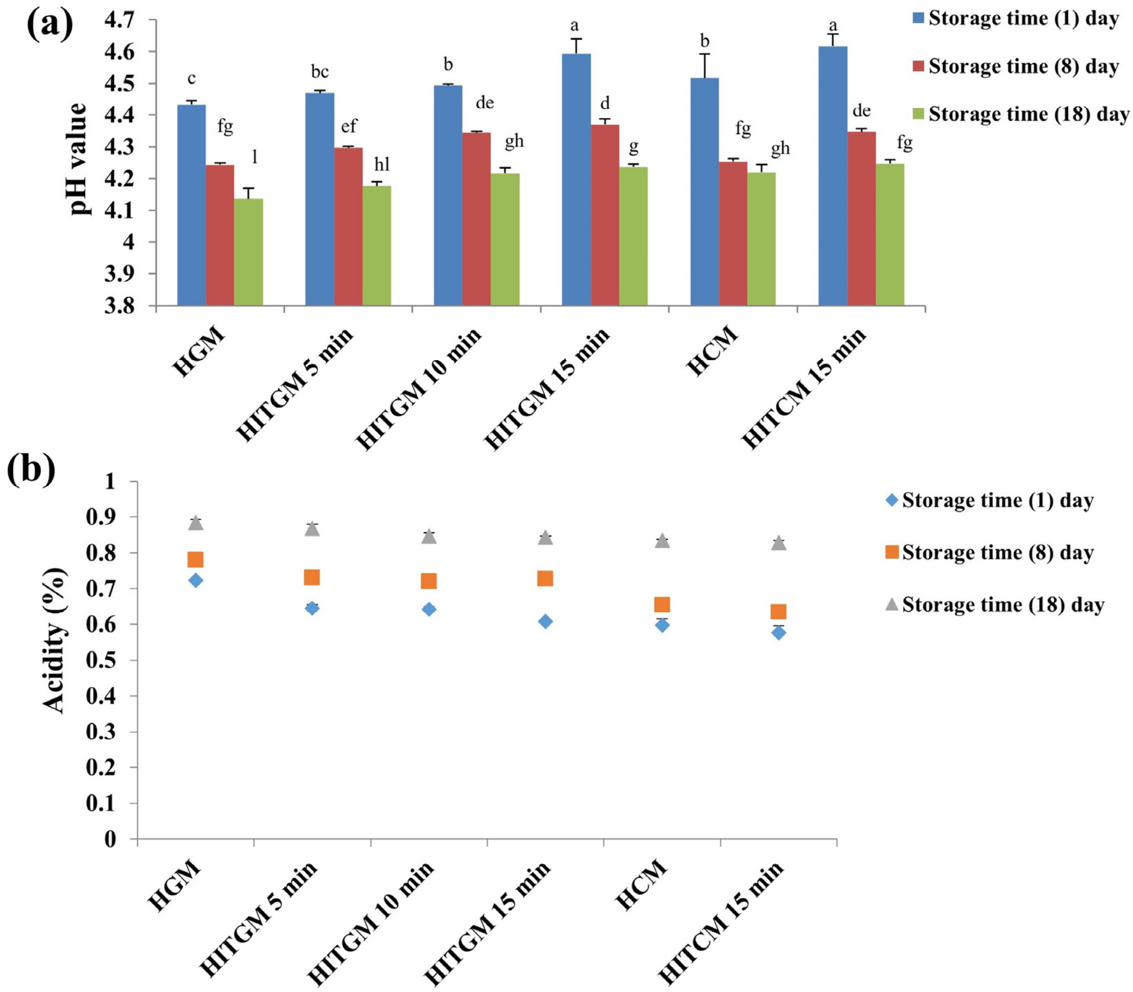

Figure 4. The change in the pH values (**a**) and acidity as lactic acid (%) (**b**) of the stirred yoghurt samples during storage. HGM, homogenized goat milk; HITGM 5 min, goat milk, high-intensity thermosonicated for 5 min; HITGM 10 min, goat milk, high-intensity thermosonicated for 10 min; HITGM 15 min, goat milk, high-intensity thermosonicated for 15 min; HCM, homogenized cow milk; HITCM 15 min, cow milk, high-intensity thermosonicated for 15 min. Data points not connected by the same letter are significantly different from each other ($p < 0.05$).

