



The Impact of Ultrasonic Treatment on the Physiochemical and Microbial Properties of Iraqi Soft Cheese

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ARTICLE INFO	ABSTRACT
Article History: Received: 2024/9/15 Accepted: 2024/12/15	In recent decades, there has been a shift towards using alternative methods to enhance traditional milk processing techniques and their derivatives. One such method is the application of ultrasonic treatment as a substitute for conventional pasteurization. The chemical content of milk treated with ultrasonic waves (US) for durations of 5 and 10 minutes at a frequency of 20 kHz and a temperature of 50°C showed increases in protein, fat, and acidity levels after 5 minutes of US pasteurization, registering at 3.50%, 3.65%, and 0.19% respectively, while the ash content was 0.73%. The logarithm of total microbial counts in milk samples showed no growth post-US pasteurization. The active compounds in the milk samples were identified using GC-MS, revealing the highest concentrations after 5 and 10 minutes of US treatment were of Hydroxy-2,8-bis(trifluoromethyl)quinoline, 2-methylpropionate 4- and Succinic acid, 3-methylbut-2-yl 3-chlorophenyl ester, at concentrations of 31.826% and 35.318% respectively. Samples treated for 10 minutes exhibited superior firmness, cohesiveness, and elasticity in soft cheese, with values of 179.9, 0.66, and 4.38, respectively. The lowest pH observed in these samples was 4.60, with the highest acidity at 1.68%. Moreover, ultrasonic treatment enhanced the sensory characteristics of the cheese, demonstrating that ultrasound waves can improve the microbial, physical, chemical, and sensory properties of white cheese.
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1-Introduction

For thousands of years, humans have relied on pasteurization to process milk intended for consumption or manufacturing, considering it a principal technology for reducing microbial contamination in the food industry, especially in milk. It extends the shelf life of derived products due to its ability to eliminate harmful microorganisms without compromising food safety [1]. Recent advancements in food sciences have led to the production of high-quality products using new techniques that save time, effort, and cost, including alternative methods to thermal pasteurization. Despite its effectiveness in eradicating pathogenic bacteria, thermal pasteurization can degrade essential nutrients and produce burnt flavor problems due to its reliance on heat [2]. An alternative technique is ultrasonic treatment, known for its high-frequency waves. This method is divided into two categories: low-intensity non-destructive ultrasound, which does not affect the chemical properties of milk but causes molecular vibrations, used at an intensity less than 1 watt/cm² and a frequency above 100 kHz with low energy, and high-intensity ultrasound, which is more than 10 watts/cm² at frequencies between 20 and 100 kHz [3]. High-energy ultrasonic waves contain sound energy bundles that induce physical and chemical changes in the material by providing high temperatures and pressure. They are used in the homogenization and quality control of dairy products and the pasteurization processes of milk for cheese making [4]. Recent research has shown that ultrasonic treatment of milk plays a role in enhancing fat production when manufacturing dairy products by improving the emulsification of fats in milk, homogenization, stability, and enhancing the activity of lactic acid bacteria by modifying

the metabolic process, as well as improving quality characteristics such as water retention, texture, and structure [5]. Ultrasonic waves increase the temperature and pressure on the milk, reducing microbial contamination and causing physical changes, including cracking of the milk fat globule membrane and changes in casein formations and fat decomposition into triglycerides [4]. [6] High-intensity ultrasonic waves homogenize milk, reduce microbial growth, and form volatile flavor compounds, benefiting curd formation in treated dairy products thereby enhancing the quality characteristics and sensory properties of the final product [7]. Ultrasonic high-intensity waves cause changes in milk components, including reducing its viscosity, crystallizing lactose, homogenizing milk fat, and improving whey filtration, as well as the use of treated milk in cheese-making and increasing coagulation potential due to the waves' ability to break down fat and protein particles into smaller sizes, which accelerates milk coagulation and improves curd qualities [4]. To our knowledge, there are no published studies on the impact of ultrasound on spoilage microorganisms or the potential effects of ultrasound on the physical and chemical properties of white cheese during maturation. Therefore, this work aimed to study the effect of different ultrasonic frequencies on milk, compare it with thermal pasteurization and investigate the physicochemical, microbial, and sensory properties of Iraqi soft white cheese during the maturation period.

