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Assessment of Quality and Microbial Characteristics and Fatty Acid Changes in Mozzarella Cheese Produced from Buffalo Milk and Fortified with Lactic Acid Bacteria

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ABSTRACT

Background: Modern technological advances have enabled production of dairy products that can support the biological role of lactic acid bacteria, contributing to the improvement of fortified Mozzarella cheese. This study assessed the quality and microbial characteristics and fatty acid changes in Mozzarella cheese produced from buffalo milk and fortified with lactic acid bacteria.

Methods: Mozzarella cheese was prepared from Iraqi buffalo milk and fortified with lactic acid bacteria. Physicochemical and microbiological tests were conducted on the cheese over four storage periods of 0, 4, 7, and 10 days. Fatty acids were identified using GC-MS technique.

Results: After 10 days of storage, the pH, acidity, protein, fat, moisture, and ash values were 5.1, 0.67, 20.57, 17.08, 46.15, and 2.92%, respectively. Total bacterial counts, protein and fat analyzing bacterial count, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* counts showed an increase with the storage period. After 10 days of storage, the logarithmic values of these bacterial count reached 5.74, 1.96, 1.78, 2.80, and 2.81 CFU/g, respectively. Several fatty acids and active compounds were identified with the highest concentration at 9.5822% area concentration after manufacturing as tribute acetyl citrate, and 1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl at 10.5025% area concentration after 10 days of manufacturing.

Conclusion: Sensory evaluation revealed acceptance in terms of color, flavor, taste, texture, and overall acceptance for Mozzarella cheese when fortified with lactic acid bacteria.

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Introduction

The world's production of buffalo milk ranks second after cow milk production, and it is considered an important source of essential nutrients because it contains high levels of proteins, fats, lactose, and minerals. It can be consumed either in liquid form or processed into cheeses, including Mozzarella cheese (1). Mozzarella cheese is considered a high-nutritional-value food product that is used in the production of various ready-made foods, such as pastries and pizzas, It is classified as a traditional Italian cheese that involves specific manufacturing steps, including curdling the cheese using starters or adding acid and rennet, and stretching the curd with hot water to achieve a smooth texture. The moisture content of low-moisture Mozzarella ranges between 45% and 54%; while a high-moisture cheese has a moisture content of 56% (2).

In the traditional method of manufacturing Mozzarella cheese, a starter culture of lactic acid bacteria is used. This is done to ferment lactose, lower the pH, accelerate curd formation after adding rennet, and impart a good flavor to the cheese, in addition to giving it the appropriate composition and texture (3). There are several types of bacteria used as starters, including *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *L. Helveticas*. The type of starter can determine the type of cheese and its characteristics (4). Lactic acid bacteria possess anti-pathogenic activity and can lead to a decrease in total cholesterol, triglycerides, low-density lipoprotein, an increase in high-density lipoprotein and result in reduction of the heart and vascular diseases risks when used in fortifying of dairy products (5).

Mozzarella cheese is widely used in pizza production due to its stretch ability, melt ability, flexibility, viscosity, and the release of free oil, attributed to its content of saturated milk fats (6). Mozzarella cheese is classified as a fermented dairy product and falls under the category of soft, white, unrepented cheeses. It is known for its high-fat content, ranging from 30-45%, which gives it the characteristic of stretch ability. There is a preference for production of buffalo milk rather than cow's milk due to its white color and high concentration of solids (7). The pH and acidity of Mozzarella cheese made from buffalo milk were demonstrated to be 4.9 and 0.92%, respectively (8). The protein, fat, and ash content were determined to be 25.26%, 22.00%, and 2.33%, respectively. The calcium, potassium, and sodium content were illustrated to be 72.16%, 8.60%, and 12.33%, respectively. So the chemical content of buffalo milk gives Mozzarella cheese excellent quality characteristics due to the presence of fatty acids, calcium, and vitamin A. Additionally, feeding green grasses to the buffalo diet was exhibited to improve the milk quality, and increase the health-enhancing particles and the proportion of long-chain polyunsaturated fatty acids as bioactive components, thus improving the health properties of the cheese (9).

High saturated fatty acids content in mozzarella cheese can help reduce the cholesterol level in the blood and provide a protection against heart and vascular diseases. This is due to its dairy fat content, as well as its inclusion of unsaturated fatty acids (8). The physical and chemical characteristics of Mozzarella cheese made from cow's milk, goat's milk, and a mixture of both were compared in a study. When cheese was stored for 30 days, significant amount of calcium, sodium, phosphorus, and potassium were demonstrated; while the pH values ranged 4.82-5.23, 4.83-5.13, 4.93-5.21. It was shown that most of the chemical components of the cheese were not affected by storage (10). So our study aimed to evaluate the quality and microbial characteristics and fatty acid changes in Mozzarella cheese produced from Iraqi buffalo milk fortified with lactic acid bacteria.

