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The effect of ultrasonic processing on physical and chemical properties of milk-based soft, brine cheese

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ABSTRACT Many earlier studies have documented pasteurization problems in the dairy industry. As a result, ultrasonic processing has been researched as a non-heat alternative to pasteurization. In this study, milk-based soft cheese was treated using various sonication times (0, 1, and 3 min) at a set frequency (22 kHz) with an amplitude of 60% of 630 W and different ripening periods (0, 15, 30, and 60 days) in brine (15%), stored at 4 °C, to reduce heat treatment and increase yield. The physicochemical parameters of white cheeses were examined over next 60 days and compared with a control cheese. The result showed that ultrasound had no significant effect on the cheeses in terms of their fat and protein content on storage. Compared to the control sample, ultrasound treatment improved the taste and aroma ratings due to increased lipolysis and proteolysis. In terms of overall acceptability, the ultra-filtrate cheese sonicated for 3 min received the highest marks compared to the control. Sonication for 3 min treated fresh milk showed the maximum yield (190.5 g/L milk) compared to untreated raw milk yields (150.32 g/L).

KEYWORDS brine cheese, dairy products, ultrasound, yield cheese

1. Introduction

Currently, fresh national cheeses from many countries are more widely available on the consumer market and included in people's diets (Akdeniz and Akalın 2019). The diverse dairy raw materials, which are often made from cow, sheep, and goat milk, have a high nutritional value and outstanding flavor thanks to national technology (Munir et al. 2019). The food industry has become more interested in using new technologies to produce tasty, safe, nutritious meals with minimal processing, or they have found alternative methods for pasteurizing and sterilizing milk (Augustin et al. 2016). These methods are becoming more important as a result of rising customer demand for novel food processing technologies that have a far smaller impact on nutrient content and whole food value. There has been a surge in demand for alternative thermal treatments because traditional thermal processes can cause the creation of disagreeable tastes and the loss of essential nutrients (Fox et al. 2017). Ultrasound (US) is a technology that alters the structural integrity of fat cells, speeds up fermentation, and produces flavor compounds as a result, making it potentially useful for making cheese (Abesinghe et al. 2019). The mechanical forces at play during US therapy result in a process known as cavitation, which causes the formation and collapse of bubbles in the fluid, the main active force. The bubbles compress and smash in response to the expansion of the sonic waves, sending shock waves into the atmosphere (Xu et al. 2013). The shock waves result in high temperature and pressure zones, which lead to microbial lysis. Milk treated with US underwent a number of physical changes, including a reduction in the size of fat globs, disruption and cracking of the milk fat globule membrane (MFGM), the emergence of casein micelles, and the production of tiny structures made of triacylglycerol micro-droplets (Yanjun et al. 2014; Shanmugam et al. 2012). When compared to other technologies, ultrasound equipment is not expensive, and the main operational expense of ultrasound systems is electricity, making it more economical and environmentally friendly than thermal pasteurization (Sfakianakis and Tzia 2013). High power US combine with heat provides the necessary outcomes in microbial deactivation while retaining the nutritional properties of the food. Thermo sonication (US + heat) may also be a cost-effective method since ultrasonication reduces the process temperature compared to thermal pasteurization (Sango et al. 2014). The chemical and physical impacts of ultrasound cause changes in milk ingredients, which have a substantial impact on milk and dairy product qualities. Ultrasound has been used in the food and dairy industries for a number of objectives, such as improving whey ultrafiltration, functional food extraction, reducing product viscosity, homogenizing milk fat

globules, crystallizing ice and lactose, and cutting cheese blocks (Ashokkumar et al. 2010). High-intensity ultrasound has been used to create cheese by pasteurizing milk. Additionally, adding heat intensification units (HIU) into the milk that makes more likely to curdle because HIU reduces fat globules to smaller pieces and modifies their membrane. These changes speed up the curd's curdling process and increase the curd's firmness.