2-Materials and Methods

2-1 Experimental design

Raw cow's milk was prepared from the Agricultural Research Station at the University of Basra and divided into three

parts. One part was treated with ultrasonic waves (milk US) at a temperature of 50°C for 5 minutes and another for 10 minutes using Korean-made Ultra-Sonic device, with a frequency of 20 KH, power of 600 watts, and voltage of 220 volts. The third part was pasteurized at a high temperature of 72°C for 15 seconds and then cooled to 4°C. The fourth sample was a control sample, with three replicates for each sample. All samples were stored at 4°C for 4 hours post-milking, and physicochemical tests were conducted [8].

2-2 Cheese Manufacturing

Iraqi soft cheese was made using the microbial, fungal rennet *Rhizomucor pusillus*, produced by Meito Sengyo Co., LTD of Japan, within its shelf life and according to the company's recommendations. The process was repeated three times for each type of raw milk: US cheese, thermal pasteurized cheese, and control cheese, following the method described in [2].

2-3 Milk Testing

2-3-1 Physicochemical Tests

The percentages of protein, fat, lactose, ash, and moisture in the milk were estimated using the Eko milk analyzer. The pH of the milk was measured using a Sartorius pH meter made in Germany, and acidity was determined by titration with 0.1N NaOH [9].

2-3-2 Microbiological Tests

The total bacterial count in the milk was performed using Nutrient Agar prepared by Oxoid, following the manufacturer's recommendations using the pour plate method [10].

2-3-3 Estimation of Active Compounds by GC-MS Technique

Active compounds in the milk samples were identified using a GC-MS device, utilizing an

HP-5ms column and helium gas at a flow rate of 1 ml/sec. The injection temperature was 290°C, and the GC oven program started at 40°C. It was raised to 300°C over 20 minutes at a rate of 10°C per minute. Separated peaks were matched with the spectral database from the NIST 2014 library [11].

2-4 Cheese Testing

2-4-1 Physical and Rheological Tests

The pH of the cheese was measured using a Sartorius pH meter made in Germany, and acidity was determined by titration with 0.1N NaOH. Texture properties such as elasticity, cohesiveness, and firmness were measured using a Texture Analyzer from CT3 4500 Brookfield, USA, according to the method followed [2].

2-4-2 Sensory Tests

The sensory properties of the Iraqi soft cheese samples processed from the treated milk, such as color, appearance, texture, odor, taste, and overall acceptance, were evaluated by 10 experts in the field of food [2].

2-5 Statistical Analysis

Statistical analysis was conducted using the statistical software SPSS (version 12, 2006). The data were analyzed using the CRD and LSD tests at a 0.05 probability level [12].

3- Results and discussion

3-1 Physicochemical Tests of Milk

Table (1) presents the percentage composition of milk derived from cow's milk, along with its pH and titratable acidity for raw milk samples (Control), milk treated with ultrasonic waves for 5 and 10 minutes, and milk pasteurized using thermal pasteurization. The statistical analysis ($P < 0.05$) indicated significant differences in protein, fat, lactose, ash, moisture content, pH, and acidity. The 5-minute US-treated

milk samples showed superior protein and fat percentages compared to other treatments. Specifically, protein content increased from 3.20% in raw milk to 3.50% after 5 minutes of US treatment, decreased slightly to 3.47% after 10 minutes, and was 3.25% post-thermal pasteurization. Fat content decreased to 3.46% following thermal pasteurization from 3.50% in raw milk. However, it reached its highest at 3.65% after 5 minutes of US treatment. The highest lactose content was recorded at 4.87% after 10 minutes of US treatment. The acidity percentage for the milk treated with ultrasonic waves for 5 minutes was 0.19% with a pH of 6.21. Ash content for thermal pasteurization and 5 and 10-minute US treatments were 0.70%, 0.73%, and 0.78%, respectively, compared to 0.70% in raw milk. The highest moisture content was observed in milk post-thermal pasteurization at 87.96%, and the lowest was 87.28% after 10 minutes of US treatment. Ultrasonic waves enhance the enzymatic hydrolysis of whey proteins, producing biologically active peptides, transforming particulate casein into soluble casein, and reducing the size of these particles, unlike conventional pasteurization, which does not alter the structure of casein particles [13]. Additionally, ultrasonication improves lactose crystallization, reduces