Materials and Methods

The research was conducted in the laboratories of Food Science Department, College of Agriculture, University of Basrah, Iraq. Iraqi buffalo milk was obtained from a farmer in Al-Karma area, Basrah province. A starter culture containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was added to the milk. The fungal microbial rennet *Rhizomucor pusillus* (Meito Sengyo Co., Japan) was used within its expiration date and according to the recommended instructions provided by the company. The acidity was adjusted using citric acid (BDH, UK). Mozzarella cheese was made following the method described by others (11). The buffalo milk was pasteurized at 65°C for 15 minutes, then cooled to 45°C, and the acidity was adjusted to a pH of 5.9 using citric acid. A starter culture containing lactic acid bacteria and rennet was added to the milk according to the instructions provided by the company.

The mixture was left to rest for 15 minutes in a warm place, the curd was cut into cubes and allowed to rest for another 15 minutes, the temperature of the curd was raised to 41°C, it was drained using the whey, the temperature was raised to 85°C, and it was finally placed in hot whey with continuous kneading. The previous process was repeated with continuous kneading to obtain a stretchy cheese. The cheese was then stored at 4°C, while physical and chemical and microbiological analysis were conducted during storage. The physicochemical analysis included measuring the pH value by a German-made Sartorius pH meter. The acidity was determined by titration with 0.1N NaOH using phenolphthalein as an indicator. The protein content was estimated using the Kjeldahl method for total nitrogen determination, multiplied by a protein factor of 6.38. The fat content was determined using the Gerber method, while the ash content was determined by combustion (12).

Estimation of fatty acids and active compounds in Mozzarella cheese was undertaken by GC-MS technique. The fat was extracted from the cheese by homogenizing it with a chloroform and methanol solvent mixture in a ratio of 1:2 v/v on a magnetic stirrer. Then, 5 drops of 3% NaCl solution were added to separate the fat. The fat was later filtered and dried by placing it in an oven at a temperature of 50°C for 4 hours (13). The fatty acids present in Mozzarella cheese were analyzed using GC-MS device with an HP-5 ms column and helium gas as the carrier gas at a flow rate of 1 mL/second. The injector and detector temperatures were set at 290°C, and the GC oven program was set at 40°C for 5 minutes. Then it was increased to 300°C for 20 minutes at a rate of 10°C/minute and the obtained spectra were compared with the spectral library, and the separated peaks were identified using the NIST 2014 library database (14).

For microbiological analysis, samples of cheese were prepared according to the method described before (15). So 11 grams of the samples were mixed with 99 mL of prepared Ranker solution (BDH Co., UK) using a sterilized glass electric blender. Decimal dilutions were prepared and the total bacterial counts and lipolytic bacteria were calculated and incubated at 37°C for 24-48 hours utilizing the pour plate method and nutrient agar culture media prepared (Oxoid Co.) (16). The proteolytic bacteria were calculated using caseinate agar medium (Himedia Co., India), based on the method described previously with some modifications. The incubation was done at a temperature of 32°C for 2-5 days, according to the recommendations of the company (17).

For enumeration of lactic acid bacteria, the method described in APHA was followed to estimate the counts of *L. bulgaricus* in Mozzarella cheese using MRS agar medium, and *S. thermophiles* bacteria using M17 medium. The plates were incubated under anaerobic conditions at a temperature of 37°C for 48 hours (18). The sensory evaluation of Mozzarella cheese samples was conducted in the laboratory by 10 specialized assessors from the Department of Food Science, College of Agriculture, University of Basrah, Basrah, Iran. The evaluation included determining the attributes such as color, flavor, taste, texture, and overall acceptability as explained before (11). For statistical analysis, CRD was used to analyze the inspected parameters and their effects on the different qualities. To compare the significant differences between the average values by choosing the least difference ($p<0.05$), the premade program LSD was employed (19).

Results

Regarding physicochemical analysis of Mozzarella cheese for pH and acidity, the results immediately after manufacturing and after 4, 7, and 10 days of manufacturing were presented in Table 1. No significant difference in pH values and significant differences in acidity values were noticed at a significance level of $p\leq0.05$. The pH values decreased and the acidity increased in the Mozzarella cheese made from buffalo milk due to the use of acid and starter bacteria and the coagulant with values of 6.6 and 0.14% in the milk, respectively. The pH decreased and the acidity increased with an increase in storage time, while the lowest pH value of 5.1 and the highest acidity value of 0.67% after 10 days of storage were visible. The highest pH value and the lowest acidity value immediately after manufacturing were 5.8 and 0.60%, respectively.

The findings of the chemical composition of the percentage ratios of the components of buffalo milk and Mozzarella cheese were demonstrated in Table 2 including protein, fat, moisture, and ash. The statistical analysis showed significant differences in the values of protein. No differences were observed in fat, moisture, and ash values ($p\leq0.05$). The moisture content decreased in Mozzarella cheese fortified with lactic acid bacteria when compared to milk, while protein, fat, and ash content increased. Furthermore, the moisture content decreased with an increase in storage period; while directly after production, it was 47.40%, and after 10 days, it decreased to 46.15%. Protein, fat, and ash content increased with the increase in the storage period; while directly after production, they were 20.00%, 16.96%, and 2.75%, respectively; and after 10 days, they increased to 20.57%, 17.08%, and 2.92%, respectively.

Table 1: pH and acidity values of Mozzarella cheese samples fortified with lactic acid bacteria during the storage period.

