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Textural, Rheological, and Sensory Modifications in Oaxaca Cheese Made with Ultrasonicated Raw Milk

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Abstract: In this research, we evaluated the effects of different frequencies (25 and 45 kHz) and times (15 and 30 min) of high-intensity ultrasound (HIU) applied to fresh raw milk on the textural properties of Oaxaca cheese. When HIU was applied for 15 min, the cheese melting time was reduced by up to 23.4 s compared to the control. The cheeses produced with ultrasonicated milk at 45 kHz presented larger melting and exudate areas. They were softer, but consumer acceptability was lower. Frequencies of 25 and 45 kHz (15 or 30 min) reduced the temperature of the solid–liquid transition by 1.9–3.6 °C and 0.6–1.8 °C, respectively, compared to controls. Consequently, the melting time in ultrasonicated cheese was significantly reduced. The strands were perfectly aligned and well organized in the direction of stretching with 25 kHz ultrasonication. In addition, these cheeses were more consistent, chewy, and rubbery. Consequently, they were preferred and more accepted in flavor, aroma, and texture, with high general acceptability. HIU is a promising technology to improve the textural and rheological properties of Oaxaca cheese made with raw milk, as HIU produced cheeses with better sensory acceptability by consumers and with a high melting capacity and thread formation.

Keywords: Oaxaca cheese; high-intensity ultrasound; microstructure; textural properties; sensory acceptability

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1. Introduction

Oaxaca cheese is a fresh product of soft and spun paste and highly commercialized in Mexico. Nevertheless, it presents high variability in its characteristics due to a non-standardized manufacturing process. This is mainly due to its traditional production with naturally acidified raw milk from cows and the use of rennet of animal origin. Further, its production is largely empirical, requiring skills and experiential knowledge. Although Oaxaca cheese is made throughout the national territory, it has been scarcely studied [1,2]. Traditionally made Oaxaca cheese varies in its physicochemical characteristics (protein 15.0–24.1%, fat 17.0–25.3%, pH 4.9–5.8, NaCl 0.8–3.6%, water 12.4–61.1%, and ash 1.8–4.2%) [1]. Industrially, this type of cheese is made with pasteurized milk by using thermophilic lactic cultures or the direct acidification of milk with organic acids [3]. However, its textural and physicochemical properties can be greatly affected. Furthermore, the pasteurization of milk is not a suitable option because the heat may remove beneficial microorganisms from the milk that determine the flavor and aroma of artisanal Oaxaca cheese. This may detract from the cheese's sensory qualities, such as milky, buttery, sour, and fermented flavor notes [4,5]. In this regard, Morales-Celaya et al. [6] reported that cheeses made with pasteurized milk have higher protein and moisture contents and yield but lower calcium content, regardless of the type of acidification (starter culture or acetic acid), compared to cheeses made with raw milk. Cheeses made from raw milk exhibited a structure characterized by relatively long and thin casein strands aligned in the direction of stretching, whereas milk pasteurization produced arrangements of layered casein strands with smaller fat globules. However, structural changes due to the reorganization of milk components derived from pasteurization are difficult to appreciate [6].

Moreover, the chemical and functional properties of Mozzarella cheese are significantly affected by the type of acid used for the pre-acidification of the milk [7]. Unfortunately, Mexican legislation prohibits the commercialization of artisan cheeses made with raw milk. Hence, alternative production technologies are mandatory to preserve the sensorial characteristics of traditional Mexican cheeses such as Oaxaca cheese. High-intensity ultrasound (HIU) is considered a non-thermal technology that is able to produce chemical and physical effects on foods due to acoustic cavitation [8]. HIU reduces the particle size and modifies the structure of milk components (mainly protein and fat), generating positive changes such as an increase in the firmness of casein gels and a reduction in syneresis [9–11]. Negative changes induced by HIU in Iranian ultrafiltered feta-type cheese have also been reported, such as the oxidation of lipids and the development of off-flavors, depending on the ultrasound exposure intensity, frequency, time, and temperature [12]. HIU accelerates lipolysis and proteolysis in cheese during ripening and enhances organoleptic, microbial, and physicochemical properties [12]. HIU can be an adequate technology to protect the production of genuine Oaxaca cheese manufactured from raw milk, since it complies with current Mexican regulations regarding microbial loads [13]. However, information on the effects of HIU on the properties of Oaxaca cheese made with raw milk is scarce. To standardize and improve the artisan manufacturing process of Oaxaca cheese together with the ultrasound technology application, this research focused on the effects of the ultrasound frequency and application time applied to fresh raw milk on the textural, sensory, and rheological characteristics of Oaxaca cheese.

2. Materials and Methods

2.1. Sample Preparation

Raw milk produced in the dairy unit of the Department of Animal Science and Ecology of the University of Chihuahua was used in this study. The milk was obtained from healthy Holstein cows under an intensive production system (herd of 30 cows). The milk was kept in a refrigerated storage tank (4 °C) for 6 h after milking. Afterward, the milk was analyzed (LactoScan LW, Milkotronic Ltd.®, Sliven, Bulgaria) in triplicate to determine the basic physicochemical parameters: titratable acidity (determined by titration with NaOH 0.1 N), pH, protein, fat, lactose, and non-fat solids (Table 1) [13]. Subsequently, the milk was assigned to one of the six evaluated treatments. The study was a randomized complete block design to decrease the variability in the experimental units (components of fresh raw milk). On this premise, the ultrasonication time (three levels: 0, 15, or 30 min) and HIU frequency (25 or 45 kHz) effects on the cheese were studied (N = 3, n = 18).

Table 1. Physicochemical values of fresh raw milk before ultrasonication [13].

Block	Titratable Acidity (°Dornic)/pH	Fat (%)	Protein (%)	Lactose (%)	Non-Fat Solids (%)
1	17/6.56	3.64	3.19	4.91	8.81
2	17/6.57	3.57	3.22	4.95	8.88
3	17/6.57	3.59	3.18	4.89	8.78

The six evaluated treatments were as follows: control/25 kHz, 15 min HIU/25 kHz, 30 min HIU/25 kHz, control/45 kHz, 15 min HIU/45 kHz, and 30 min HIU/45 kHz (time/HIU frequency). Ultrasonication was carried out with Elmasonic® Xtra ST 800 H equipment (Singen, Germany), which had a minimum operating capacity of 40 L. The equipment was operated in dynamic mode, so the alternate "scanning" and "pulse" functions worked automatically. These increase the ultrasound performance by 20% and ensure the uniform distribution of acoustic waves. The initial temperature of the milk before ultrasonication was 4 ± 1.2 °C. Once HIU was applied, the temperature of the raw milk was increased to 3.1 ± 0.2 and 3.9 ± 0.2 °C for 15 and 30 min of HIU, respectively. The milk was used for Oaxaca cheese production immediately after ultrasonication.

2.2. Oaxaca Cheese-Making Process

The manufacturing of artisanal Oaxaca cheese was carried out following the methodology described by Villegas [14]. Figure 1 shows the cheese-manufacturing process [13]. Total cheese production, from the receipt of the milk for all treatments to the packaging of the final products, was completed in approximately 4 h. Five liters of milk was used for each treatment. Firstly, the temperature of the milk was adjusted to 32–34 °C. Glacial acetic acid (10 % v/v) in water was added to the milk to increase the acidity of the curd from 15–16 °Dornic to 37 °Dornic (pH of 5.8–5.9). Later, microbial rennet (10 % v/v. Cuamix®, CHR Hansen A/S, Hørsholm, Denmark) diluted in water was added to promote coagulation. After 20 min, the coagulum was cut into cubes (2.5 ± 0.6 cm^3) using a wooden shovel. The cubes were gently stirred (15 min) and left to rest (5 min), after which $\frac{3}{4}$ parts of the exuded were removed by syneresis. The curd was settled for 30–45 min until the acidity of the exuded whey reached 32 °Dornic. The thread-forming test was carried out at this point. The test consisted of kneading the paste with water at 70 ± 2 °C to form a thread with a "gum" appearance. When the strand test was positive, the entire paste was kneaded, forming a cylinder that was stretched to form a thin strip or ribbon of approx. 5 cm wide. This was placed in water at 4 °C for the next 5 min. Afterward, the ribbon was rubbed with NaCl (2% w/w) to form a traditional Oaxaca cheese ball, which consisted of rolling the ribbon following a triangular pattern around an imaginary center. The cheese balls were placed in plastic bags and stored at 4 °C for 24 h. Subsequently, the response variables were determined, including the volume of whey exuded (mL), after refrigerated storage.

