

PAPER • OPEN ACCESS

Effect of Encapsulated Bacteria *Limosilactobacillus reuteri* on the Physicochemical, Microbial, and Sensory Characteristics of Yogurt Produced from Sheep's Milk

To cite this article: Asaad SH. Atta *et al* 2025 *IOP Conf. Ser.: Earth Environ. Sci.* **1487** 012112

View the [article online](#) for updates and enhancements.

You may also like

- [Ultrasonic velocity profiling rheometry based on a widened circular Couette flow](#)
Takahisa Shiratori, Yuji Tasaka, Yoshihiko Oishi *et al.*
- [Effect of Fruit Lemon Juice Addition to The Content of Protein, Fat, Lactose and Probiotic on Soy Yogurt](#)
F M T Supriyanti, Zackiyah and N Azizah
- [Fermentation properties of yogurt with fermented broth of *Paecilomyces cicadae*](#)
Hua Xiong, Qin-Lan Guan, Ming-Fu Gong *et al.*



ECS The Electrochemical Society
Advancing solid state & electrochemical science & technology

247th ECS Meeting

Montréal, Canada
May 18-22, 2025
Palais des Congrès de Montréal

ECS UNITED

Unite with the ECS Community

**Register to
save \$\$
before
May 17**

Effect of Encapsulated Bacteria *Limosilactobacillus reuteri* on the Physicochemical, Microbial, and Sensory Characteristics of Yogurt Produced from Sheep's Milk

Asaad SH. Atta¹, Haider I. Ali² and Alaa Kareem Niamah³

¹⁻³Department of Food Science, College of Agriculture, University of Basrah, Basra City, Iraq.

¹E-mail: asaad.shameel@uomisan.edu.iq

²E-mail: haider.ali@uobasrah.edu.iq

³E-mail: alaa.niamah@uobasrah.edu.iq

Abstract. Yogurt is one of the most widely consumed dairy products worldwide. It can be produced from different types of milk. Sheep milk produced yogurt by adding encapsulated and unencapsulated *Limosilactobacillus reuteri* and starter bacteria. It was divided into four groups: yogurt is produced from a yogurt starter (T_C); yogurt is produced from the regular starter with 3% free *Limosilactobacillus reuteri* (unencapsulated) (T_f); and T_{1%} and T_{3%} are yogurt samples produced from the regular starter with 1% and 3% encapsulated *Limosilactobacillus reuteri*, respectively. During a 21-day refrigerated storage period, yogurt samples' physicochemical, microbial, and sensory characteristics were assessed. The results on the first day of storage showed that the pH values ranged from 4.51 to 5.61, while the total acidity percentage was between 0.84% and 0.98%. The control group's water-holding capacity (WHC) increased in the second treatment group (T_{3%}). In contrast, whey excretion decreased in samples T_{1%} and T_{3%} compared with samples from the control group (T_C) and T_f, after 21 days of storage. The percentage reduction in *L. reuteri* was 26.76%, 1.00%, and 0.98% for samples T_f, T_{1%} and T_{3%}, respectively. Adding encapsulated *L. reuteri* bacteria to yogurt improved the sensory properties compared with the control sample (T_C) and the sample containing free bacteria (T_f). Adding encapsulated bacteria to yogurt enhances its physicochemical properties, the viability of bacteria, and sensory qualities. The encapsulation process also decreases the rate of reduction of bacteria used as a starter for producing yogurt.

Keywords. Microencapsulation, Probiotic bacteria, Yoghurt probiotic, sheep milk, *Lim. reuteri*.

1. Introduction

Yogurt is one of the most widely consumed dairy products worldwide. It can be produced from different types of milk [1]. Yogurt is manufactured by fermenting milk with a starter mixture of lactic acid bacteria, including *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Its validity period yogurt is determined by the quality of the milk, the formation of lactic acid, and the yogurt production process [2]. Yogurt is a dairy product with a smooth texture, thick consistency, and a pleasant sour taste. It is produced through the bacterial fermentation of cow, buffalo, sheep, and goat milk using symbiotic cultures of bacteria. It typically contains species from the genera *Lactobacillus*



and *Streptococcus thermophilus*, which contribute to its specific flavor characteristics [3]. Yogurt has positive health effects, such as lowering cholesterol levels and blood pressure and reducing the risk of cardiovascular disease and diabetes [4].