In general, the acidity of all samples increased gradually after the first day of storage over the 18 days. The extent of acidity in the stirred yoghurt samples increased noticeably during the storage period (Figure 4b). During storage, the stirred yoghurt of the homogenized goat milk sample had the highest titratable acidity value, which ranged from 0.72% on the first day to 0.88% at the end, while there was no significant ($p > 0.05$) difference in the titratable acidity value of the stirred yoghurt obtained from cow milk at the end of the storage period (at 18 days). The continued development of acidity in all samples was derived from microbial growth, which can result in syneresis during storage [33,34]. The acidity and the pH level of a fermented product are directly associated with the microbial load and activity conditions; thus, the thermosonication processing reduced the microbial activity [14].

3.6. Confocal Scanning Laser Microscopy (CSLM)

The microstructures of the stirred yoghurt prepared from HIT and homogenized milk samples are illustrated by CSLM in Figure 5. The stirred yoghurt of the homogenized milk samples had much larger clusters of protein aggregates and a few contact points between the clusters with bigger pore sizes (Figure 5a,e). However, the stirred yoghurt of HIT milk was similar with small pores (increased connectivity between the aggregates) and thin strands (Figure 5b–d,f).

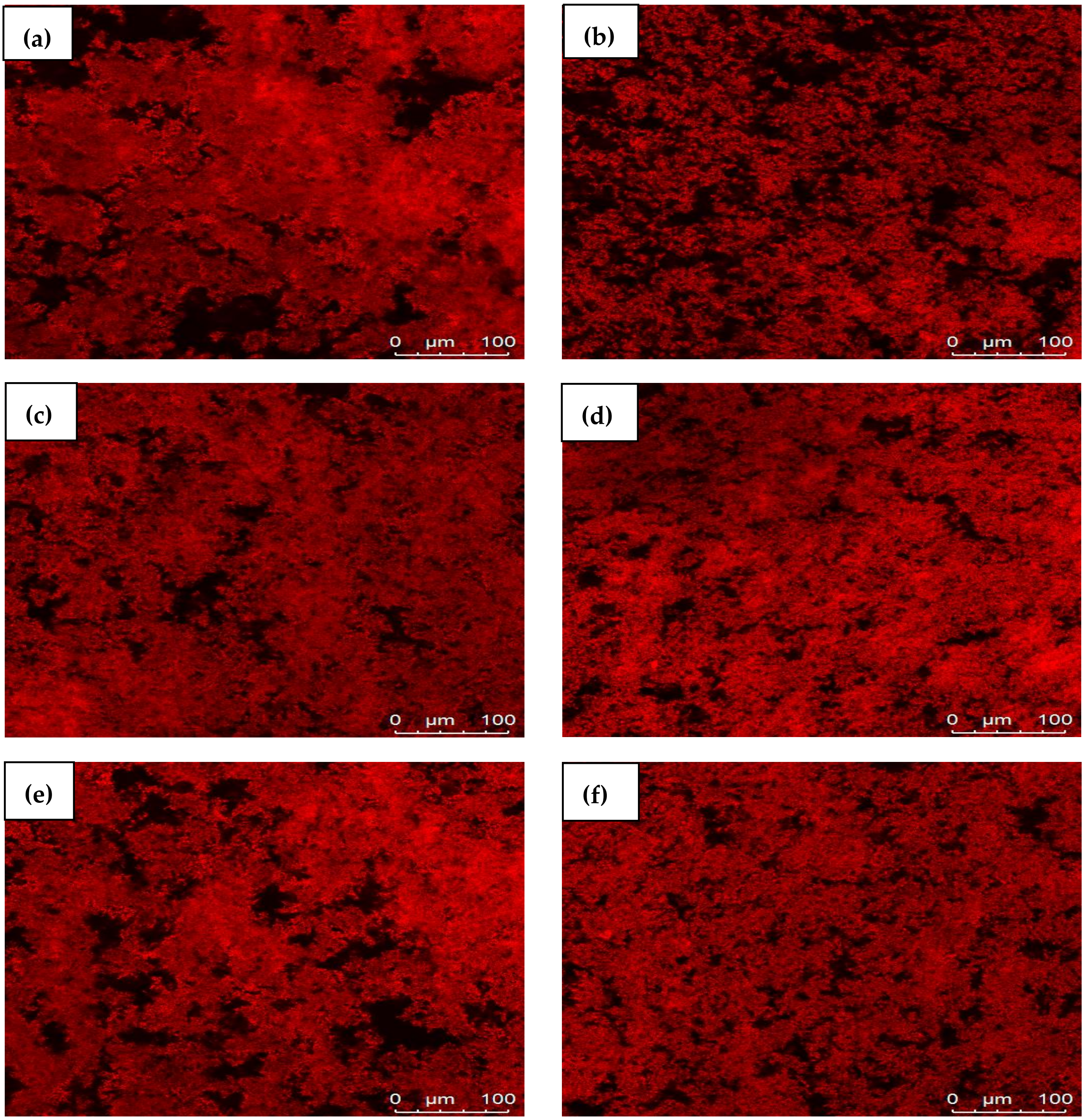

Figure 5. Microstructure of the homogenized and high-intensity thermosonicated stirred yoghurts of the homogenized goat milk (**a**), goat milk, high-intensity thermosonicated for 5 min (**b**), 10 min (**c**) and 15 min (**d**), homogenized cow milk (**e**) and cow milk, high-intensity thermosonicated for 15 min (**f**), captured by confocal laser scanning microscopy (CLSM) on the first day.

The microstructure of yoghurt made by thermosonication for 10 min at an ultrasound amplitude of 24 kHz., which displayed a honeycomb-like network and exhibited a more porous nature throughout the structure, was examined by Riener et al. [12]. Furthermore, Ragab et al. [34] discovered that yoghurt produced by homogenized milk contained much larger protein aggregate clusters and pores with a honeycomb microstructure, while yoghurt produced by the thermosonication of milk (at an ultrasound frequency of 20 kHz and output power of 4000 W, from 5 to 15 min) clearly displayed densely cross-linked network structures with a high level of interconnections and numerous tiny pores dispersed throughout the structures.