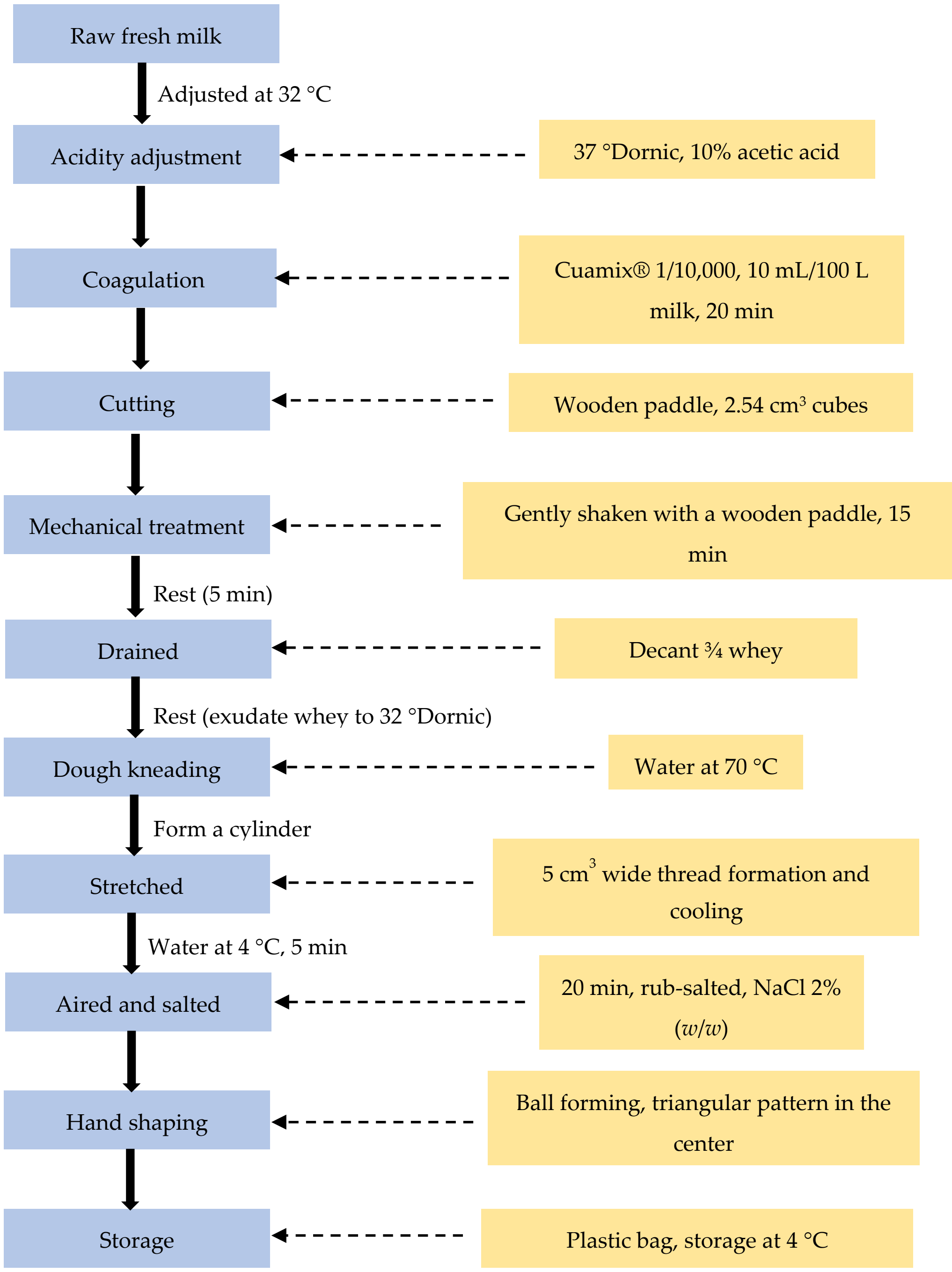


Figure 1. Flow chart representing the production of Oaxaca cheese [13].

2.3. Strand and Melt Tests

An empirical test was designed to evaluate the thread-forming capacity. This consisted of applying a tensile force to cheese samples with 5 cm length × 1 cm width × thickness of the strip in the direction of the threads. The threads that formed the cheese sample were then visualized by separating the threads longitudinally. The applied tension force was not quantifiable because it was performed with the thumbs and index fingers of both hands. However, it was performed slowly and carefully so as not to break the formed threads. The meltability test for Mozzarella cheese [15] was applied to evaluate the melting capacity of Oaxaca cheese. However, a disadvantage of this method was that disk formation from compression with a plunger at a constant distance produced uneven disks. Therefore, a test called the "tortilla test for the evaluation of melting in Oaxaca cheese" was designed.

Briefly, 5 g of cheese was compressed with a 34 mm diameter plunger to homogenize and soften the sample. A sphere was formed and placed between two Petri dishes. Both boxes were pressed to form a disk or "tortilla" of 5 cm diameter. The "tortilla" was then placed in the center of an electric grill previously heated to 125 °C. The time (min) from the placement of the sample to the completion of melting (when the expansion ends and whey exudate appears around the melted cheese) was recorded. The diameter of the formed circle was measured, and the areas of cast and exuded whey were determined using Image J software (Wayne Rasband, National Institute of Health, Bethesda, MD, USA).

2.4. Texture and Rheological Properties

The texture profile analysis (TPA) of the cheese was performed using a texture analyzer (TA.XTplus, Stable Micro System, London, UK) following the procedure described by Nájera-Domínguez et al. [16] with slight modifications. Briefly, three rectangular sections (1.5 cm height and 1.0 cm width) of each cheese were compressed twice with a cylindrical flat-ended probe (P/2 SL, 2 cm diameter) to 75% of their original height, with a delay of 5 s before the second compression. The speed conditions of the texture analyzer were 0.5 mm/s as the pre-test speed, 1.0 mm/s as the crosshead speed, and 5.0 mm/s as the post-test speed. Texture exponent software (Stable Micro System) was used for data analysis. The evaluated parameters of the texture were hardness, which is the maximum peak of force that occurs during the first compression (N); springiness, measured as the degree to which the sample returned to its original shape during the first compression (mm); cohesiveness, described as how well the product withstands a second deformation relative to its resistance during the first deformation (%); gumminess, characterized by the hardness * cohesiveness (N); chewiness, determined as the gumminess * springiness (J); resilience, described as how well the product fights to regain its original height; and adhesiveness, measured as the work needed to overcome the attractive force between food and other surfaces or the negative work between the two cycles (N*s).

The viscoelastic properties of the samples were evaluated with an AR-2000EX rheometer (TA Instruments, New Castle, DE, USA) with an aluminum (0° angle) plate. A dynamic small-amplitude oscillatory test was conducted on each cheese sample. Sample disks (40 mm diameter and 2–3 mm thick) were glued to the rheometer platform to prevent slippage, and stress sweeps were carried out with the following test parameters: strain 0.1%, logarithmic mode, frequency 1 Hz, and temperature ramp from 10 to 70 °C with an increase of 2 °C per min. The storage modulus (G'), loss modulus (G"), and complex viscosity (η *) were recorded during the temperature sweep.

2.5. Microstructural Analysis

Three samples (2 cm long × 0.5 cm wide × 0.3 ± 0.1 cm thick) per treatment were obtained in the direction of the cheese strands for morphostructural analysis. They were fixed in 2.5% glutaraldehyde in Sorensen's phosphate buffer pH 7.2. Sections of the samples were dehydrated in a graded series of ethanol from 30% to 100%. Subsequently, they were dried at the critical point using CO_2 and mounted both transversally and longitudinally on nickel holders with copper tape. The samples were then coated with a layer of gold–palladium, aiming to facilitate electronic conduction. The samples were viewed using a scanning electron microscope (SEM-JSM-6390, Jeol, Tokyo, Japan) operated at 10 kV.