As a literature which provides the information to those result, the purpose of this study was to evaluate the impact of various ultrasonic frequencies on the physicochemical and sensory properties of cheese throughout the ripening stage and the yield of cheese. Analysis of prepared cheese studied using gross composition, sensory analysis, and determination of the cheese yield. The statistical analysis of the results was conducted to ensure their validity.

2. Materials and Methods

Fresh raw cow milk was obtained from the farms in the Argayash district of the Chelyabinsk region. The milk was received from healthy cows at 4 °C, four hours after milking. The U-sonic ultrasound with power of 630 W of amplitude 60%, frequency at 22±1.65 KHz with tip diameter 22 mm was used in all experiment. Distilled water was used for carried out experiment.

2.1. Experimental design

2.1.1 Analysis of fresh raw milk

The raw milk was treated with HIU. The physicochemical quality of the fresh raw milk was analyzed using the LactoScan LW Milk Analyzer prior to milk treatment. Titratable acidity, fat, protein, lactose, and non-fatty solids were among the milk characteristics that were analyzed (SNG). The duration of the ultrasonic treatment (0, 1, and 3 min), the frequency (60 kHz), and the ripening period were all completely random in this experiment (0, 15, 30, and 60 days).

2.1.2 Cheese-making

The ultra-filtrate (UF) milk retentate was pasteurized (72, 15 sec) and then ultrasonicated at frequencies of 22 kHz (for 0, 1, and 3 min at a 60% intensity of 630 W) using U-sonic. The booster horn and probe of the sonicator were disassembled, washed completely, and sterilized at 121 °C for 20 min by using an autoclave before each treatment. Schematic formation of cheese as shown in Figure 1

2.1.3 Gross composition, pH and salt analysis for cheese

The pH of the cheese was measured by direct insertion of an electrode (model HI98103, Hanna Instruments, Romania) into grated cheese after calibration with standard buffers pH 4 and 7 at 22 to 31 °C. Using the technique outlined by Sahingil et al. (2014), titratable acidity was calculated (g/100 g of lactic acid). By oven drying the cheese samples at 102±2 °C, the dry matter (DM) concentration was determined. Using an $AgNO_3$ titration, Shabbir et al. (2021) evaluated the cheese's salt and fat contents. The fat content of cheese was determined by analysis of cheese that had been diluted and blended with a dissolver at 65 °C. Analysis of the cheese plus dissolver achieved analytical repeatability that was comparable to that achieved with the Mojonnier method testing milk Salt content was determined by back-titration of the remaining unreacted silver using potassium thiocyanate with ferric ammonium indicator. Cheeses were heated and digested with nitric acid and potassium permanganate in the presence of a known number of moles of silver nitrate. The acid digestion allows the chloride in the sample to be freed and reacted with the silver to form AgCl. Total nitrogen (TN), WSN, and NPN were measured using the Kjeldahl method (Jalilzadeh et al. 2020). Using a stainless steel cylinder cutter, the cheese was carefully sliced into 20 mm tall by 20 mm wide cylinders and stored at room temperature (20 °C). The ash content of cheese samples was determined using the method described in (Shabbir et al. 2021).

2.1.4 Sensory Analysis

After the ripening process, a descriptive sensory analysis was done in 60 days. Each evaluator received the three kinds of cheese simultaneously, each with a different number assigned to it. The panel consisted of fifteen experts who rated the following characteristics in two consecutive sessions using shapeless scales with anchors at the split ends: smell, shade, the appearance of physique, elasticity, mouth feel, cream, bitter and salty taste, and other organoleptic characteristic (Potoroko et al. 2020).