The prebiotics in yogurt stimulates lactic acid bacteria, leading to increased production of volatile compounds, thus positively affecting the sensory properties[5]. The FAO and the WHO define probiotics as microorganisms that are safe for human consumption and provide health benefits when consumed in appropriate concentrations. The number of bacteria must not be less than 10^6 colony-forming units per gram or millilitre [6,7]. Lactic acid bacteria (LAB) are widely used in the preparation of fermented foodstuffs. They are increasingly being added to a growing number of food items such as cheese, yogurt, cereals, fruit, and vegetable juices [8]. The most common strains of probiotics used in food products belong to the genera *Lactobacillus* and *Bifidobacterium* [9].

Studies have shown that dairy products made from sheep's milk are suitable for delivering probiotics to the host [10]. Sheep milk has high digestibility, low sensitivity, high productivity, and high nutritional value. It contains proteins, fats, vitamins, and minerals in higher concentrations than the milk of other animals, making it a competitive product [10]. Moreover, sheep milk also provides prebiotic compounds that stimulate the growth of strains of probiotic bacteria [11,12].

Limosilactobacillus reuteri (*L. reuteri*) has been utilized in numerous fermented foods because of its capacity to release metabolic substances that enhance human health, such as reuterin. Reuterin is a fermentation byproduct of glycerine metabolism. Its function extends beyond that, as it possesses powerful antimicrobial properties and has broader effectiveness in food preservation [13]. *L. reuteri* has been identified as one of the types of bacteria belonging to the Lactobacillaceae family. It can manufacture bioactive compounds by producing vitamin B₂, which plays a crucial role in cellular metabolism [14]. It can also convert linoleic acid into conjugated linoleic acid (CLA), a fatty acid that has a wide range of health benefits, including anti-cancer and anti-inflammatory properties [15].

The current study aimed to use microencapsulated *L. reuteri* with yogurt starter in the production of probiotic yogurt from sheep milk. The study also aimed to investigate its physical, chemical, and sensory characteristics.

2. Materials and Methods

2.1. Materials

Sheep milk from the Agricultural Research Station of the College of Agriculture/University of Basra operation with laboratories in The Ministry of Science and Technology Ethical approval No. ISO 17025 was used in the production of yogurt. The isolated microencapsulated *L. reuteri* bacteria were prepared using whey proteins and Arabic gum sourced from the Dairy Chemistry and Technology Laboratory of the Department of Food Science, College of Agriculture, University of Basrah. The culture media MRS agar, MacConkey agar, and Mannitol salt agar were used to calculate the numbers of *L. reuteri* bacteria. MRS-T media, consisting of MRS agar supplemented with 0.9 mg/ml tetracycline (Sigma Company, Germany), was used. The food media was sterilized in the autoclave at 121°C for 15 min. All chemicals used in this study are of analytical grade.

2.2. The Physicochemical Properties of Milk

Sheep milk's chemical content and physical properties were estimated using the Lacto Flash device (Benny Impex Private Limited, New Delhi/ India). The total acidity was estimated by weighing 10 g of the sample (sheep milk), adding three drops of phenolphthalein reagent, and titrating until the neutralization point was reached and the pink color appeared. The percentage of total acidity, estimated based on lactic acid, was calculated using the following equation:

$$\text{Total acidity (\%)} = [(\text{Milliliters of NaOH} \times 0.1 \text{ N} \times 0.0098) \div \text{weight of sample}] \times 100$$

A device (EUTECH pH meter, Model No: pH700) was used for measuring pH, at laboratory temperature [16].

2.3. Yogurt production

The milk was heated to 90°C for 10 min and then cooled to 42°C. Next, 3% (log 8.25 CFU/gm) starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Italy, Sasco) and the encapsulated *L. reuteri* bacteria were added at rates of 0%, 1%, and 3%. The logarithm count was 9.12 CFU/gm for the T_C, T_{1%}, and T_{3%} samples. In addition, a sample containing non-encapsulated *L. reuteri* bacteria with 3% (log 9.01 CFU/gm) was added and denoted as T_f. The samples were then incubated at 42°C until the pH reached 4.6 ± 0.2. After the completion of the coagulation process; the samples were stored in a refrigerator until the required tests were conducted at specific intervals (1, 7, 14, and 21 days) [17].