2.6. Consumer Sensory Analysis

A preference ordering test and an acceptance test with consumers were carried out. Both tests used the same consumers (n = 100). The consumer sample consisted of students, teachers, and administrative staff of the Department of Animal Science and Ecology. The gender ratio was 34:66 men to women, and the age range was 21 to 47 y old. Consumers were recruited based on the following criteria: (a) their liking for fresh cheese, (b) their consumption of fresh cheese at least once a week, and (c) their interest in participating in the study. The procedure was individually explained in detail to the participants, who signed a free and informed consent form. The analysis was carried out in 12 booths with white light (fluorescent) and controlled temperature (20 °C) at the Sensory Analysis Laboratory of the University.

The cheese samples (5 cm long × 2 cm wide × the thickness of the ribbon, approximately a bite size) were served in glass dishes at 20 °C to favor the release of volatile compounds for the evaluation of aroma and flavor. Ten sessions were held with 10 different consumers in each session. The panelists were informed before the test about the definition of each evaluated attribute to ensure the consistency and reproducibility of the trials. The attributes evaluated were aroma (volatiles associated with the characteristic dairy aroma in genuine fresh cheese made with raw milk), texture (firmness or resistance of the sample to chewing, soft–hard), flavor (perception associated with the characteristic flavor of Oaxaca cheese in terms of sweet, salty, sour, or bitter), and general acceptability (acceptance of the product in terms of all perceived sensory attributes). Each consumer evaluated a total of six cheese samples (two sets of three samples) in a different order to avoid first-sample and carryover effects [17]. The preference for the products was evaluated using the preference test by ordering them according to the degree of liking (1 = lowest preference, 3 = highest preference) to compare the preference for the ultrasonicated samples against the control samples [18]. They were asked to rinse their mouths with low-salt water between each tested sample [18]. The acceptance of attributes was evaluated by means of a 7-point hedonic scale (1 = dislike extremely and 7 = like extremely) for the score and the degree of global satisfaction [19].

2.7. Statistical Analysis

The data were analyzed using a factorial design with 3 × 2 randomized blocks, with the HIU time factor at three levels (0, 5, and 10 min of USAI) and the HIU frequency factor at two levels (25 and 45 kHz). There were two controls, which corresponded to HIU time 0 in the design. The values from the acceptance test were transformed into numbers according to the assigned category (1 = dislike extremely to 7 = like extremely). The results are reported as means ± standard deviations. The means were compared using Tukey's test ($p < 0.05$). For the preference-ordering test, a multiple comparison procedure involving the Kramer rank sum was performed for the analysis of ordinal data. The analysis of the data from the rank order test compared ultrasonicated samples (15 and 30 min HIU treatments) with the control (HIU time 0) [19,20]. The statistical program SAS System 9.4 (SAS Institute Inc., Cary, NC, USA) was used to perform the statistical analysis.

3. Results and Discussion

3.1. Strand and Melt Tests

The exudate volume increased significantly ($p < 0.0001$) with an ultrasound frequency of 45 kHz and a long application time (30 min) (Table 2). However, the higher exudate volumes occurred with 30 min of ultrasonication, regardless of the frequency, and with 15 min and 45 kHz ultrasonication (Figure 2a). HIU tended to increase the amount of exudate in fresh cheese due to the high retention of whey in the phosphocasein matrix (higher water retention capacity) and the slowing down of the syneresis process [21,22]. HIU apparently increases the denaturation of whey proteins and the contents of soluble minerals (calcium and phosphorus, which pass from the micellar phase to the aqueous phase) in milk. This produces an increase in the crosslinking of gels (small and uniform pores with more interconnections) and an improvement in the coagulation and water retention properties [23]. Therefore, the increase in the surface area due to the decrease in the particle size could be associated with a higher capacity to retain water.

Table 2. Effects of blocks, frequency, and application time of ultrasonication on the volume of exudate and melting of Oaxaca cheese.

Factor	Exudate *	Melting **		
Block	**Volume (mL)**	**Time (s)**	**Cheese Area (cm²)**	**Exudate Area (cm²)**
1	27.1 ± 6.8 [a]	59.7 ± 12.5 [a]	22.0 ± 3.3 [a]	5.84 ± 1.7 [a]
2	28.6 ± 8.2 [a]	59.1 ± 11.8 [a]	21.9 ± 2.9 [a]	5.67 ± 1.5 [a]
3	27.7 ± 7.1 [a]	59.2 ± 9.2 [a]	21.6 ± 3.1 [a]	5.73 ± 1.6 [a]
HIU Frequency (kHz)	**Volume (mL)**	**Time (s)**	**Cheese Area (cm²)**	**Exudate Area (cm²)**
25	25.2 ± 7.1 [b]	59.9 ± 6.8 [a]	20.1 ± 1.5 [b]	6.15 ± 1.8 [a]
45	30.4 ± 1.1 [a]	58.7 ± 13.9 [a]	23.5 ± 3.0 [a]	5.34 ± 1.1 [b]
HIU Time (min)	**Volume (mL)**	**Time (s)**	**Cheese Area (cm²)**	**Exudate Area (cm²)**
0	22.8 ± 0.6 [c]	71.1 ± 4.2 [a]	19.3 ± 0.6 [c]	7.05 ± 0.5 [a]
15	27.9 ± 9.8 [b]	47.7 ± 5.8 [c]	22.4 ± 3.6 [b]	5.82 ± 1.7 [b]
30	32.8 ± 2.8 [a]	59.1 ± 1.0 [b]	23.7 ± 1.8 [a]	4.36 ± 0.6 [c]

* Volume of exudated whey in the package of the cheese 24 h after processing. ** Melting capacity of the cheese due to the effect of temperature (125 °C) until the maximum expansion and the appearance of exudate around the melted cheese. [a,b,c] Different letters within the same column indicate significant differences between treatments (Tukey's multiple range tests, assuming a significant difference at $p < 0.05$, $n = 18$).