fermentation time, lowers pH through enhanced lactose hydrolysis, increases fat globule breakdown, improves emulsifying properties, raises the level of free fats, and thereby increases its susceptibility to oxidation [13,14]. Observations [15] indicated an increase in moisture and protein content and a decrease in carbohydrate and fat percentages in thermal pasteurized milk compared to raw milk (87.46%, 3.33%, 4.54%, and 3.94% vs. 87.40%, 3.16%, 4.74%, and 3.97%, respectively). The reduction in protein particle size due to structural disruption caused by ultrasonic waves enhances the gelation and foaming properties due to effects on hydrogen bonds and Van der Waals forces between the three-dimensional structures of proteins [16]. Ultrasonic waves break down fat globules in milk and homogenize them, reducing their diameter sizes due to repeated collisions [7]. Observe [17] an increase in protein content in milk treated with ultrasonic waves and its interaction with larger fat globules of unhomogenized milk, observed an increase in fat content post-treatment and homogenization, increased surface area of fat globules, and disrupted their membranes, and noted a slight increase in lactose content that does not affect acidity.

Table 1. Chemical composition of US ultrasound-treated and thermal pasteurized milk

Milk	Protein %	Fat%	Lactose %	Ash%	Moisture%	Ph	Acidity %
Control	3.20 ^d	3.50 ^c	4.64 ^c	0.70 ^c	87.96 ^a	6.23 ^b	0.18 ^b
Thermal pasteurization	3.25 ^c	3.46 ^d	4.63 ^c	0.70 ^c	87.96 ^a	6.17 ^d	0.20 ^a
US(5min)	3.50 ^a	3.65 ^a	4.82 ^b	0.73 ^b	87.30 ^b	6.21 ^c	0.19 ^b
US(10min)	3.47 ^b	3.60 ^b	4.87 ^a	0.78 ^a	87.28 ^c	6.25 ^a	0.17 ^c

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the probability level ($P < 0.05$).

3-2 Microbial Tests for Milk

Ultrasound treatment significantly reduces the microbial content in milk, surpassing the efficacy of conventional pasteurization. Table (2) illustrates the logarithmic total counts of bacteria present in milk processed using ultrasound for 5 and 10 minutes, pasteurized using the thermal pasteurization method, and in raw milk. Statistical analysis results, with a significance level of $P < 0.05$, indicate significant differences in the logarithmic total bacterial counts. It was observed that the logarithm of the counts in thermal pasteurization pasteurized milk reached 2.34 cfu/ml, and no growth was noted post-ultrasound treatment of raw milk. [18] Illustrate that ultrasound treatment markedly inhibits the growth of microorganisms, with the extent of inhibition varying based on the amplitude, duration of

exposure, and temperature; it was noted that this method resulted in the lowest logarithmic number of Enterobacteriaceae bacteria at 1.06151 cfu/ml. [17] noted that conventional pasteurization processes hinder the growth of some microorganisms, excluding pathogenic bacteria such as *E. coli*, which can proliferate in pasteurization equipment. However, ultrasound treatment results in lethal and inhibitory effects on pathogenic microorganisms due to the generated pressure in the milk, leading to the puncture and destruction of microbial cell membranes. While thermal treatments reduce the microbial counts of Psychrotrophs, ultrasound treatment significantly reduces microbial counts, including coliform bacteria, molds, and yeasts, thereby extending the shelf life of products manufactured from ultrasound-treated milk [19].

Table 2. Logarithm of the total numbers of bacteria present in milk processed using the US method and thermal pasteurized milk

Milk	Total plate count (cfu/ml)
Control	7.29 ^a
Thermal pasteurization	2.34 ^b
US(5min)	< 1 ^c
US(10min)	< 1 ^c

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the probability level ($P < 0.05$).

3-3 Analysis of Active Compounds in Milk by GC-MS Technique

Numerous active compounds were detected in milk treated with ultrasound, as indicated in Table (3) and Figure (1), which illustrates

the chromatogram of the active compounds, their retention times, and concentrations in milk processed by ultrasound for 5 minutes. This includes volatile compounds as well as fatty acids and active peptides with health benefits. It was observed that the highest

concentration was of Hydroxy-2,8-bis(trifluoromethyl)quinoline, 2-methylpropionate 4-, at 31.826% after 34 minutes from the start of separation. Conversely, results from Table (4) and Figure (2) for milk treated with ultrasound for 10 minutes show a decrease in the concentrations of active compounds compared to the 5-minute treatment, with the highest concentration being Succinic acid, 3-methylbut-2-yl 3-chlorophenyl ester at 35.318% after 49 minutes. Additionally, various compounds appeared at different ratios, such as maltol, catechol, alcohols, organic acids, phenolic compounds, and benzene, among others, contributing to the flavor-enhancing volatile compounds. . [20] pointed out that raw milk contains many desirable volatile compounds like ethyl butanoate, 1-octen-3-ol, and phenylethanol, which impart a distinct aroma to milk but are lost after thermal or ultrasound processing due to physical and chemical changes that