In general, the microstructure of the stirred yoghurt samples was closed firmly and more complex with increased viscosity. Similarly, Tabatabaie [30] observed that the microstructure of ultrasound-treated milk yoghurt had a more stable surface and more uniform size distribution, resulting in improved gel texture and viscosity.

3.7. Color Measurements

The color values (L^*, a^* and b^*) of the stirred yoghurt samples were evaluated over 18 days and are listed in Table 2. There was a significant ($p < 0.05$) increase in the L^* values on the first day of the stirred yoghurt produced from the HIT milk samples, and these values also increased over the 18-day storage period for all samples, especially the HIT samples, while the a^* values of the samples showed insignificant ($p > 0.05$) changes during storage (1 and 18 days). The b^* values of the stirred yoghurt in the HIT samples declined significantly ($p < 0.05$) as compared to the homogenized samples during the storage period. The color values (a* and b*) of the stirred yoghurt samples in our study were similar to those reported by Bermúdez-Aguirre et al., [35] for whole milk, after thermosonication treatment, stored at 4 °C for 16 days. Unlike our results, Gursoy et al. [20] reported that insignificant differences in L^*, a^* and b^* values were found between yoghurt drinks produced with milk thermosonicated at different ultrasound powers in comparison to the control yoghurt drink.

Table 2. Color values of stirred yoghurt samples on the first and 18th day of storage (n = 12).

Samples	Color Values					
	Storage 1 Day			Storage 18 Days		
	L^*	a^*	b^*	L^*	a^*	b^*
HGM	97.92 ± 0.11^{d}	-2.03 ± 0.08^{ab}	9.48 ± 0.1^{cd}	99.52 ± 0.07^{a}	-3.01 ± 0.36^{c}	8.51 ± 0.32^{f}
HITGM 5 min	98.77 ± 0.52^{bc}	-3.19 ± 0.45^{c}	9.38 ± 0.09^{d}	99.58 ± 0.06^{a}	-2.75 ± 0.17^{c}	8.91 ± 0.12^{e}
HITGM 10 min	98.38 ± 0.06^{cd}	-1.87 ± 0.05^{a}	7.76 ± 0.21^{gh}	99.17 ± 0.25^{ab}	-1.89 ± 0.44^{a}	7.4 ± 0.22^{h}
HITGM 15 min	99.15 ± 0.04^{ab}	-1.96 ± 0.01^{a}	8.15 ± 0.23^{fg}	99.43 ± 0.36^{a}	-1.79 ± 0.06^{a}	7.69 ± 0.05^{h}
HCM	98.32 ± 0.03^{cd}	-2.64 ± 0.06^{bc}	10.24 ± 0.3^{a}	98.51 ± 0.39^{c}	-2.86 ± 0.05^{c}	10.05 ± 0.02^{ab}
HITCM 15 min	98.69 ± 0.14^{bc}	-2.83 ± 0.02^{bc}	9.22 ± 0.21^{de}	99.33 ± 0.39^{a}	-2.01 ± 0.34^{ab}	9.78 ± 0.07^{bc}