Storage period/day	pH	Acidity%
Milk	6.6 ± 0.15^{a}	0.14 ± 0.02^{d}
0	5.8 ± 0.15^{a}	0.60 ± 0.02^{d}
4	5.6 ± 0.15^{a}	0.62 ± 0.02^{c}
7	5.2 ± 0.15^{a}	0.65 ± 0.02^{b}
10	5.1 ± 0.15^{a}	0.67 ± 0.02^{a}

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($p<0.05$). Values were expressed as mean±SD of three replicates.

Table 2: Chemical composition of buffalo milk and Mozzarella cheese fortified with lactic acid bacteria during storage period.				
Storage period/day	**Protein %**	**Fat%**	**Moisture%**	**Ash %**
Buffalo milk	3.66±3.91[e]	3.71±0.006[a]	87.10±0.004[a]	0.75±0.009[a]
0	20.00±3.91[d]	16.96±0.006[a]	47.40±0.004[a]	2.75±0.009[a]
4	20.21±3.91[c]	16.98±0.006[a]	46.88±0.004[a]	2.77±0.009[a]
7	20.54±3.91[b]	17.00±0.006[a]	46.46±0.004[a]	2.90±0.009[a]
10	20.57±3.91[a]	17.08±0.0068[a]	46.15±0.004[a]	2.92±0.009[a]

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability ($p<0.05$). Values are expressed as mean±SD of three replicates.

Table 3: Fatty acids and active compounds in Mozzarella cheese fortified with lactic acid bacteria.			
Peak	**R.T.**	**Area Pct**	**Library/ID**
Immediately after manufacturing			
1	4.9056	4.9031	N,N-Dimethylaminoethanol
2	9.8323	3.6052	Ethanol, 2,2’-oxybis-
3	17.9673	2.2797	2,4-Di-tert-butylphenol
4	21.9647	2.9901	1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester
5	22.4883	4.147	Hexadecanoic acid, methyl ester
6	24.1699	3.5526	11-Octadecenoic acid, methyl ester
7	24.7378	3.2443	1-Propene-1,2,3-tricarboxylic acid, tributyl ester
8	25.6302	9.5822	Tributyl acetyl citrate
9	26.4636	6.2672	9-Octadecenamide, (Z)-
10	27.2601	5.839	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-
11	27.9608	3.7008	Bis(2-ethylhexyl) phthalate
12	28.0345	5.0374	1H-Indole, 2-methyl-3-phenyl-
13	28.1304	8.2926	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide,-
14	28.1894	3.0106	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide,
15	28.2632	2.5941	1H-Indole, 2-methyl-3-phenyl-
16	29.4211	7.2877	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl)
17	29.6571	8.4957	9-Octadecenamide, (Z)-
18	31.1543	3.3723	Furan, 2-butyltetrahydro-
19	32.209	8.2795	Cholesterol
20	32.4671	3.519	Pyrrolidine-2,4-dione
After 10 days of manufacturing			
1	4.729	3.6506	Ethanol, 2-methoxy-, acetate
2	8.925	2.2619	1,2-Cyclopentanedione
3	12.075	5.9744	2-Oxo-n-valeric acid
4	22.481	6.52	Hexadecanoic acid, methyl ester
5	24.17	4.236	11-Octadecenoic acid, methyl ester
6	25.63	4.5744	Butyl citrate
7	26.464	2.8262	9-Octadecenamide, (Z)-
8	27.26	6.7282	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-
9	27.57	2.4674	Carbonic acid, 2-ethylhexyl octyl ester
10	27.954	4.995	Bis(2-ethylhexyl) phthalate
11	28.027	6.5816	Methadone N-oxide
12	28.131	10.5025	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-
13	28.19	3.8741	1H-Indole, 5-methyl-2-phenyl-
14	28.263	3.6976	Methadone N-oxide
15	29.082	2.5159	Carbonic acid, decyl 2-ethylhexyl ester
16	29.414	9.3497	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)
17	29.65	3.6842	13-Docosenamide, (Z)-
18	31.154	3.868	Propanoic acid, 2-methyl-, 1,2,3-propanetriyl
19	32.209	6.6895	Cholesterol
20	32.467	5.0029	Hexanethioic acid, S-propyl ester