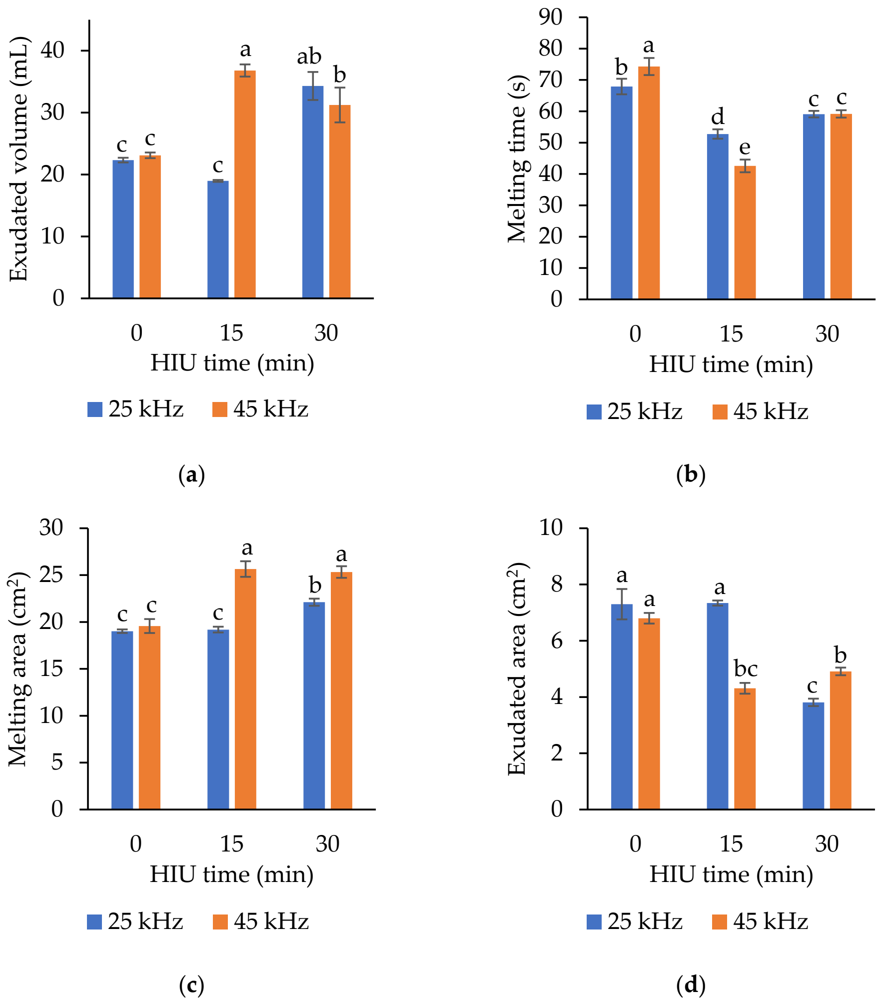
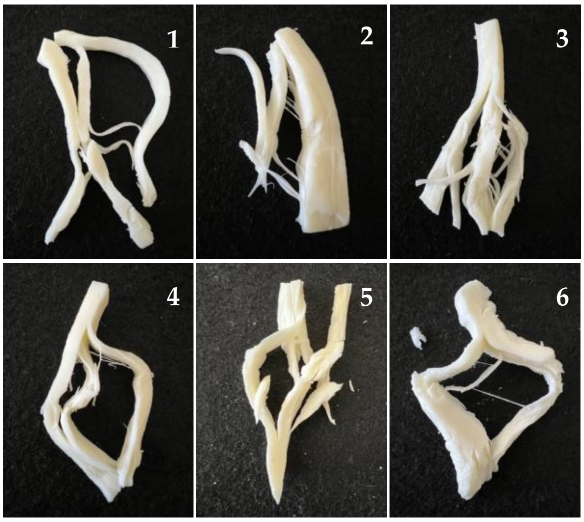


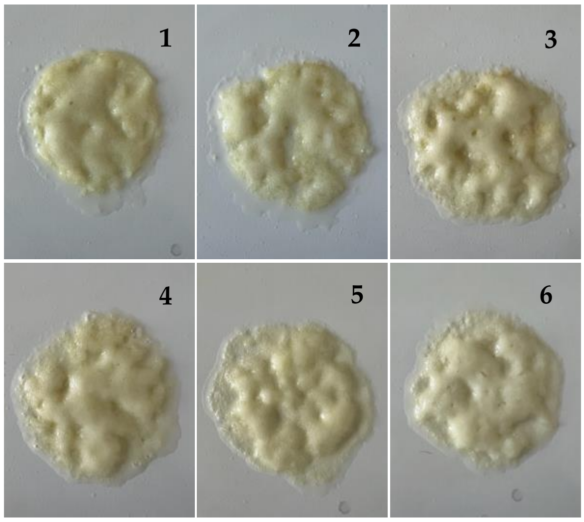
Figure 2. Effects of HIU frequency x time of application to fresh raw milk on (**a**) exudate volume, (**b**) melting time, (**c**) melt area, and (**d**) exudate area in Oaxaca cheese. Data are expressed as

mean ± S.D. (n = 3). [a,b,c] Different letters in columns within the same graph indicate differences among the treatments (Tukey's multiple range tests, assuming a significant difference at $p < 0.05$, n = 18).

Regarding the time for the complete melting of the cheese, the intermediate sonication time (15 min) shortened ($p < 0.0001$) the melting time by up to 23.4 s compared to the controls (Table 2), regardless of the frequency (Figure 2b). Controls without HIU took the longest time to melt (Figure 2b). In addition, the cheeses ultrasonicated for 15 and 30 min produced larger melting areas ($p < 0.0001$, Table 2). Therefore, HIU treatment increases the meltability of the cheese (Figure 3). Ultrasonication at a frequency of 45 kHz produced larger melting areas regardless of the time of HIU application (15 or 30 min, Figure 2c). According to McMahon et al. [24], cheese pH is closely related to the functional properties during melting and solidification. The range between 5.0 and 5.35 produces softer cheeses with a higher melting capacity due to the more extensive hydration of the protein network caused by the migration of micellar calcium to the serum phase in the cheese.



(**a**)



(**b**)

Figure 3. Thread-forming capacity (**a**) and meltability (**b**) of Oaxaca cheese made with ultrasonicated raw milk: (1) 25 kHz for 0 min, (2) 25 kHz for 15 min, (3) 25 kHz for 30 min, (4) 45 kHz for 0 min, (5) 45 kHz for 15 min, and (6) 45 kHz for 30 min.

The solubility of micellar calcium is higher with the addition of acid compared to natural acidification [6]. pH values below 5.0 cause the loss of casein solubility and consequently the loss of elasticity (or stretch) and melting capacity [6,13]. The lower pH values of Oaxaca cheese produced with ultrasonicated raw milk explain the higher melting capacity and the shorter time required to complete melting compared to controls [13]. It has been reported that HIU increases enzymatic activity and results in the hydrolysis of phosphoric esters. HIU also promotes the release of fatty acids, nitrites, nitrates, hydrogen peroxide, and other free radicals, favoring a drop in the final pH of the cheese [11,25–27]. Cheeses obtained with ultrasonicated milk have higher moisture content [13]. Gels retain more water because the surface becomes more hydrophilic due to the interaction of casein micelles with whey proteins. McMahon and Oberg [28] reported that less energy (heat) is required to release the water from the protein matrix when the cheese has a higher moisture content, so the cheese tends to melt easily [13]. Consequently, Oaxaca cheese made with ultrasonicated raw milk melts faster and has a higher melting capacity. The cheeses with the largest melted areas were those with the smallest exudate area ($p < 0.0001$). Controls and the sample sonicated at 25 kHz for 15 min released more whey and had lower melt areas (Figure 3b, subfigures 1, 2, and 4). This inverse relationship between the cheese area and whey release during cheese melting is closely related to whey release after storage.

The cheeses that released the least amount of whey after refrigerated storage (controls and sample sonicated at 25 kHz for 15 min) also released the highest amount of whey during cheese melting. Therefore, the application of HIU at 45 kHz for 15 or 30 min and 25 kHz for 30 min can be beneficial to increase the meltability of Oaxaca cheese, reducing the melting time, increasing the melt area, and releasing less whey.

Regarding the empirical test of the thread-forming capacity, ultrasonication at 25 kHz resulted in greater thread formation (Figure 3a), regardless of the HIU time (15 or 30 min, Figure 3a, subfigures 2 and 3, respectively). The samples sonicated at 45 kHz for 15 or 30 min (Figure 3a, subfigures 5 and 6, respectively) formed fewer threads, the same as the controls without HIU (Figure 3a, subfigures 1 and 4, respectively). Mozzarella cheese is very similar to Oaxaca cheese, as both are made of spun paste, and the curd is stretched in hot water, producing a fibrous texture [29]. There must be a suitable combination of pH and calcium content in the curd to achieve this, so the curd must be sufficiently acidified (pH between 5.2 and 5.4) and demineralized [30]. In addition, hot water facilitates the plasticization and unidirectional organization of the fibers. The protein matrix in the curd is interwoven with fat globules and is maintained by primary bonds with calcium bridges, electrostatic interactions, and hydrogen bridges [31]. The low thread formation in the controls could be associated with the low level of acidification in the cheese, since the pH values were higher than 5.5, even though the same level of acidification of the paste was applied in all treatments during production. Meanwhile, ultrasonication could have favored the excessive loss of calcium, since the cheeses made with milk ultrasonicated at 45 kHz (15 or 30 min) had a low mineral content [13].