2.1.5 Determination of the cheese yield (Y)

Determination of the theoretical yield (Y) of soft white cheese using only the milk composition and a modified version of the VAN SLYKE yield equation:

$$Y = \frac{(0.93F + C - 0.1)1.09}{1 - M} \quad (1)$$

Where, C is the concentration of casein and F of fat in the cheese milk, and M is the concentration of moisture in the cheese. Values are usually expressed in terms of weight.

$$C = (0.833P) - 0.018 \quad (2)$$

Where C is the concentration of casein, %; P is the protein concentration in cheese milk, % Then we have to determine the actual yield and do compression with Theoretical Yield (Fox et al. 2017)

2.1.6 Field Emission-Scanning Electron Microscopic (FE-SEM) analysis of cheese

FE-SEM determines the external microstructure and morphology of cheese samples of 5×5 mm^2. After being submerged in liquid nitrogen to remove any remaining mois-

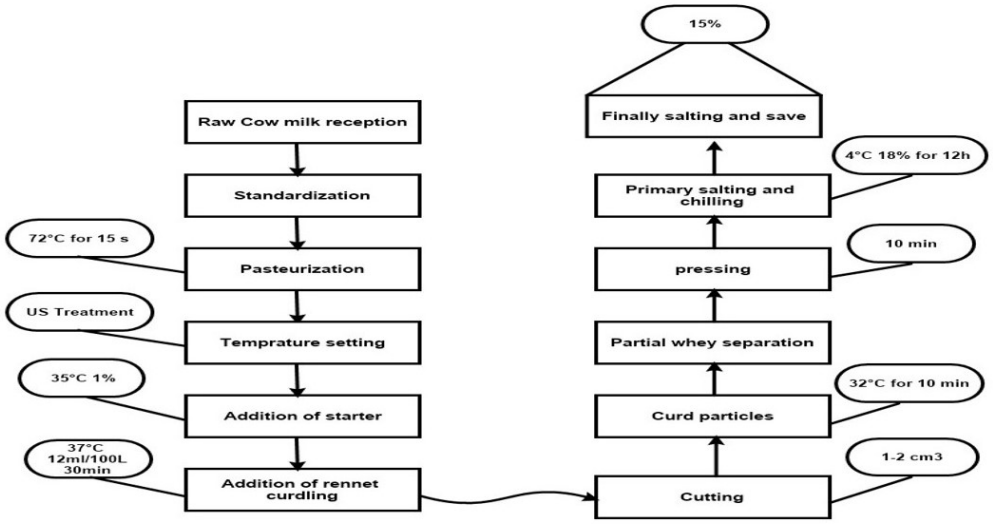

FIGURE 1 Cheese making process using cow milk

ture, the cheese burst. Then, using an ion sputter, gold was sucked into the ruptured cheese. Finally, a sample maintained the FE-SEM slot with imaging at 20 kV (Jeol JSM-7001F, Moscow).

2.1.7 DPPH assay for antioxidant activity of cheese analysis

On the basis of scavenging the steady DPPH, the antioxidant activity of cheese was assessed. According to the procedure described by Potdar et al. (2022), 0.1 g of the Cheese model was placed in a bottle with 2 mL of ethanol and shaken for 2 min before being centrifuged at 8000 rpm for 30 minutes. After being collected, 0.5 mL of the superior elucidation was added to 2 mL of methanol DPPH solution and 1 mL of ethanol. The solution was protected and vortexed for 30 minutes in total darkness at room temperature. Cheese was omitted from the same previously prepared controls sample. The absorbance of sample at 517 nm wavelength was measured using a UV spectrophotometer (Shimadzu UV-2700, Japan), and it was compared to a control sample. The following equation was used to determine DPPH scavenging activity.

DPPH scavenging activity (%)

$$= \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \qquad (3)$$

2.2. Statistics analysis

The results are described as means ± standard deviations. To observe the effect sonication time (0, 1, and 3 min) and ripening period, data were collected and using Excel ANOVA calculated the mean deviation difference of significant ($p < 0.05$).