2.4. Yoghurt Tests

2.4.1. Physicochemical Tests

Conducting physicochemical tests for yogurt samples during the refrigerated storage period, including measurements of total acidity, pH, and water holding capacity (WHC), was estimated according to the method described by [18]. To do this, weigh 10 gm of yogurt, centrifuge it at 5000 RPM for 15 min, and then weigh the filtrate. The value of the water binding capacity was estimated using the following equation.

$$\text{WHC}\% = 1 - (W1/W2) \times 100$$

W1 = weight of the filtrate after the centrifugation process, W2= weight of the yogurt.

Susceptibility to syneresis (STS) of yogurt samples was also estimated by placing 10 ml of the sample on filter paper (Whatman No.1). The size of the exudate was calculated after 6 h using a graduated cylinder. The following equation was applied to calculate the whey exudation rate:

$$\text{STS}\% = 1 - (V1/V2) \times 100$$

V1 = Volume of the descending sphincter. V2 = Volume of the yogurt sample [19].

2.4.2. Microbial Tests

A sequence of decimal dilutions was performed on yogurt samples using 0.01% peptone water, which was prepared by dissolving 1 g of peptone in 1 L of distilled water. Subsequently, the total count of lactic acid bacteria was determined after these samples were cultured on MRS agar media and then incubated at 37°C for 48 h under anaerobic conditions. In addition, the quantities of total coliform bacteria and Staphylococci bacteria were assessed following cultivation on MacConkey and Mannitol salt agar, respectively. The plates containing the cultures were then placed in an incubator set at 35°C for 24 h. Moreover, for the estimation of encapsulated *L. reuteri* bacteria. The selective medium MRS-T was employed to enumerate these specific bacteria, with the plates incubated at 37°C for 48 h as specified [20]. To determine the percentage of reduction in the live bacterial population, a specific formula was used for calculation.

$$\text{Reduction ratio}\% = \frac{\text{Logarithm of the number of viable bacteria at the end of the storage period} - \text{Logarithm of the number of viable bacteria at the beginning of the storage period}}{\text{Logarithm of the number of viable bacteria at the beginning of the storage period}} \times 100$$

2.4.3. Sensory Tests

The sensory assessment was conducted on yogurt samples on the first and 21st days of storage, involving a panel of 10 experts affiliated with the Department of Food Sciences at the College of Agriculture, University of Basrah. The attributes evaluated encompassed appearance, aroma, consistency, flavor, and overall likability. Ratings were assigned on a scale of 0 to 9 for each attribute [21].

2.5. Statistical Analysis

A ready-made statistical program (SPSS, 2006), version 12, was used to analyze the data, using Complete Randomized Design (CRD). The means were compared using the modified least significant difference (LSD) test at the 0.05 probability level.

3. Results and Discussion

3.1. Chemical Content of Sheep Milk

Illustrated the proportions of fat, total solids, moisture, total acidity, density, and freezing point in sheep milk. A reduction in fat and total solid percentages, alongside an elevation in moisture percentage, is observed, which is attributed to the quality of sheep milk utilized in the sector. The acidity level falls within the typical range for fresh milk, aligning with the findings of [22] regarding the chemical composition of sheep milk, which includes total solids (17.32 gm/100 gm), total protein (5.86gm/100gm), casein proteins (4.46 gm/100 gm), whey proteins (1.08 gm/100 gm), fat (7.28gm/100gm), ash (0.93 gm/100 gm), and lactose (3.41 gm/100 gm). In contrast, indicated that buffalo milk surpasses cow and goat milk in terms of total solids, fat, protein, carbohydrates, calculated energy, calcium, phosphorus, sodium, magnesium, and iron content [23]. Furthermore, goat milk exhibits a higher potassium content than cow or sheep milk, the data presented in Table (1).

The fat content is highest in sheep milk, followed by the milk of buffalo, cow, camel, goat, mother, horse, and donkey [24]. The good palatability and ease of intestinal absorption of calcium necessary for infant bone growth are attributed to the high lactose content [25]. The protein content of sheep's milk is also higher compared with the milk of buffalo, cows, goats, camels, horses, donkeys, and mother's milk. Ash has the highest percentage in buffalo milk, followed by the milk of sheep, camels, cows, goats, horses, donkeys, and mother's milk. Milk is a good source of water in the diet, as it makes up 87% of the diet. The percentage of water in donkey milk is highest, followed by the milk of mothers, horses, camels, cows, goats, buffalos, and sheep. The variation in the proportions of milk components is largely due to genetic, physiological, and nutritional factors, the time and repeated milking operations each day, and environmental conditions. Most changes in milk composition occur during lactation [26]. The highest amount of casein was observed in goat milk, while the lowest percentage of whey protein was observed in cow's milk. The content of whey protein and casein in cow's milk is similar to that of camel, buffalo, goat, and sheep milk [27].