HGM, homogenized goat milk; HITGM 5 min, goat milk, high-intensity thermosonicated for 5 min; HITGM 10 min, goat milk, high-intensity thermosonicated for 10 min; HITGM 15 min, goat milk, high-intensity thermosonicated for 15 min; HCM, homogenized cow milk; HITCM 15 min, cow milk, high-intensity thermosonicated for 15 min. Different letters indicate statistically significant differences across the table ($p < 0.05$).

The color of the stirred yoghurt obtained from HIT milk was whiter; therefore, HIT is an available method that can improve color and appearance due to enhanced homogenization of milk. The observations were consistent with those of Riener et al. [16], who found that thermosonication improved the appearance of yoghurt and exhibited slightly higher L^* values than conventionally heated milk. As is well known, the white color of milk is attributed to the presence of fat globules and casein micelles, which disperse light in the visible spectrum [35,36]. The highest ultrasound power with a long treatment time (10 min) achieved excellent homogenization of milk, due to the reduction in fat globule size [27].

3.8. Electronic Nose

Figure 6 shows the PCA of the stirred yoghurt samples on the first day and after 18 days of storage. In comparison to the homogenized treatment, the HIT process had a significant effect on the odor volatiles in the stirred yoghurt. On day one (Figure 6a), the first axis (PC1) represented 99.2%, while the second axis (PC2) represented 0.66%. Moreover, the stirred yoghurts produced by HITGM for 15 min, HCM and HITCM for 15 min treatments are on the left side, while the rest of the samples of the stirred yoghurt from HGM, HITGM for 5 min and HITGM for 10 min are on the right side. It was noted that the stirred yoghurts prepared by treatments from HITGM for 15 min, HCM and HITCM for 15 min were close to each other and showed fewer differences and similar behaviors between them, as they differed significantly from the first three treatments, which were also similar to each other.