3. Results and Discussion

In the first stage of the study, cow's milk samples were tested for a set of indicators and the rapidity of coagulation by rennet, which allow to assessing the ratio of the primary nutrients and the degree of aggregation of whey proteins and establishing the favorable environment for the development of microorganisms (Uymaz et al. 2019; Cuffia et al. 2015). In table 1, it show the mean values of the results of analyzing the quality and cheese suitability indicators of cow's milk samples from various lactation phases.

3.1. Gross composition, pH and salt analysis for cheese

The average statistics for the UF white cheese samples during the ripening phase for pH, titratable acidity, DM, fat in DM, salt in moisture, and protein. Table 2 displays the average outcomes for pH, titratable acidity, DM, fat, ash, salt in moisture, and protein in samples of soft white cheese tested at 0, 15, 30, and 60 days of ripening time under various ultrasonic treatment times (0, 1, and 3 min). All sonicated samples had pH levels that were lower than the control after the storage time. The activity of lactic acid bacteria is the main factor determining pH fluctuation during

TABLE 1 Average results of assessing the quality and cheese suitability of cow's milk

Indicator name	Test samples of cow's milk
Acidity, ° T	17±0,5
Density, kg / m3	1028±0.41
Mass fraction of protein, %	3.44±0.05
CMass fraction of fat, %	4.03±0.03
% Solids-non-fat (SNF)	9.11
Mass fraction of calcium, %	130±0.8
Fermentation test	I / Good

TABLE 2 Effect of different ultrasound time (0, 1 or 3 min) as pretreatment on the physicochemical properties of cheeses during ripening period

	Type of samples	Time(days)			
		0	15	30	60
Ash,%	Without US	1.53±0.09a	7.98±0.18b	11.92±0.35a	10.51±028c
	US (1min)	166±011b	778±022h	844±029d	889±03b
	US (3min)	212±014b	811±007c	868±014f	902±041a
DM,%	Without US	3946±044c	4434±054b	4511±091g	4429±039c
	US (1min)	3877±081a	4354±06k	43,91±063g	4372±022c
	US (3min)	3864±091d	4392±011m	4412±077k	4364±033b
PH	Without US	631±001f	6±0016n	59±0019h	611±0012c
	US (1min)	631±001f	595±003f	585±005c	571±007g
	US (3min)	631±001f	624±006a	591±004c	567±007g
Salt,%	Without US	084±001c	588±009a	697±0019b	898±006b
	US (1min)	084±001d	668±011a	843±0044b	1081±0045c
	US (3min)	084±001a	654±006c	798±008c	1079±0034c
Acidity	Without US	023±0027a	027±0067b	029±0073d	04±0045e
	US (1min)	029±0057d	033±0073a	036±0089g	047±0050d
	US (3min)	026±0073f	03±0052c	033±0041f	042±0052e
Protein,%	Without US	1911±09a	1896±06a	1681±089h	1592±019b
	US (1min)	1888±101f	1765±04f	1691±091h	1555±031g
	US (3min)	1767±088d	1712±022n	163±032g	1511±062i
Fat,%	Without US	2086±065f	2129±095d	2111±141e	2071±066i
	US (1min)	1967±121g	2012±101f	1922±109d	1834±091f
	US (3min)	1911±098a	1923±19i	1877±088e	1821±112d