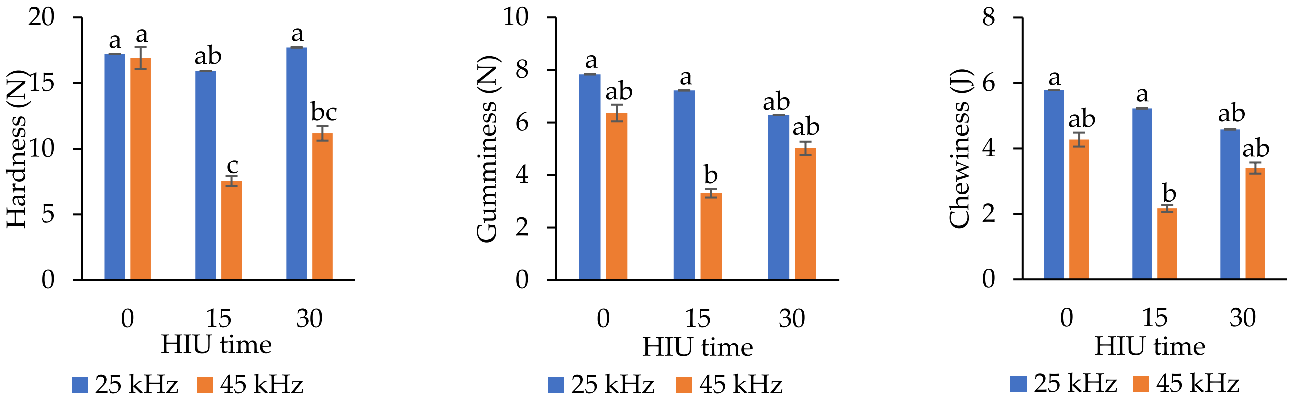
3.2. Texture Profile and Rheometry

According to the texture profile analysis (Table 3), significant differences were found in the hardness of Oaxaca cheese due to the effects of the ultrasound frequency ($p = 0.0002$) and time ($p = 0.0022$). The combination of factors ($p = 0.0003$, Figure 4a) showed a significant decrease in the hardness of the cheese treated at 45 kHz, regardless of the ultrasonication time (15 or 30 min). On the contrary, the highest hardness values were obtained at a frequency of 25 kHz and a sonication time of 0 min (control). The increase in the sonication time in the bath system significantly decreases the hardness of fresh cheese [32]. Koca and Metin concluded that the textural properties of kashar cheeses can be modified when the fat content is modified. The use of fat substitutes decreases hardness, elasticity, gumminess, and chewiness [33]. Previous studies showed that the fat content in Oaxaca cheese is not modified by the effect of the frequency or the time of treatment with HIU [13], so the changes in hardness seem to be related to the effects of HIU on other milk components. Consistent with our results, Jalilzadeh et al. [12] found a significant decrease in the hardness of Iranian ultrafiltered white cheese 24 h after processing with frequencies of 20 and 40 kHz. However, these differences were not significant after 60 d of aging. The hardness increased due to the decrease in moisture content at 30 d of aging. Meanwhile, at 60 d of aging, the decrease in hardness was associated with progressive proteolysis. The decrease in hardness could be related to the acceleration of the proteolysis of casein due to ultrasonication and the increase in the solubility of whey proteins [12,34]. On this topic, Gaya et al. [35] reported that the degradation of p-kappa and beta caseins is more intense in cheese made with raw milk compared to cheese made with pasteurized milk. So, ultrasonication could further enhance the degree of proteolysis (amount of free amino acids) during the storage of cheese made with raw milk [36]. Another factor that can reduce the hardness of cheese is pH. For Cheddar cheese, Pastorino et al. [37] reported that pH values higher than 5.0 lead to calcium solubilization and a reduction in protein–protein interactions, resulting in a softer cheese. The decrease in the hardness of Oaxaca cheese made with ultrasonicated raw milk could be related to this effect, since these cheeses had lower pH values compared to the controls [13].

Table 3. Effects of blocks and HIU frequency and time on the texture profile of Oaxaca cheese.

Treatment	Texture Profile Analysis *						
Block	**H (N)**	**S (mm)**	**C (%)**	**G (N)**	**Ch (J)**	**R**	**A (N*s)**
1	14.2 ± 4.7^{a}	0.68 ± 0.0^{a}	0.40 ± 0.0^{a}	5.61 ± 1.9^{a}	3.9 ± 1.5^{a}	0.20 ± 0.0^{a}	-0.13 ± 0.0^{a}
2	14.4 ± 4.8^{a}	0.69 ± 0.1^{a}	0.45 ± 0.1^{a}	6.38 ± 2.0^{a}	4.47 ± 1.7^{a}	0.23 ± 0.0^{a}	-0.18 ± 0.0^{a}
3	14.6 ± 3.4^{a}	0.72 ± 0.0^{a}	0.42 ± 0.1^{a}	6.02 ± 1.7^{a}	4.34 ± 1.3^{a}	0.23 ± 0.0^{a}	-0.2 ± 0.0^{a}
HIU Frequency (kHz)	**H (N)**	**S (mm)**	**C (%)**	**G (N)**	**Ch. (J)**	**R.**	**A (N*s)**
25	17.0 ± 1.2^{a}	0.73 ± 0.0^{a}	0.42 ± 0.1^{a}	7.11 ± 1.2^{a}	5.19 ± 0.9^{a}	0.23 ± 0.0^{a}	-0.16 ± 0.0^{a}
45	11.9 ± 4.5^{b}	0.66 ± 0.0^{a}	0.44 ± 0.1^{a}	4.9 ± 1.6^{b}	3.28 ± 1.2^{b}	0.20 ± 0.0^{a}	-0.18 ± 0.0^{a}
HIU Time (min)	**H (N)**	**S (mm)**	**C (%)**	**G (N)**	**Ch. (J)**	**R.**	**A (N*s)**
0	17.1 ± 0.6^{a}	0.70 ± 0.0^{a}	0.41 ± 0.0^{a}	7.09 ± 0.8^{a}	5.02 ± 0.9^{a}	0.21 ± 0.0^{a}	-0.19 ± 0.0^{a}
15	11.7 ± 4.8^{b}	0.69 ± 0.1^{a}	0.45 ± 0.1^{a}	5.27 ± 2.4^{b}	3.69 ± 1.8^{a}	0.23 ± 0.0^{a}	-0.13 ± 0.0^{a}
30	$14.5 \pm 4.2^{a,b}$	0.70 ± 0.1^{a}	0.40 ± 0.1^{a}	$5.65 \pm 1.5^{a,b}$	3.99 ± 1.2^{a}	0.22 ± 0.0^{a}	-0.19 ± 0.0^{a}

* H, hardness; S, springiness; C, cohesiveness; G, gumminess; Ch, chewiness; R, resilience; A, adhesiveness. ^{a,b,c} Different letters within the same column indicate significant differences between treatments (Tukey's multiple range tests, assuming a significant difference at $p < 0.05$, $n = 18$).



(**a**) (**b**) (**c**)

Figure 4. Effects of HIU frequency *x* application time of raw milk on the hardness (**a**), gumminess (**b**), and chewiness (**c**) of Oaxaca cheese. Data are expressed as mean ± S.D. ($n = 3$). ^{a,b,c} Different letters in columns within the same graph indicate significant differences between treatments (Tukey's multiple range tests, assuming a significant difference at $p < 0.05$, $n = 18$).

The frequency of ultrasonication had a significant effect on the gumminess and chewiness of Oaxaca cheese (Table 3, $p = 0.002$ and $p = 0.0009$, respectively). The ultrasonication time had a significant effect on gumminess ($p = 0.0439$). The combination of factors was also significant ($p = 0.0072$ and $p = 0.0056$, respectively). According to Jalilzadeh et al. [12], the cohesiveness and elasticity parameters are closely related to the fat content. Consequently, since there were no significant differences ($p > 0.05$) in the fat content of cheese caused by the effect of the frequency and time of HIU [13], these properties were not significant (Table 3). In general, in matured cheeses, the cohesion and elasticity properties tend to remain constant at the end of the storage period [12,38]. Chewability is a property that results from the product of hardness, cohesion, and elasticity. As with hardness, a frequency of 25 kHz produced higher chewiness values (Figure 4c). An increase in the frequency of ultrasonication in milk produces smaller and more uniform fat globules [39]. Carrillo-Lopez et al. [8] reported that the application of frequencies between 20 and 60 kHz modifies the protein structure and favors the formation of casein–fat-globule aggregates due to the reduction in particle size. Therefore, the increase in the hardness and chewiness of Oaxaca cheese could be related to greater interaction between these components in the cheese matrix, producing higher firmness, hardness, and chewiness.