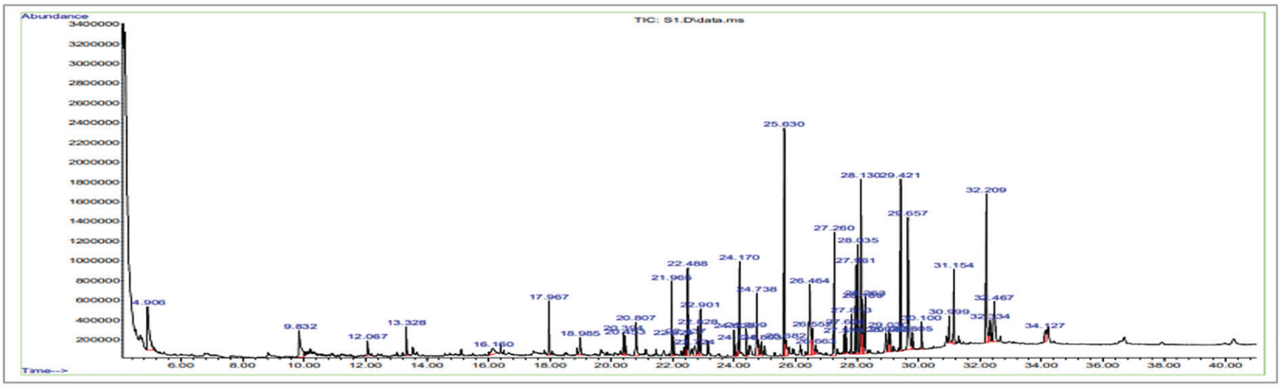

Figure 1: Chromatogram the fatty acids and active compounds in Mozzarella cheese fortified with lactic acid bacteria immediately after manufacturing.

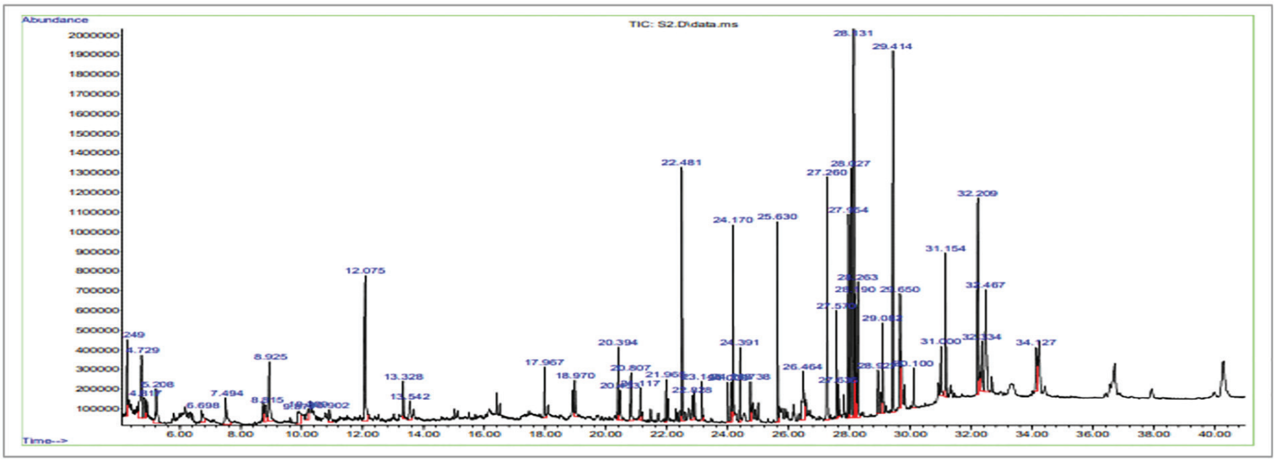

Figure 2: Chromatogram of the fatty acids and active compounds in Mozzarella cheese fortified with lactic acid bacteria after 10 days of manufacturing.

The fatty acids and active compounds in the manufactured Mozzarella cheese made from buffalo milk were diagnosed by GC-MS. There was variability in these compounds and their concentrations based on the differences in the activity of the starter cultures of lactic acid bacteria used to fortified the Mozzarella cheese, as well as the duration required for fermentation, This led to variations in the cheese's characteristics evidenced by the appearance of several acids with varying concentrations. Table 3 shows the fatty acids and active compounds, and Figure 1 presents the acids and compounds in Mozzarella cheese immediately after production. The findings showed that the compound with the highest concentration was tri butyl acetyl citrate, with an Area% concentration of 9.5822%, while the compound with the lowest concentration was 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, with an Area% concentration of 2.2797%. Additionally, several compounds were noticed at different concentrations. Table 3 refers to fatty acids and active compounds, and Figure 2 reveals the acids and compounds in Mozzarella cheese after 10 days of production. The results demonstrated that the highest concentration of compounds was 1-propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl with a concentration of 10.5025% area. Many other compounds, including butyl citrate and cholesterol appeared at different concentration in terms of area percentage.

Table 4 shows the results of the microbiological analysis of Mozzarella cheese fortified with lactic acid bacteria, including the total bacterial count and the counts of proteolytic and lipolytic bacteria. No significant differences in the total bacterial count and the counts of proteolytic and lipolytic bacteria were visible at a significance level of $p \leq 0.05$. The total bacterial count and the counts of proteolytic and lipolytic bacteria increased when the storage period was extended. The respective logarithmic values of the mentioned bacterial count at the beginning of the storage period were 5.20, 1.50, and 1.43 CFU/g. The numbers increased after 10 days of storage to 5.74, 1.96, and 1.78 CFU/g at a relative humidity of 50±1%.

Table 5 shows the total counts of *L. bulgaricus* and *S. thermophiles* in Mozzarella cheese at four storage periods. The results of the statistical analysis indicated no significant differences for both types of lactic acid bacteria at a significance level of $p \leq 0.05$. The bacterial counts increased with longer storage periods. Immediately after production, the count was 2.12 and 2.22 CFU/g and after 10 days, they reached 2.80 and 2.81 CFU/g, respectively.