cheese ripening, according to de Lima Alves et al. (2018). Previous research found that ultrasonic pretreatment with varying amplitudes and periods considerably ($p > 0.05$) lowered the pH of milk, which is consistent with the current findings (Jalilzadeh et al. 2018). However, several investigations discovered that the pH of the ultrasound-treated milk (20 kHz) and untreated control samples did not vary significantly. Our research showed that ultrasonic pretreatment increased the acidity of cheese at the end of the ripening phase (Figure 1). There was no linear relationship between sonication time and acidity on day 60 of ripening, which was consistent with the reference data. Acidity was higher in all sonicated samples than in the control samples (Zhao et al. 2014) related to the hydrolysis of triglycerides during ultrasonic treatment, which is consistent with research published by Ashokkumar (2014). ANOVA analysis revealed a significant ($p > 0.05$) impact of ultrasonic therapy on the DM content of cheese samples, but no linear association between time and treatment length ($p > 0.05$). The solubility and movement of several cheese components into the whey may be the cause of the small decrease in DM content for all samples throughout ripening (Yangilar and Yildiz 2016). These findings are in line with the study (Shanmugam and Ashokkumar 2014), which found that increasing whey protein, calcium, and phosphorus solubility can minimize DM by allowing them to be more easily removed from the cheese matrix during ripening. On the other hand, the decrease in DM can be attributed to cheese's increased high moisture content. Figure 1 shows the influence of sonication on cheese's ripening fat content. This effect was not statistically significant ($p > 0.05$). The study found that sonicating raw and pasteurized milk decreased the size of fat globules and modified the surface area of those globules. In our study, milk was homogenized on the production line before being sonicated. Similar to our findings, Marchesini et al. (2012)'s findings revealed that ultrasonic treatment had no appreciable impact on the fat level of milk or cheese. During 60 days of storage, sonication had no discernible ($p > 0.05$) impact on the protein level of cheese samples (Table 1). However, the protein content of all treatments decreased during ripening. Reduction in protein content can be due to the transfer of soluble compounds from cheese curd to whey during storage. It was also reported that sonication (30 kHz and 300 W) had no effect on the protein content of Mahoncheese. Studies conducted by Marchesini et al. (2012), showed that ultrasound pretreatment did not affect the protein content of milk measured by the Kjeldahl method. The results of our study are inconsistent with the findings of the (Carrillo-Lopez et al. 2020).

TABLE 3 Dynamics of the organoleptic qualities of cheese samples after 60 days of exposure in salt brines

No. Sample	Type of sample	Score in points			
		Appearance	Taste and aroma	Consistency, color	Overall quality score
1	Without US	519±016	531±014	593±039	54767
2	US (1min)	711±022	592±049	617±044	64
3	US (3min)	723±031	671±031	753±015	71567

3.2. Sensory analysis

Ultrasonication had a substantial negative impact on the organoleptic properties of cheese samples ($p > 0.05$) (Table 3). The progression of proteolysis and lipolysis during storage resulted in an increase in sensory ratings (Shanmugam and Ashokkumar 2014). After receiving US treatments, the color and appearance of UF cheese significantly improved ($p > 0.05$). The control sample received the lowest color and appearance ratings, and there were no differences across sonication treatments that were significant ($p > 0.05$). Sonication alters the color of milk and influences the final color of dairy products like cheese or yogurt, claim (Ashokkumar et al. 2010). Compared to the control sample, ultrasound treatment improved taste and aroma ratings due to increased lipolysis and proteolysis. The untreated control sample scored the lowest overall and the UF cheese that had been sonicated for three minutes scored the highest. The cheese sample cubes maintained their leading roles and had a dense, uniform structure in the section. The evaluation of samples of soft white brine cheeses using physicochemical markers revealed that processing with recirculation in the milk system had a beneficial impact on the full set of characteristics, demonstrating the potential of these technical advancements.

3.3. Determination of the cheese yield (Y)

Determination of the theoretical yield (Y) of soft white cheese using only the milk composition and a modified version of the VAN SLYKE yield equation:

$$Y = \frac{(0.93F + C - 0.1)1.09}{(1 - M)}, C = (0.833P) - 0.018, C = 2.65; \quad (4)$$

Theoretical yield: Y = 15.14%.