Conversely, Bahri and Kenari [32] found that ultrasonication reduced the chewiness and increased the softness of fresh white cheese, despite the fact that harder samples generally have higher chewiness scores. Gumminess, expressed in units of force (N), describes the energy required to disintegrate food before swallowing, so it is the product of hardness and cohesiveness. Thus, the increase in the hardness of the samples sonicated at 45 kHz produced greater gumminess (Figure 4b). Similar values have been reported for Iranian feta cheese [12] and goat milk gels produced with ultrasonicated milk [23]. The decrease in particle size and whey protein denaturation and the increase in the calcium and soluble phosphorus contents in ultrasonicated milk produced a significant increase in the firmness of the gels, the strength of the clots, cohesion, the ability to retain water, and the crosslinking of the gels as the sonication time increased [21,23].

The oscillation temperature ramp test (Figure 5) was conducted from 10 °C to 70 °C with an increase of 2 °C per min. Oscillatory viscograms of manufactured cheese samples are shown in Figure 5, where the complex viscosity (η*) and G cross-over point of the storage modulus (G′) and loss modulus (G″) are highlighted. At high temperatures, Oaxaca cheese behaves like a viscous liquid. The G cross-over point has been previously correlated with the turning point between the viscoelastic solid and liquid. This means the cheese melting and/or re-solidification phases [40,41]. As can be observed, in both ultrasound frequency treatments (25 and 45 kHz), the G cross-over point of the cheese presented a slight non-significant decrease ($p > 0.05$) from time 0 (56.2–58.9 °C) to 15 min of ultrasound exposure in samples (54.5–55.3 °C) and, finally, a non-significant increase (55.6–57.1 °C) after 30 min of sonication (Figure 5). In other words, at 56.2–58.9 °C, Oaxaca cheese controls (without ultrasound) undergo a liquid-to-solid transition where G′ equals G″. This can also be described as the gelation point. Below this temperature, G′ is higher than G″, indicating that cheese is in a solid state. The temperature for the solid–liquid transition decreased significantly by up to 1.9–3.6 °C and 0.6–1.8 °C, respectively, with ultrasound frequencies of 25 and 45 kHz. This behavior explains the significant decrease in the melting time of Oaxaca cheese in ultrasonicated samples. Samples sonicated for 15 min (regardless of frequency) had significantly shorter melting times (Figure 2b) and lower temperatures for the solid–liquid transition (Figure 5c,d). As explained in the previous section, the low pH and high moisture content of Oaxaca cheese made with ultrasonicated raw milk explain this behavior.

Chandrapala et al. observed that after a few minutes of milk sonication (20 kHz), the break-up of casein–whey protein aggregates and a fat globule reduction occurred, which can lead not only to a small increase in soluble whey protein and fat contents but also to a decrease in milk viscosity [10]. Additionally, Almanza-Rubio et al. mentioned that the melting behavior of fat could be modified by the ultrasound-induced altering of triglyceride properties [42]. The change in fat structure or content was previously related to rheological properties and cheese melting [28,43,44]. Moreover, McMahon and Oberg mentioned that there is a correlation between moisture and cheese meltability, where the higher the moisture, the lower the energy needed to release water from the protein matrix, and so the cheese melts more easily [28]. Additionally, an exponential reduction in complex viscosity (η*) when increasing the temperature to 45 °C is observed (Figure 5), which can be partially associated with the liquefaction of the fat phase in pasta filata cheeses [45]. That is, the rise in G′ and G″ at temperatures below 45 °C is associated with the crystallization of the fat component in cheese.

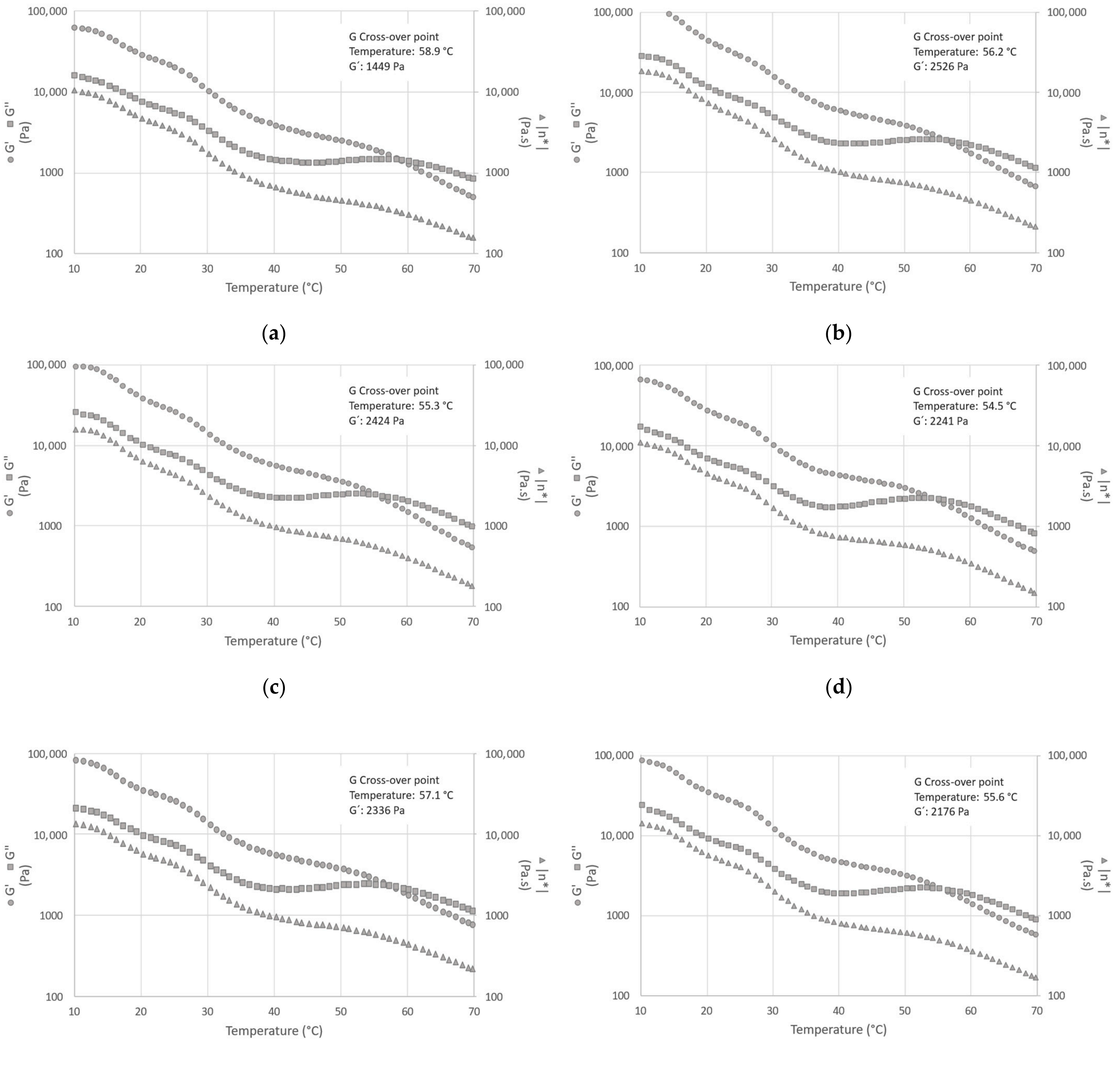


Figure 5. Influence of temperature on viscosity of cheese produced with milk treated with sonication at (**a**) 25 kHz for 0 min; (**b**) 45 kHz for 0 min; (**c**) 25 kHz for 15 min; (**d**) 45 kHz for 15 min; (**e**) 25 kHz for 30 min; and (**f**) 45 kHz for 30 min.