Table 4: Microbiological analysis in lactic acid bacteria fortified Mozzarella cheese during storage period.

Storage period/day	Total count of bacteria CFU/mL	Proteolytic bacteria count	Lipolytic bacteria count
0	5.20±0.007^{a}	1.50±0.007^{a}	1.43±0.006^{a}
4	5.22±0.007^{a}	1.62±0.007^{a}	1.50±0.006^{a}
7	5.25±0.007^{a}	1.88±0.007^{a}	1.60±0.006^{a}
10	5.74±0.007^{a}	1.96±0.007^{a}	1.78±0.006^{a}

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability (p<0.05). Values were expressed as mean±SD of three replicates.

Table 5: Total counts of lactic acid bacteria in Mozzarella cheese fortified with lactic acid bacteria during the storage period.

Storage period/day	Total count of bacteria *Lactobacillus bulgaricus* CFU/g	Total count of bacteria *Streptococcus thermophiles* CFU/g
0	2.12±0.007^{a}	2.22±0.007^{a}
4	2.53±0.007^{a}	2.54±0.007^{a}
7	2.72±0.007^{a}	2.74±0.007^{a}
10	2.80±0.007^{a}	2.81±0.007^{a}

*Different letters indicate the presence of significant differences, and similar letters indicate no significant differences between the treatments at the level of probability (p<0.05). Values were expressed as mean±SD of three replicates.

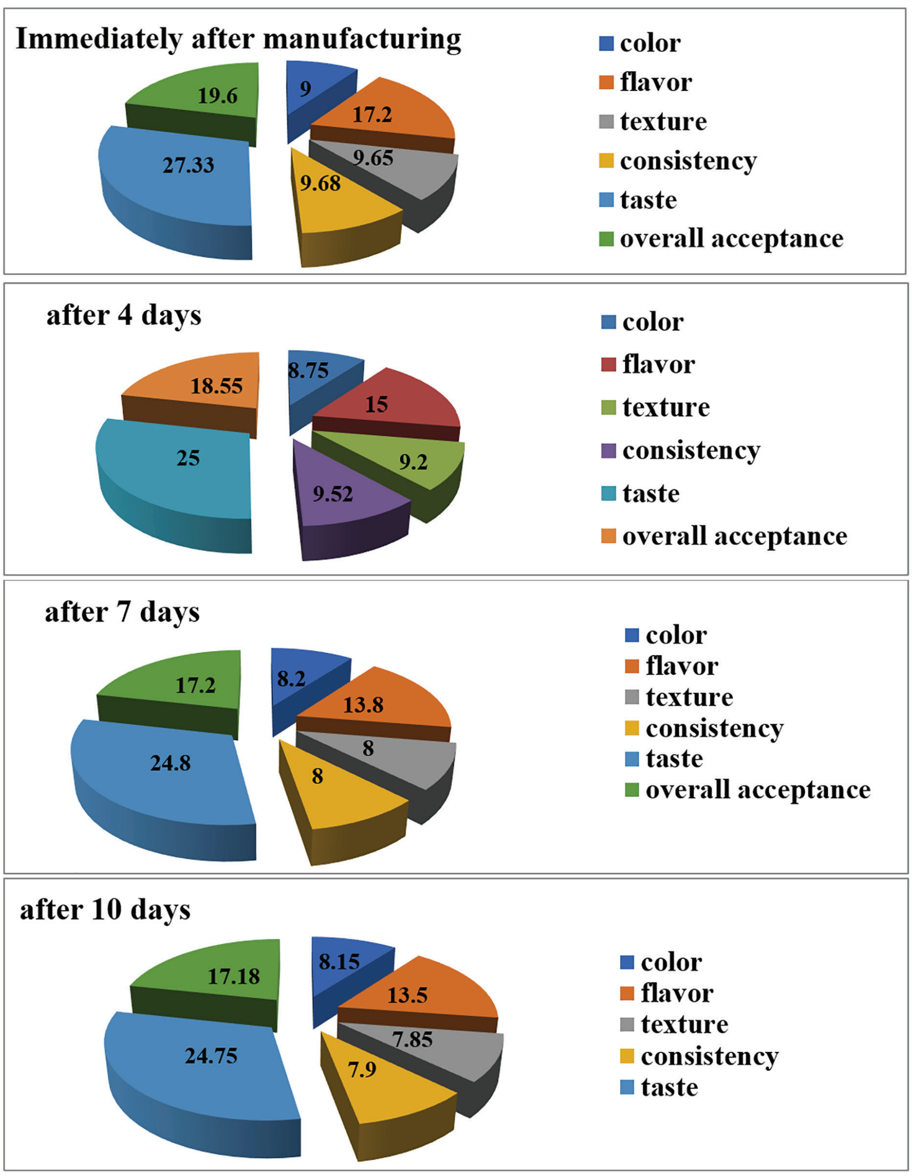

Figure 3: Sensory evaluation of Mozzarella cheese fortified with lactic acid bacteria during the storage period.

Figure 3 shows the sensory evaluation results of Mozzarella cheese revealing that Mozzarella cheese outperformed in terms of overall acceptance, flavor, and uniform color. It also obtained high scores for taste, texture, and consistency due to the high-fat content and other solids in buffalo milk.