adversely affect the flavor of milk by producing undesirable compounds from increased treatment impact. Ultrasound treatment leads to an increase in volatile compounds compared to thermally pasteurized milk, with notable appearances of compounds such as aldehydes from the oxidation of unsaturated fatty acids by ultrasound, and maltol, an active aromatic compound, along with organic acids such as acetic, hexanoic, and butyric, formed by the rupture of fat globule membranes, along with benzene and toluene, and some aliphatic compounds [19]. The formation of volatile compounds is linked to the degree of lipid and protein decomposition by enzymes and by ultrasound, which promotes the formation of free fatty acids, active peptides, and amino acids, as well as free radicals that cause oxidation, leading to the production of undesirable compounds like methyl ketones, aldehydes, esters, secondary alcohols, or sulfur compounds [20].

Table 3. Active compounds, retention time, and concentration of milk treated with ultrasound for 5 minutes

Peak	RT.	Area%	Library/ID
1	10.333	0.468	1,2-Dimethyl-3-isopropylidiaziridine
2	11.29	0.452	[(2-Amino-3-hydroxypropanoyl)amino]acetic acid
3	11.548	0.505	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione
4	11.847	1.055	1H-Imidazole-4-carboxylic acid, methyl ester
5	12.105	1.094	Allyl 2-methylbutyrate
6	12.335	0.382	Maltol
7	12.519	0.491	Cyclopentasiloxane, decamethyl-
8	12.722	0.366	2-Propanamine, N-methyl-N-nitroso-
9	12.899	1.527	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
10	13.218	0.876	2-Methylheptanoic acid
11	13.713	1.09	Catechol
12	14.155	11.529	5-Hydroxymethylfurfural
13	15.058	0.646	Cyclohexasiloxane, dodecamethyl-
14	15.35	1.165	1,3-Butadiene-1-carboxylic acid
15	16.022	1.371	2,3-Pentadienoic acid-, ethyl ester
16	16.476	3.866	3,4-Altrosan

17	17.284	0.37	Cycloheptasiloxane, tetradecamethyl-
18	17.902	4.009	2,3-Pentadienoic acid-, ethyl ester
19	19.423	1.265	Furan, 3-(4,8-dimethyl-3,7-nonadienyl)-, (E)-
20	21.541	0.516	Cyclo(L-prolyl-L-valine)
21	22.525	0.357	Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione
22	22.905	1.555	n-Hexadecanoic acid
23	23.224	0.338	Hexadecanoic acid, ethyl ester
24	24.596	1.358	9-Octadecenoic acid, (E)-
25	24.806	0.616	Octadecanoic acid
26	27.922	0.81	Bis(2-ethylhexyl) phthalate
27	29.164	0.344	4-tert-Butylphenol, TMS derivative
28	29.816	0.593	Tris(tert-butyl dimethylsilyloxy)arsane
29	30.814	0.329	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
30	31.167	1.465	4-(7-Methyloctyl)phenol, TMS derivative
31	32.498	1.757	Cholest-5-en-3-ol (3.β.), tetradecanoate
32	34.032	31.826	4-Hydroxy-2,8-bis(trifluoromethyl)quinoline, 2-methylpropionate
33	36.014	17.363	2-Ethylbutyric acid, 2,7-dimethyloct-5-yn-7-en-4-yl ester
34	42.606	2.05	4-tert-Octylphenol, TMS derivative
35	49.503	6.195	Cyclotrisiloxane, hexamethyl-

Table 4. Active compounds, detention time, and concentration of milk treated with ultrasound for 10 minutes