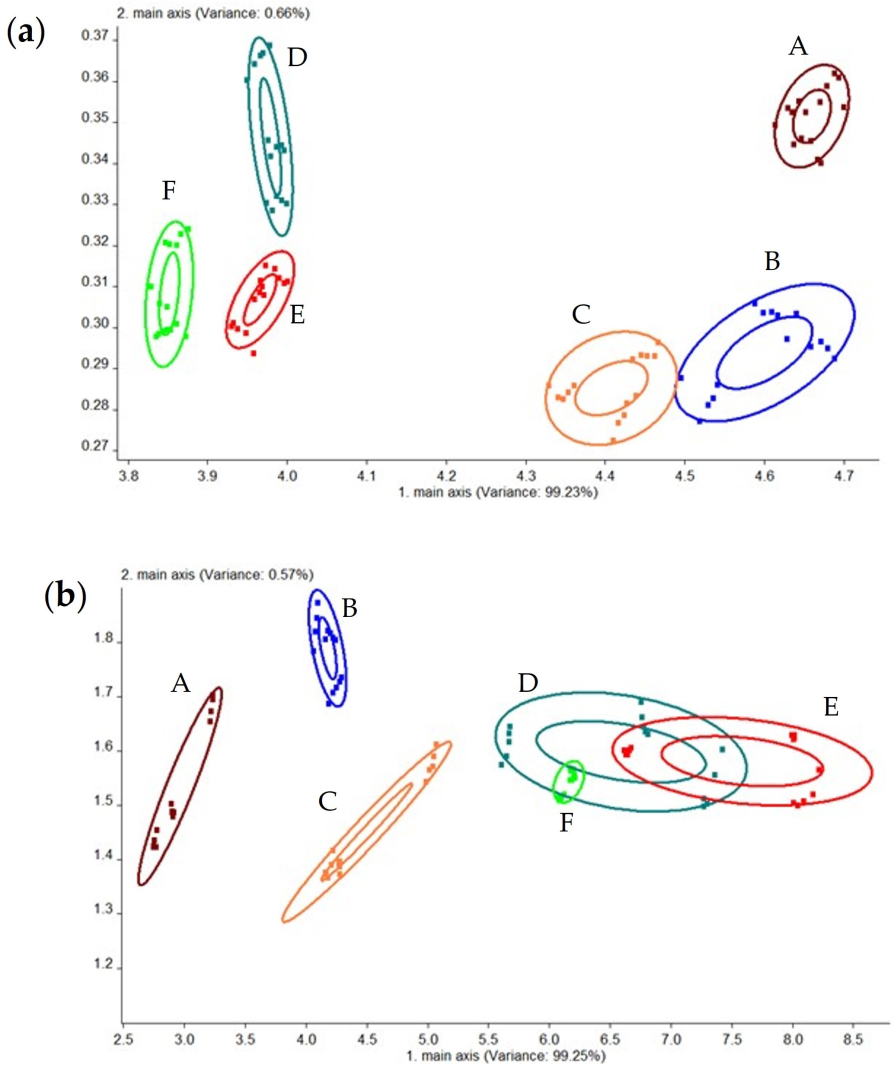

Figure 6. Principal component analysis (PCA) based on electronic nose data produced by different stirred yoghurt samples of the homogenized goat milk (A), goat milk, high-intensity thermosonicated for 5 min (B), 10 min (C), 15 min (D), homogenized cow milk (E) and cow milk, high-intensity thermosonicated for 15 min (F) on the first day (**a**) and on the 18th day (**b**).

On the 18th day of storage (Figure 6b), the first axis (PC1) was 99.3%, while the second axis (PC2) was 0.57%. There was a clear difference in the results; the treatments on the right side became closer to each other than the treatments on the left. Likewise, the right-side treatments were more closely related than their counterparts on the first day.

The sensory quality was somewhat superior in yoghurt produced from thermosonication milk compared to that from conventionally heated, and the odor of the yoghurt made by thermosonication was better [11].

3.9. Sensory Evaluation

Table 3 shows the appreciable significant differences found by the taste panelists regarding the following sensory characteristics of stirred yoghurt samples: appearance color, consistency, odor, taste, acidity and total acceptability on the first day, and after 8 and 18 days of storage. The data in Table 3 show that the HIT process improved the stirred yoghurt whether made from goat or cow milk. The sensory characteristics of the stirred yoghurt produced from HITGM gradually increased, with the prolonging of the treatment time. The stirred yoghurt produced from HITGM for 15 min had sensory characteristics similar to those of the stirred yoghurt produced from HCM. Higher sensory scores were obtained for the stirred yoghurt produced by the HITGM when compared to the homogenized goat milk. Nevertheless, the best sensory score was awarded to the stirred yoghurt produced from the HITCM. The sensory characteristics of all samples improved during storage until the end of storage at 18 days compared to the first day, especially for the stirred yoghurt obtained from the HIT milk samples. The sensory properties of the thermosonicated fermented milk (ayran samples) were better than the thermal-treated samples after storage. However, at the end of 30 days of storage of the thermosonication samples at 60 °C, the sensory properties were better than the others [18]. The set yoghurt prepared from thermosonication milk prior to culturing displayed superior qualities compared to that from the conventional heat treatment of milk [16].

Table 3. Sensory evaluation of stirred yoghurt samples during the storage period (panelists: n = 15).