Discussion

A decrease in Ph was observed in our study. The reason for the decrease in pH and increase in acidity can be due to the manufacturing conditions, such as cooking temperature, curd washing, and pressure. The washing process affects the reduction of lactose, ash, and minerals content, as well as the production of lactic acid during fermentation, which increases acidity and decreases pH (20-22). Ayesha et al. pointed out that the acidity of cheese is affected by the milk source, the fat content in the milk used for manufacturing, the type of lactic acid bacteria used, and the ripening period. It was noticed that the acidity is not affected by the milk source, whether it is from buffalo, cow, or the fat content. However, the acidity is influenced by the type of starter used in the manufacturing process and increases with increasing ripening period due to moisture's impact on lactic acid production (23).

An increase in fat content was visible in our research. The reason for the increase in fat content in cheese can be the reduction in moisture content compared to the milk, and it depends on the level of protein degradation during heating, allowing the fat to accumulate. The reason for the difference in moisture content is related to the temperature and manufacturing process of Mozzarella cheese, as well as the type of acidic materials used to lower the pH and ferment the cheese, resulting in the formation of a dry protein layer on the surface of the cheese that prevents moisture from escaping, and leads to an increase in moisture content and ash (24, 25). It was shown that using buffalo milk in the production of Mozzarella cheese can cause the moisture, protein, and fat content to reach 47.49%, 28.56%, and 17.91%, respectively. In comparison, using a mixture of cow and buffalo milk in a ratio of 1:1, the component ratios were 48.76%, 27.89%, and 17.37%, respectively. The type of lactic acid bacteria starter used in the production also could affect the component ratios. When traditional starters were used, the component ratios were 48.40%, 28.07%, and 17.47%, respectively, and when commercial starters were used, the component ratios were 47.85%, 28.39%, and 17.56%, respectively (23).

There are many by-products of lipid hydrolysis through initiators or heating that enhance flavor, such as alcohols, aldehydes, acetals, carboxylic acids, and esters. Fermentation was shown to play a significant role in the development of the aroma of Mozzarella cheese (26). It was found that Mozzarella cheese contains lower amounts of secondary metabolites, such as short-chain carboxylic acids like acetic, propionic, and nonanoic acids, and palmitic acid, due to metabolic, microbiological, thermal treatments, and initiators differences (27). Feeding animals with green forage was demonstrated to increases long-chain fatty acids and aldehydes in milk, and lead to an enhanced flavor. Some of these compounds include alcohols like 2-Ethyl-1-hexanol and 1-Butanol, aldehydes like Hexanal and Nonanal, esters like propyl acetate and ethyl butanoate, and fatty acids like acetic acid and tetra decanoic acid (28).

It was shown that active peptides, organic acids, and spicy fats are bioactive and these bioactive compounds are responsible for antimicrobial activities such as pyrrole (1,2-a) pyrazine-1,4-dione, ethanol, threitol, phenol, cycloheptane, benzoic, dichloroacetic, undecanoic, and succinic acids which act as active antimicrobial agents (29). The reason for the decrease in bacterial counts in Mozzarella cheese made from buffalo milk in comparison to Mozzarella cheese made from cow milk is attributed to the low moisture content and this low moisture content impacts bacterial growth (11). The increased maturation of Mozzarella cheese during storage was demonstrated to be due to the proteolytic activity of the used probiotic bacteria (30). These bacteria produce lipolytic microorganisms that are responsible for the hydrolysis of triglycerides and the production of free fatty acids, which can later lead to flavor development or cheese spoilage (31). It was found that microorganisms have an impact on the physicochemical and sensory properties of stored Mozzarella cheese at a temperature of 4°C for 30 days. This can lead to an increased level of free fatty acids and acidity and result in development of a pungent odor, an increased bitterness, a color change, and the texture alteration (32).

The decrease in lactic acid bacterial counts in Mozzarella cheese is attributed to the heat treatment during production, while the counts increase with longer storage periods (30). Lactic acid bacteria have health benefits, as fortifying Mozzarella cheese with *S. thermophiles* and probiotic bacteria can improve sensory, physical, and chemical characteristics (33). Lactic acid bacteria produce enzymes that enhance protein degradation and increase the production of active peptides and amino acids; while protein degradation increases with longer storage periods (34).

The increase in sensory acceptance of Mozzarella cheese is attributed to proteolysis in cheese, the fat content in the milk used in manufacturing, and the use of starters and coagulants that improve the cheese's texture, decrease the product's hardness to proteolysis and the nature of microorganisms used in coagulation, and finally cause the hydrolysis to increase. Also, the interaction between sugars and proteins in cheese enhances the structure, stability, and sensory characteristics of the product (30, 35). It was shown that fat content can affect the sensory properties of Mozzarella cheese, as the hardness decreases by using milk with a fat content of 2.5% to 6.50 when compared to using milk with a fat content of 1.5%, which reached 6.73. The smoothness of the product increased with an increase in fat content and reached 5.99 and 5.81 for the mentioned fat percentages of milk, respectively (23). It was found that color, acceptance, and texture values for the control sample immediately after production were 55.23, 8, and 7.33, respectively. These values decreased with increased storage period, and reached 47.23, 6.96, and 5.33 after 90 days, respectively (8).