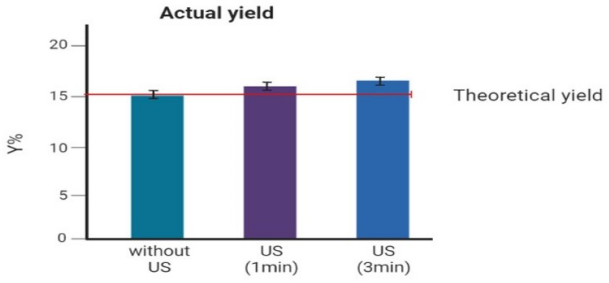

FIGURE 2 Dynamics of changes in the actual yield of cheese samples

The actual yield of the cheese sample from the sonicated milk (1 minand 3 mins) wereas 171.1 g/L milk and (3 min) was 190.5 g/L milk, respectively, while the weight of the cheese sample from the nonuntreated ultrasound treatment (0) milk was 150.32 g/L milk.

Significant differences obtained in yield of cheese after 24 h at 4 °C, when the effect of sonication time ($p < 0.05$) on fresh raw milk studied (Figure 2). Highest yield of cheese (190.5 g/L milk) was obtained when fresh raw milk was sonicated at an amplitude of 60% for 3 min compared to milk was treated with an amplitude of 60% (171.1 g/L milk) for 1 min. The lowest yields were obtained using non treatment fresh raw milk (control, 0 min, 150.32 g/L). It indicated that fat and protein decomposition was higher in the samples treated with ultrasound. It is important to note that using an innovative approach to the technology of cheese production has made it possible to ensure a good yield of curd grains. So the mass of the product obtained with the use of the US. This effect was provided primarily due to cavitation, which occurs during USI, which changes the hydration properties of milk proteins and the structure of water in their composition.

Therefore, time of 1 and 3 min of ultrasonication treatment of fresh raw milk increased significantly yield of cheese; results of our study are consistent with the findings of the (Carrillo-Lopez et al. 2021).

3.4. FE-SEM analysis of Cheese

The FE-SEM examination of a sample of US-treated cheese during the ripening process is shown in Figure 3. Figures 3a and 3b, which relate to the sensory evaluation of cheese, reveal that US-treated cheese control has a smooth surface of the protein-casein network with less porosity after 30 days of ripening. This demonstrates that the structural integrity of the cheese sample has not changed, negating any impact on the cheese's organopletic qualities. In contrast, figure 3c shows some roughness and porosity following the 60-days ripening phase. This suggested that there had been some modifications in the cheese's flavor and color. This could be as a result of the cheese's sonication treatment, which eventually breaks the protein-casein network after 90 days of ripening.

3.5. DPPH scaving antioxidant activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is a free radical used as scavenging material in the presence of antioxidants by observing hydrogen molecules. The antioxidant activity of the sample cheese is analyzed by using a radical scavenging method in terms of DPPH. All the re-