Samples	Days	Stirred Yoghurt					
		Appearance/Color	Consistency	Odor	Taste	Acidity	Acceptability
HGM	1	7.14 ± 0.64	5.25 ± 0.43	4.42 ± 0.49	4.43 ± 0.49	5.50 ± 0.50	4.57 ± 0.40
	8	7.57 ± 0.72	5.40 ± 0.48	5.29 ± 0.69	4.43 ± 0.49	4.85 ± 0.34	5.29 ± 0.45
	18	8.00 ± 0.76	5.20 ± 0.74	4.71 ± 0.45	4.71 ± 0.45	6.00 ± 0.75	5.43 ± 0.49
HITGM 5 min	1	7.00 ± 0.53	5.50 ± 0.50	5.43 ± 0.90	5.00 ± 0.75	5.86 ± 0.34	5.29 ± 0.45
	8	7.86 ± 0.83	6.40 ± 0.80	5.57 ± 0.72	5.86 ± 0.83	6.00 ± 0.76	6.29 ± 0.45
	18	8.14 ± 0.83	6.60 ± 0.48	6.42 ± 0.49	6.42 ± 0.49	7.00 ± 0.75	6.29 ± 0.45
HITGM 10 min	1	7.57 ± 0.49	5.75 ± 0.43	6.57 ± 0.90	6.29 ± 0.69	6.28 ± 0.69	6.43 ± 0.49
	8	8.00 ± 0.75	6.80 ± 0.74	6.71 ± 0.69	6.43 ± 0.72	7.33 ± 0.47	6.43 ± 0.49
	18	8.00 ± 0.76	7.20 ± 0.75	6.85 ± 0.63	7.14 ± 0.64	7.42 ± 0.49	7.00 ± 0.75
HITGM 15 min	1	7.57 ± 0.49	7.25 ± 0.43	7.28 ± 1.03	7.14 ± 0.63	6.57 ± 0.73	7.42 ± 0.49
	8	8.14 ± 0.63	8.00 ± 0.63	7.71 ± 0.69	7.28 ± 0.69	7.71 ± 0.45	7.57 ± 0.49
	18	8.29 ± 0.45	8.20 ± 0.40	7.85 ± 0.64	7.71 ± 0.45	7.71 ± 0.45	8.00 ± 0.53
HCM	1	7.14 ± 0.35	7.50 ± 0.50	7.71 ± 0.69	8.00 ± 0.01	6.86 ± 0.83	7.71 ± 0.69
	8	8.43 ± 0.49	8.00 ± 0.63	7.71 ± 0.69	8.00 ± 0.01	8.43 ± 0.49	7.71 ± 0.45
	18	8.43 ± 0.49	8.20 ± 0.40	8.00 ± 0.53	8.00 ± 0.53	8.42 ± 0.49	8.14 ± 0.34
HITCM 15 min	1	7.71 ± 0.69	8.25 ± 0.47	8.00 ± 0.53	8.29 ± 0.45	7.43 ± 0.73	8.00 ± 0.53
	8	8.86 ± 0.34	8.60 ± 0.48	8.28 ± 0.45	8.28 ± 0.45	8.57 ± 0.45	8.29 ± 0.45
	18	8.86 ± 0.34	8.60 ± 0.49	8.42 ± 0.49	8.28 ± 0.45	8.71 ± 0.45	8.57 ± 0.49

Scores vary between 1 (extremely poor) and 9 (excellent). Means are averages from two independent trials. HGM, homogenized goat milk; HITGM 5 min, goat milk, high-intensity thermosonicated for 5 min; HITGM 10 min, goat milk, high-intensity thermosonicated for 10 min; HITGM 15 min, goat milk, high-intensity thermosonicated for 15 min; HCM, homogenized cow milk; HITCM 15 min, cow milk, high-intensity thermosonicated for 15 min.

4. Conclusions

Our results showed that the HIT process used on goat milk could reduce the diameter of fat globules as well as making them smaller than those in conventionally homogenized milk. Overall, compared to homogenized milk, milk processing with an HIT treatment before culturing increased viscosity while decreasing the syneresis values in stirred yoghurt. It could be successfully used to enhance the sensory characteristics such as appearance/color, consistency, odor, taste, acidity and total acceptability during the storage period compared to the stirred yoghurts of homogenized milk, while the sensory properties of stirred yoghurt obtained from HIT samples were better than those of homogenized samples during storage. The microstructure of yoghurt made from HIT-treated milk had more interconnected chains. A longer 15 min exposure time improved the ultrasound homogenization effect, reducing the fat globules and particle size. Therefore, HIT could be used as an alternative process to homogenization treatment because of its positive effects in promoting stirred-yoghurt quality during storage.

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