Conclusion

Mozzarella cheese is considered a major source of proteins and essential fatty acids. It has a higher content of saturated fatty acids and is a primary source of essential fatty acids that have beneficial effects on health. Iraqi buffalo milk was shown to be one of the best sources of milk used in the production of Mozzarella cheese due to its high content of solids and fatty acids, which transfer to the cheese or are influenced by the fortification with lactic acid bacteria. This fortification could improve the sensory, nutritional, and technological qualities of the product, as well as the production of other fatty acids and active compounds with functional properties, which gave the product a physicochemical composition and microbial content, as well as sensory acceptance, to meet consumer preferences. Consequently, it enhanced the value of local products, promoted the dairy sector, and finally supported the growing trend of consuming healthy food.

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Finding

No fund was received for this study.

Authors' Contribution

NHA: Suggested a title of the research, sample preparation. RSA: Laboratory methodology, statistical analysis and writing the manuscript. RRA: Analyzed data, statistical analysis, and laboratory methodology.

Conflict of Interest

The authors have no conflict of interest.

References

1 Di Paolo M, De Stefano M, Polizzi G, et al. Effect of a new sustainable cooling system used during firming and brining on the microbiological, chemical, and sensory characteristics of buffalo mozzarella cheese. *Ital J Food Saf.* 2023;12:11290. DOI: 10.4081/ijfs.2023.11290. PMID: 37680315.

2 Akarca G, Atik A, Atik İ, et al. A comparison study on functional and textural properties of mozzarella cheeses made from bovine and buffalo milks using different starter cultures. *Int Dairy J.* 2023;141:105622. DOI:10.1016/j.idairyj.2023.105622.

3 Banks JM. The technology of low fat cheese manufacturing. *Int J Dairy Technol*. 2004;57:199-207. DOI:10.1111/j.1471-0307.2004.00136.x.

4 Coppola S, Blaiotta G, Ercolini D, et al. Molecular evaluation of microbial diversity occuring in different types of mozzarella cheese. *J Appl Microbiol.* 2001;90:414-420. DOI:10.1046/j.1365-2672.2001.01262.x. PMID: 11298237

5 Nasser EK, Majeed KR, Ali HI. Effect of some strains of lactic acid bacteria and their mixture on the level of fats and cholesterol in albino rats (Rattus norvegicus) male with hypothyroidism induced using carbimazole. *Basrah J Agric Sci.* 2021;34:139-146. DOI:10.37077/25200860.2021.34.1.12

6 Akhtar A, Nasim I, ud Din MS, et al. Effects of different fat replacers on functional and rheological properties of low-fat mozzarella cheeses: A review. *Trends Food Sci Technol*. 2023;139:104136. DOI:10.1016/j.tifs.2023.104136

7 Akhtar A, Khan R, Khalid N. Formulation and evaluation of functional attributes of low-fat mozzarella cheese using okra mucilage as a fat replacer. *Int J Food Sci Technol*. 2022;57:6237-6244. DOI:10.1111/ijfs.15952.

8 Gulzar N, Rafiq S, Nadeem M, et al. Influence of milling pH and storage on quality characteristics, mineral and fatty acid profile of buffalo Mozzarella cheese. *Lipids Health Dis*. 2019;18:33. DOI:10.1186/s12944-019-0976-9. PMID: 30696457.

9 Di Paolo M, Vuoso V, Ambrosio RL, et al. Role of Feeding and Novel Ripening System to Enhance the Quality and Production Sustainability of Curd Buffalo Cheeses. *Foods*. 2023;12:704. DOI:10.3390/foods12040704. PMID: 36832779.