Peak	RT.	Area%	Library/ID
1	10.333	0.562	Cyclohexanol, 4-methyl-
2	11.84	0.425	3-Furancarboxylic acid, methyl ester
3	12.132	3.198	5-tert-Butyl-1,2,3,4,5,6,7-[1,2,3]triazolo[4,5-e][1,2,3,4]tetrazine-1,3,7-trione
4	12.899	0.785	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
5	13.306	1.368	L-Serine, N-methyl-, methyl ester
6	13.761	1.706	Catechol
7	14.162	2.756	5-Hydroxymethylfurfural
8	14.657	0.45	Butyrolactone, 3-cyano-4,4-dimethyl-
9	15.058	0.676	Cyclohexasiloxane, dodecamethyl-
10	15.424	0.454	3-Octadecene, (E)-
11	16.551	1.72	Pyrazole-5-carboxylic acid, 3-methyl-
12	19.253	0.361	Cyclooctasiloxane, hexadecamethyl-
13	20.828	0.655	Tetradecanoic acid
14	21.554	0.823	Cyclo(L-prolyl-L-valine)
15	21.873	0.376	1,3-Cyclohexanedione, 2-propyl-

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Retention Time (min)	Approximate Abundance
10.333	100,000
11.256	150,000
11.840	200,000
12.405	250,000
12.899	300,000
13.333	200,000
13.800	250,000
14.155	2,000,000
14.860	200,000
15.058	250,000
16.476	300,000
17.284	250,000
17.902	200,000
19.423	200,000
21.541	200,000
22.543	200,000
22.905	750,000
23.224	200,000
24.806	250,000
24.596	300,000
27.922	200,000
29.816	250,000
29.164	200,000
30.814	300,000
31.167	400,000
32.498	1,200,000
34.032	2,000,000
36.014	1,200,000
42.606	600,000
49.503	900,000

Figure 1. Chromatograms of active compounds, retention time, and concentration of milk treated with ultrasound for 5 minutes

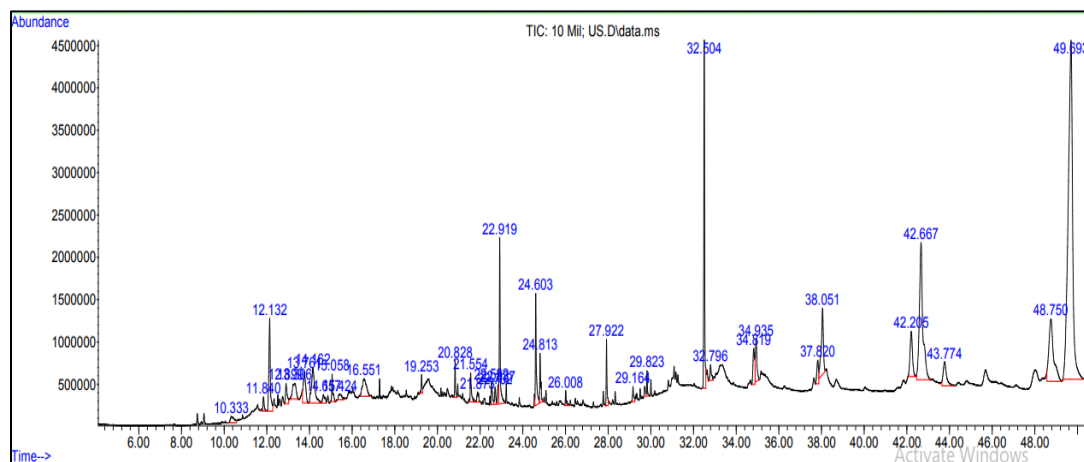


Figure 2. Chromatograms of active compounds, retention time, and concentration of milk treated with ultrasound for 10 minutes

3-4 Physical and Rheological Examinations of Cheese

Table 5 delineates the physical characteristics of Iraqi soft cheese produced from both raw and pasteurized milk using thermal pasteurization and ultrasonic (US) methods for durations of 5 and 10 minutes. Statistical analysis results ($P < 0.05$) indicate significant differences in the properties of hardness, cohesion, elasticity, acidity, and pH levels of the cheese. It was observed that samples treated using the US method outperformed other samples. Specifically, those treated for 10 minutes showed superior hardness, cohesion, and elasticity, with respective values of 179.9, 0.66, and 4.38. Additionally, the lowest pH value was recorded in cheese samples from milk treated with the US method for 10 minutes, at 4.60. The highest acidity reached 1.68%, compared to 4.72 and 1.60% for thermal pasteurization, respectively. The pH values decrease, and acidity increases post-cheese production due to the action of coagulating microorganisms, and ultrasonic treatment further reduces the pH values and increases acidity, thus