3.3. Microstructure

The microstructures of cheeses made with ultrasonicated milk were determined after 24 h of storage at 4 °C. The structural arrangements of the matrices were different depending on the ultrasound frequency and the time of application (Figure 6). Visually, the control (Figure 6a,b) cheese had casein threads that were relatively short, thick, and broken in the same direction as the stretching direction. These casein threads were disorganized and imperfectly aligned. Similar results were reported by Morales-Celaya et al. [6], who showed that the direct acidification of raw milk with acetic acid tends to produce cheeses with less ordered structures than when starter cultures are used for milk acidification. They pointed out that acidification with a starter culture gradually reduces the pH of the milk, and the decrease in protein–protein interactions is slower (moderated). They also observed short and thick casein threads, the alignment of which was less noticeable in the direction of stretching, including large and numerous fat globules trapped between strands. In this case, the direct addition of acetic acid causes a drastic drop in the pH of the raw milk, causing the solubilization of casein minerals and the consequent formation of aggregates [37].

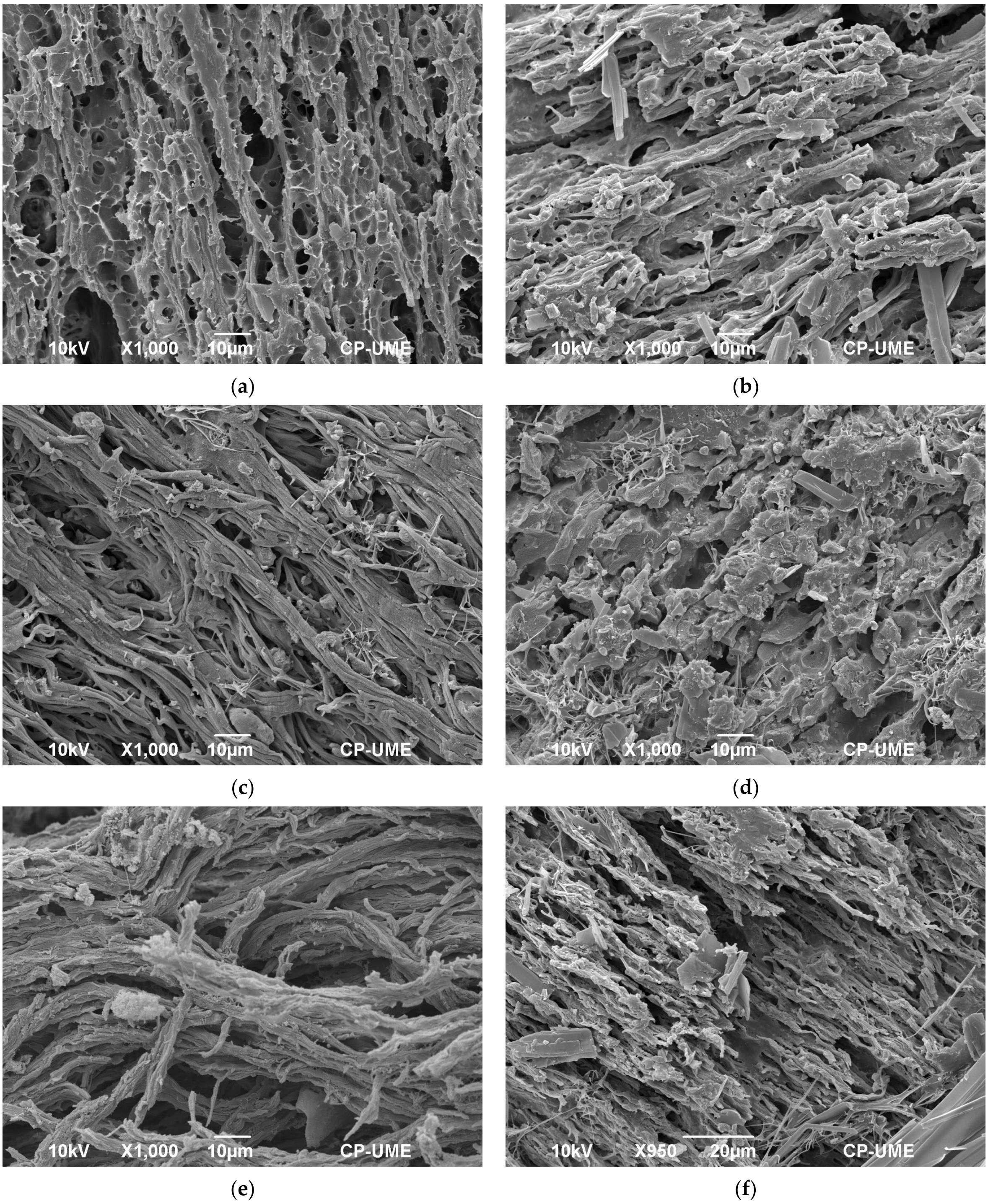


Figure 6. Scanning electron micrographs of Oaxaca cheese made with ultrasonicated raw milk (HIU) after 1 d of storage at 4 °C. (**a**) 25 kHz for 0 min HIU, (**b**) 45 kHz for 0 min HIU, (**c**) 25 kHz for 15 min HIU, (**d**) 45 kHz for 15 min HIU, (**e**) 25 kHz for 30 min HIU, (**f**) 45 kHz for 30 min HIU.

Figure 6a shows the parallel strands of casein with channels between strands containing whey, including empty spaces in the matrix. Empty spaces in the phosphocasein matrix have been associated with the absence of fat globules and whey in pasta filata cheeses (Mozzarella and Oaxaca), which were present during sample preparation [6,46]. With HIU for 15 and 30 min (25 kHz, Figure 6c,e, respectively), the structure is characterized by the formation of perfectly shaped threads. However, at 15 min, the strands are thinner, perfectly aligned, and well organized, while at 30 min, they are slightly thicker and relatively misaligned in the direction of stretching. HIU modifies milk components, reducing the size of fat globules and leading to calcium solubilization [23,37]. Thus, the spaces occupied by fat globules in the matrix of the cheeses made with ultrasonicated milk are smaller compared to those in the controls. The smaller size of the fat globules favored the aggregation of casein micelles, producing well-formed and thinner strands in cheeses made with ultrasonicated milk for 15 min at 25 kHz. At 45 kHz, short treatment times resulted in no thread formation (Figure 6d), while long treatment times (Figure 6f) hardly produced strands that were misaligned and rather thin in the direction of stretching, poorly organized, and broken. In this case, we believe that the lack of alignment of the casein strands is associated with the low ash and calcium contents in cheeses produced with ultrasonicated milk at 45 kHz [13]. They could have limited the binding capacity of the protein in the matrix, leading to the coalescence of fat globules and producing deeper and wider channels between casein strands with higher susceptibility to proteolysis [12,47,48].

In addition, cheeses produced with sonicated milk (45 kHz) had significantly higher counts of psychrophilic bacteria compared to those at 25 kHz [13]. So, during refrigerated storage, bacterial extracellular proteinases could have led to proteolysis in cheese [49]. Various authors have reported that there is a greater amount of proteolysis in cheeses made with acidified milk and with a lower calcium content [6,50].

3.4. Consumer Sensory Test

The sum of ranks in the preference-ranking test (Table 4) indicates significant differences ($p < 0.05$) for both combinations of the three treatments evaluated by the panelists (25 and 45 kHz). The control without ultrasound (0 min) and the samples sonicated for 15 min (25 kHz) were not different from each other ($p > 0.05$), and both were equally preferred. However, the samples ultrasonicated for 30 min were significantly different from the control and the samples sonicated for 15 min ($p < 0.05$), with this treatment being the least preferred by the judges. According to the comments of the panelists on the scoring sheets, the high preference for the control samples and those ultrasonicated for 15 min was because they were consistent with a pleasant aroma and flavor and higher release of moisture during chewing. Objectively, these groups of samples released a higher amount of whey during the melting of the cheese. In contrast, the less preferred samples were classified as consistent but insipid in flavor, negatively impacting flavor and aroma.