10 Sulieman EAM, Mohamed Ali RA, Razig KAA. Production and Effect of Storage in The Chemical Composition of Mozzarella Cheese. *Sci Academic Publish*. 2012;2:21-26. DOI: 10.5923/j.food.20120203.02.
11 Fasale AB, Patil VS, Bornare DT. Process optimization for mozzarella cheese from cow and buffalo milk. *Int J Food Ferment Technol*. 2017;7:165-173. DOI: 10.5958/2277-9396.2017.00018.6.
12 Ghazal MM, Al-Hilfy NAA, Ali HI. Production of functional soft cheese and studying its chemical and sensory evaluation properties. *Basrah J Agric Sci*. 2021;34:67-83. DOI:10.37077/25200860.2021.34.1.07.
13 Association of Official Analytical Chemists A.O.A.C. Official Methods of Analysis 16th ed. Association of Official Analytical Chemists International Arligton, Virginia,U.S.A. 2008.
14 Mastovska K, Lehotay SJ. Rapid sample preparation method for LC− MS/MS or GC− MS analysis of acrylamide in various food matrices. *J Agric Food Chem*. 2006;54:7001-8. DOI:10.1021/jf061330r. PMID: 16968055.
15 Diliello LR. Methods in food and dairy microbiology. Avi. Publishing Company Inc. USA. 1982.p.142.
16 Andrews W. Manuals of food quality control, 4. Microbiological analysis. FAO Food and Drug administration, Washington , DC, USA. 1997.
17 Cempirkova R, Magda M, Jan T. Counts of psychrotrophic lipolytic bacteria in cow's raw milk samples from the aspect of technological quality. *J Agrobio*. 2009;26:113-121.
18 American Public Health Association (APHA). Standard methods for examination of dairy products, 20th edition, Washington DC, NewYork. 1989.
19 Schmuller J. Statistical Analysis with R For Dummies. John Wiley and Sons.111 , River Street, Hoboken, Canada. 2017.p.438.
20 Hammam AR, Metzger LE. Characteristics of imitation Mozzarella cheese manufactured without emulsifying salts using a combination of culture-based acid curd and micellar casein concentrate. *Food Sci Nutr*. 2023;11:4616-4624. DOI: 10.1002/fsn3.3424. PMID: 37576028.
21 Masoumi SJ, Mehrabani D, Saberifiroozi M, et al. The effect of yogurt fortified with Lactobacillus acidophilus and Bifidobacterium sp. probiotic in patients with lactose intolerance. *Food Sci Nutr*. 2021;9:1704-1711. DOI: 10.1002/fsn3.2145. PMID: 33747481.
22 Azad A, Ranjbaran AR, Zareshahrabadi Z, et al. Protective effects of the probiotic bacterium streptococcus thermophilus on candida albicans morphogenesis and a murine model of oral candidiasis. *Iran J Med Sci*. 2021;46:207-217. DOI: 10.30476/ijms.2020.82080.0. PMID: 34083853.
23 Ayesha S, Anjum FM, Nuzhat H, et al. Chemical composition and sensory evaluation of mozzarella cheese: influence by milk sources, fat levels, starter cultures and ripening period. *Pakistan J Agric Sci*. 2010;47:26-31.
24 Andreatta E, Fernandes AM, Santos MV, et al. Composition, functional properties and sensory characteristics of Mozzarella cheese manufactured from different somatic cell counts in milk. *Brazilian Arch Biol Technol*. 2009;52: 1235-1242. DOI:10.1590/S1516-89132009000500022.
25 Raquib M, Borpuzar T, Hazarika M, et al. Effect of coagulating enzymes and types of milk on the physico-chemical, proximate composition and sensory attributes of iron fortified Mozzarella cheese. *Emirates J Food Agric*. 2022;34:180-187. DOI: 10.9755/ejfa.2022.v34.i3.2839.
26 Natrella G, Gambacorta G, De Palo P, et al. Evolution of volatile compounds from milk to curd during manufacturing of mozzarella. *Mljekarstvo*. 2020;70:50-58. DOI: 10.15567/mljekarstvo.2020.0105.
27 Salzano A, Manganiello G, Neglia G, et al. A preliminary study on metabolome profiles of buffalo milk and corresponding mozzarella cheese: Safeguarding the authenticity and traceability of protected status buffalo dairy products. *Molecules*. 2020;25:304. DOI:10.3390/molecules25020304. PMID: 31940896.
28 Sacchi R, Marrazzo A, Masucci F, et al. Effects of inclusion of fresh forage in the diet for lactating buffaloes on volatile organic compounds of milk and mozzarella cheese. *Molecules*. 2020;25:1332. DOI:10.3390/molecules25061332. PMID: 32183410.
29 Sharafi H, Moradi M, Amiri S. Application of Cheese Whey Containing Postbiotics of Lactobacillus acidophilus LA5 and Bifidobacterium animalis BB12 as a Preserving Liquid in High-Moisture Mozzarella. *Foods*. 2022;11:3387. DOI:10.3390/foods11213387. PMID: 36359999.
30 Akarca G, Yildirim G. Effects of the probiotic bacteria on the quality properties of mozzarella cheese produced from different milk. *J Food Sci Technol*. 2022;59:3408-3418. DOI: 10.1007/s13197-021-05324-w. PMID: 35875239.
31 Malik R, Tahir M, Pervaiz N, et al. Flavor Development in Various Forms of Cheese. *Int*

J Curr Microbiol App Sci. 2020;9:2497-2511. DOI:10.20546/ijcmas.2020.901.284.

32 Huang X, Nzekoue FK, Renzi S, et al. Influence of modified governing liquid on shelf-life parameters of high-moisture mozzarella cheese. *Food Res Int.* 2020;159:111627. DOI:10.1016/j.foodres.2022.111627. PMID: 35940766.

33 Yerlikaya O, Ozer E. Production of probiotic fresh white cheese using co-culture with Streptococcus thermophilus. *Food Sci Technol.* 2014;34:471-477. DOI:10.1590/1678-457x.6365.

34 Oliveira MEGD, Garcia EF, Queiroga RDCRDE, et al. Technological, physicochemical and sensory characteristics of a Brazilian semi-hard goat cheese (coalho) with added probiotic lactic acid bacteria. *Sci Agric.* 2012;69:370-379.

35 Ahmed NS, Abd El-Gawad MA, El-Abd MM, et al. Properties of buffalo Mozzarella cheese as affected by type of coagulate. *Acta Scie Polon Technol Aliment*. 2011;10:339-357.