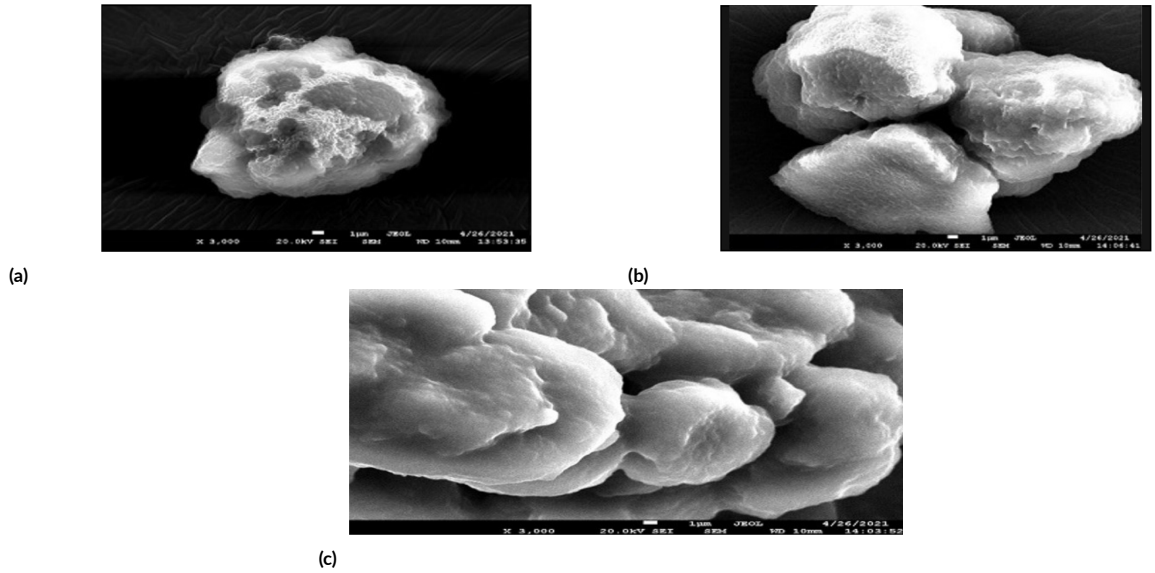

FIGURE 3 FE-SEM analysis cheese a) Control, b) 30 days ripening and c) 60 days ripening

TABLE 4 Dynamics of the organoleptic qualities of cheese samples after 60 days of exposure in salt brines

Sample	Ripening days	% Inhibition (DPPH)
0 Day Ripening		
Control	K	46.704±0.07
Sonication time	1 min	39.31±0.04
	3 min	19.60±0.33
15 Days Ripening		
Control	K	56.25±0.29
Sonication time	1 min	57.62±0.05
	3 min	51.92±0.07
30 Days Ripening		
Control	K	57.55±0.55
Sonication time	1 min	59.90±0.58
	3 min	54.96±0.28
60 Days Ripening		
Control	K	59.20±0.47
Sonication time	1 min	62.38±0.52
	3 min	57.32±0.21

sults are shown in Table 4 which is 46.70% for the antioxidant activity as control sample where 15,30 and 60 days ripening period of cheese samples showed more antioxidant activity as 56.25%, 57.50% and 59.20% respectively. Despite this, after 1 min of sonication, cheese exhibited an increase in antioxidant activity. After 1 min of ultrasonication, the values for each cheese sample are 57.62, 59.90, and 62.37% for ripening periods of 15, 30, and 60 days, respectively. Since sonication protein networks release phenolic compounds, this demonstrates the amount of phenolic compounds present in cheese samples. The Hydroxyl group presented in the phenolic compound acts as a proton supplier to free radicals. However when sonication time increase from 1 min to 3 min, there is a significant decrease in antioxidant activity (value decrease to 51.9, 54.96 and 57.32% for 15, 30 and 60 days ripening period) as more sonication action broke radical into peroxide molecules which directly attack on cheese compound for oxidation and reduce their antioxidant activity. However, overall sonication is prime reason to its antioxidant activity.

4. Conclusions

We conclude that over time, sonication decreases in the mass fraction of fat and protein in cheese compared to non-sonicated cheese samples. The acidity of the cheese samples using ultrasound was less pronounced than the control sample because the change was no longer stable. Samples of soft white brine cheese using ultrasound technology have higher organoleptic properties than the control sample.

An increased fat and protein decomposition rate leads to a better ripening of the cheese. Thus, the study results proved the positive effect of ultrasound on the stage of milk preparation for cheese production. Although sonication significantly increased the cheese yield, we note the need for further study, effect of sonication not only on yield but also safety aspect of cheese. On an industrial scale, ultrasound is conceivable to enhance the yield of cheese produced from milk, hence the amount of money made.

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Authors' contributions

AK did Conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing — original draft preparation, and writing— review, and editing, UB did formal analysis and validation, and review and revision of original draft. IP did supervision project administration. All authors have read and agreed to the published version of the manuscript. Authorship must be limited to those who have contributed substantially to work reported.

Competing interests

There are not competing interests.

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