decreasing the time required for coagulation. This effect is attributed to the fact that ultrasonic waves enhance the hydrolytic breakdown of fats, thereby impacting the characteristics of hardness, cohesion, and elasticity that vary with the frequency used in the US treatment. The increase in these values is due to reduced moisture content resulting from protein hydrolysis; ultrasonic waves increase the cheese's hardness by promoting coagulation processes [2]. Ultrasonic waves also alter the structure of proteins and stimulate hydrophobic groups on their surface, which enhances gel formation and increases its hardness [21]. The change in the size of milk components post-ultrasonic treatment improves the gel structure by increasing the gel strength and curd firmness and accelerating the coagulation process. The reduction in the size of milk fat globules due to ultrasonic vibrations enhances emulsifying properties, which is attributed to the rupture of milk fat globule membranes and the interaction of casein particles with these membranes, as well as the division of larger fat globules [13].

Table 5. Physical properties of Iraqi soft cheese made from milk treated with US ultrasound for 5 and 10 minutes and thermal pasteurization

Milk	Hardness/gm	Cohesiveness	Springiness/mm	Ph	Acidity%
Control	175.3 ^c	0.78 ^a	4.55 ^a	4.73 ^a	1.59 ^c
Thermal pasteurization	170.4 ^d	0.62 ^b	3.99 ^c	4.72 ^a	1.60 ^c
US(5 min)	177.5 ^b	0.65 ^b	4.36 ^b	4.70 ^b	1.65 ^b
US(10 min)	179.9 ^a	0.66 ^b	4.38 ^b	4.60 ^c	1.68 ^a

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the probability level ($P < 0.05$).

3-5 Sensory Tests for Cheese

Table (6) presents the sensory characteristics of soft Iraqi cheese made from raw, thermal pasteurized, and ultrasonically treated (US) milk for 5 and 10 minutes, focusing on color, texture, aroma, taste, and overall acceptance. Statistical analysis results, with a significance level of $P < 0.05$, indicate significant differences in the mentioned attributes. Cheese samples processed with ultrasound for 10 minutes excelled with the best overall score of 22.49, showing superior results in color, taste, and overall acceptance with scores of 4.78, 4.36, and 4.48, respectively. In contrast, samples made from thermal pasteurized milk exhibited the best aroma, scoring 4.50, possibly attributed to the increased formation of aromatic volatile compounds in US-treated milk compared to thermal pasteurization. Ultrasonic waves enhance the sensory quality of cheese due to their impact on fat emulsification and protein breakdown in milk, influenced by the formation of volatile compounds from enzymatic reactions of milk components, as well as the formation of complex compounds from the degradation of hydroxides in secondary oxidation processes [13]. [2] observed that ultrasonics positively affect the

sensory properties of cheese by improving its color, appearance, and texture and enhancing flavor and taste due to the breakdown of fats and proteins.

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Table 6. Sensory properties of Iraqi soft cheese made from milk treated with US ultrasound for 5 and 10 minutes and thermal pasteurization

Milk	Color /5	Texture/5	odor/5	Flavor/5	Overall acceptability/5	Total/25 mark
Control	4.00 ^d	4.75 ^a	3.05 ^d	3.30 ^c	3.39 ^d	18.49 ^d
Thermal pasteurization	4.30 ^c	4.15 ^d	4.50 ^a	4.05 ^b	4.30 ^c	21.30 ^c
US(5 min)	4.75 ^b	4.70 ^c	4.12 ^c	4.35 ^a	4.45 ^b	22.37 ^b
US(10 min)	4.78 ^a	4.72 ^b	4.15 ^b	4.36 ^a	4.48 ^a	22.49 ^a

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the probability level ($P < 0.05$).

4- Conclusion

This study highlights the potential of utilizing ultrasonic treatment for pasteurization and modifying the properties of milk prior to its conversion into dairy products. This technique provides non-destructive, non-oxidative heat to the milk. It facilitates homogenization, which enhances the sensory and physical characteristics of the resultant dairy products. Moreover, it improves the physicochemical and sensory properties, as well as the texture and consistency of these products. Additionally, ultrasonic treatment helps reduce microbial contamination. It leads to the formation of active and volatile compounds with functional health benefits, resulting from the hydrolysis of proteins and fats in the milk and its products. Consequently, this enhances the functional and health properties of these products. This technology can be widely adopted in dairy plants to reduce time, effort, and costs while achieving superior results compared to traditional pasteurization methods.

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