Table 4. Effect of the application of ultrasound at 25 or 45 kHz for 0, 15, or 30 min on the preference-ranking test for Oaxaca cheese (rank sum).

Preference	Treatment Group 1 *			Treatment Group 2 *		
	25 kHz, 0 min	**25 kHz, 15 min**	**25 kHz, 30 min**	**45 kHz, 0 min**	**45 kHz, 15 min**	**45 kHz, 30 min**
Rank sum **	108 ^{a}	106 ^{a}	86 ^{b}	112 ^{a}	87 ^{b}	101 ^{a,b}

* Groups of three Oaxaca cheese samples for evaluation in order of preference (1 = least preferred, 3 = highest preferred). ** Sum of scores in the classification of panelist samples. ^{a,b} Row values with different superscripts differ significantly ($p < 0.05$, $n = 100$).

Regarding the samples ultrasonicated at 45 kHz (Table 4), significant differences were found between controls (0 min HIU) and samples ultrasonicated for 15 min ($p < 0.05$). The panelists indicated that the least preferred samples (45 kHz, 15 min) were not very consistent (too soft) during chewing. Nevertheless, they were juicy (high whey release) and with an intense flavor and aroma. Conversely, controls and samples sonicated for 30 min were equally highly preferred. The panelists expressed that these samples were hard during chewing, and the flavor and aroma were less intense, although they were pleasant. This could be related to the higher instrumental hardness values for this group of samples and the low whey release. The results indicate that the use of ultrasound during food processing does not affect acceptance and would potentially not affect purchase intentions. Consumers had a higher preference for ultrasonically processed guava juice regardless of the high price [51]. In general, consumers' perceptions of processed foods are strongly influenced by a lack of clear and reliable information, so it is necessary to familiarize consumers with the benefits and safety of products processed with emerging technologies [52].

Unlike the preference test, where judges are forced to choose one sample over another, in the acceptance test, the panelists rank the samples based on their degree of like or dislike. Significant differences were found among treatments (Figure 7) for aroma (p = 0.0106), flavor (p = 0.0079), texture (p = 0.0024), and general acceptability (p = 0.0091). Similarly to the preference test, the control (25 kHz, 0 min) received the highest score for all evaluated attributes (Figure 7). The difference in scores in comparison to the 45 kHz control could be due to the halo effect and adaptation, since the sensory system is easily confused when multiple attributes are evaluated in a single sample at the same time. Additionally, the sensitivity to a stimulus generally decreases due to repeated exposure to the stimulus [18,53]. This type of bias is common in hedonic tests. In the present study, the judges evaluated two groups of three samples (first 25 and then 45 kHz) each. The control (45 kHz, 0 min) had higher acceptance in taste, aroma, and general acceptability compared to the samples ultrasonicated for 15 and 30 min. Samples ultrasonicated at 25 kHz for 15 min were as well accepted as the controls.

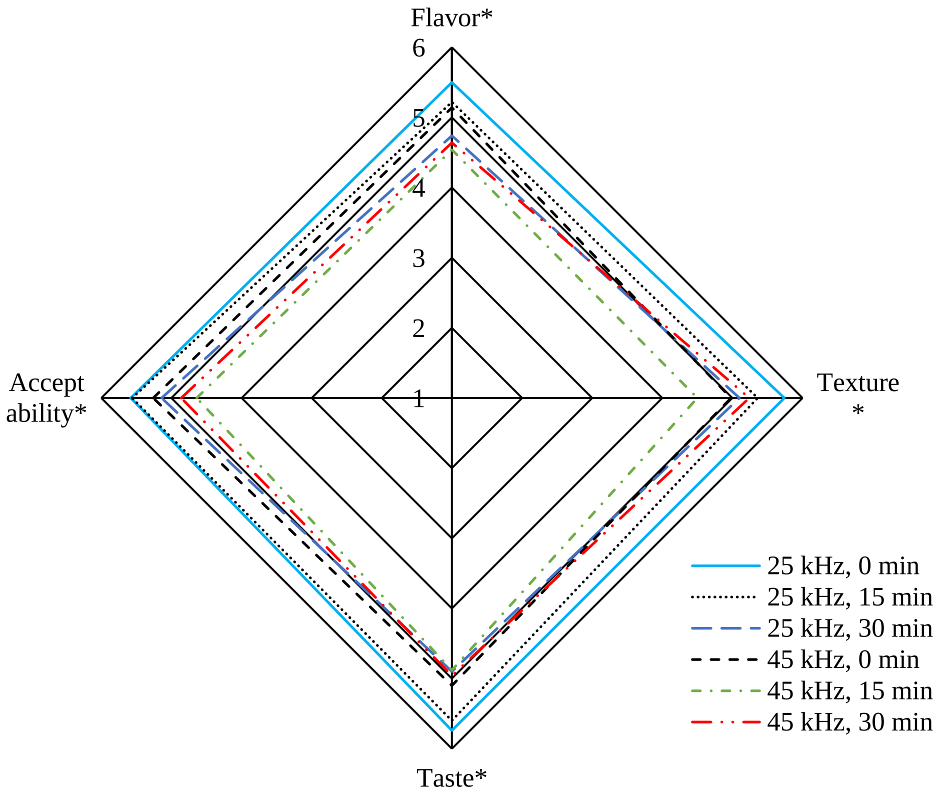


Figure 7. Effect of the application of high-intensity ultrasound at 25 or 45 kHz for 0, 15, or 30 min on the sensory acceptance of Oaxaca cheese. * Indicates significant differences between treatments ($p < 0.05$).

The sample sonicated at 45 kHz for 15 min was the least accepted. We hypothesize that this was due to its texture. The instrumental measurements indicated significantly low values of hardness, gumminess, and chewiness and a high release of exudate during refrigeration, which negatively influenced the flavor and aroma during the evaluation by consumers. On the contrary, the most accepted samples in all evaluated attributes were characterized by having high instrumental values of hardness (higher consistency), a low release of exudate during storage, and a high loss of whey during melting. Therefore, they retained a higher amount of moisture during the sensory test. The microstructures of the best-evaluated samples were characterized by the presence of well-structured strands in the stretching direction separated by whey channels. Although sensory studies are needed to evaluate the sensory acceptance of dairy products produced with the assistance of ultrasound, several studies have shown positive changes in their physicochemical properties. Carrillo-Lopez et al. showed significant increases in the yield and moisture, and protein contents of Panela cheese, and they were dependent on the exposure time and ultrasound amplitude [21]. Jalilzadeh et al. reported that sonicated Iranian feta cheese had better taste sensory scores due to increased lipolysis and proteolysis, significant improvements in color and appearance, and higher texture scores [12]. Consistent with our results, Jalilzadeh et al. [12] showed that the organoleptic property scores of Iranian feta cheese depended on the ultrasonication frequency and storage time. They observed the lowest texture scores when higher frequencies were applied to cheese. This agrees with the present study for the 45 kHz frequency. Sánchez et al. [36] studied Mahon cheese salted in the presence of an ultrasonic field and then aged for 75 d. They observed a higher degree of proteolysis (higher concentrations of free amino acids), which improved the flavor. Other authors have reported improvements in the curd firmness, texture, and yield and a reduction in protein loss in cheese with the use of ultrasound due to structural changes in milk proteins [9,10,54]. The use of ultrasound has also significantly increased the acceptance of sensory attributes such as texture and tenderness and general acceptability of meat products, such as pork loin and restructured cooked ham [55,56].

4. Conclusions

Ultrasound is a promising technology to improve the structural and sensory characteristics of Oaxaca cheese made with fresh raw milk and direct acidification. The application of ultrasound to milk can reduce the melting time and improve the thread-forming capacity of Oaxaca cheese because of the modification of its chemical composition and changes in its microstructural components. Short ultrasonication times and a frequency of 25 kHz produce the same characteristics of hardness, gumminess, and chewiness, preference, and sensory acceptance as non-ultrasonicated controls. The use of these ultrasound parameters can be recommended for the implementation of this technology at the artisanal level, since they improve the rheological and microstructural properties in comparison to the controls, producing a higher capacity for thread formation and meltability during cooking.

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