Table 1. Chemical content of sheep milk used in manufacturing yogurt.

Milk source	Percentage of contents / 100 gm milk								Freeze point	Total acidity (%)	pH
	Protein	Fat	Lactose	Ash	Moisture	Solid non-fat	Total solids	Density gm/cm ³			
Sheep milk	5.85	6.87	3.45	0.91	82.94	10.21	17.08	1.033	- 0.63	1.13	6.61

3.2. Chemical and Physical Properties of Yogurt

The results of Table (2) showed the chemical and physical tests for yogurt made from sheep's milk, supported by the addition of encapsulated *L. reuteri* bacteria in different proportions during a storage period that lasted for 21 days at a temperature of 4°C. The total acidity value of the yogurt containing the encapsulated *L. reuteri* bacteria increased at the end of the storage period, reaching 1.21% and 1.28%, estimated as the percentage of lactic acid at concentrations T1% and T3%, respectively, compared with the total acidity value on the first day of manufacturing, which amounted to (0.84, 0.94, and 0.98%) at concentrations of 0, 1, and 3%, respectively. However, the total acidity value in the T_f sample reached 0.87% and 1.07% at the beginning of manufacturing and the end of the storage period, respectively. The reason for the increase in the acidity value, as the fermentation time increases, is due to the bacteria's consumption of carbohydrates in the media, leading to higher acidity in the encapsulated bacteria relative to the higher solids compared to the control sample (T_c), stated that increasing the concentration of non-fatty solids led to an increase in the total acidity of milk; it reached 1.18, 1.22, and 1.28% at concentrations of 12, 15, and 18%, respectively.

The encapsulation of the probiotic bacteria *L. acidophilus* ATTC-4356 with sodium alginate and carrageenan during the manufacturing and storage period; affected different yogurt parameters at a significant level ($P \leq 0.5$), whey transpiration, and total acidity increased, whereas viscosity and pH decreased during 28 days of storage [25]. Preserved encapsulation improved the viability of probiotics in prepared yogurt and the gastrointestinal tract. [29] and [30] mentioned the higher acidity of sheep's milk and the yogurt made from it compared with cow's and buffalo's milk. This is attributed to its high content of mineral salts, protein, and dissolved carbon dioxide [31]; therefore, more NaOH solution needs to be consumed during titration.

The pH value reached (4.60, 4.58, and 4.51) % at concentrations of 0, 1, and 3%, respectively, on the first day of yogurt manufacturing, while it reached (4.47, 4.45, and 4.41) % at concentrations of 0, 1, and 3%, respectively, after 21 days of storage at 4°C. The reason for the decrease in pH at concentrations of 1% and 3% is attributed to the conversion of starter bacteria and therapeutic bacteria encapsulating carbohydrates found in sheep milk, buffalo whey, and Arabic gum in the bacterial shell into lactic acid. As a result, its acidity increased compared to yogurt in the TC sample, and with the T_f sample, its pH value reached 4.61% at the beginning of manufacturing and decreased to 4.46% at the end of the storage period at 4°C. These results agreed with the findings of (1), who found that the pH values in yogurt from cow's milk, buffalo's milk, sheep's milk, and goat's milk reached 4.61, 4.68, 4.51, and 4.20, respectively. These differences in acidity value may be due to the high nitrogen content in sheep milk. The high protein content plays a major role in increasing the activity of the starter, resulting in increased acidity [32]. The increase in the number and activity of starter bacteria depends on the protein content of the growth environment, especially amino acids because some of them contain more than one carboxyl group in their structure [33]. The low pH value is due to genetically modified yogurt, to the fact that the isoelectric point of genetically modified casein is at pH 4.2. Casein proteins become more closely linked and begin to aggregate and precipitate [34].

(28) found that the pH value decreased from 4.88 to 4.43 during 28 days of storage, while the ability to bind water at concentrations of 1% and 3% reached 42.21 and 35.15%, respectively, after 21 days of storage, due to the high solids content. While the lowest WHC reached 33.17% at the end of the storage period at 0% concentration, as for the yogurt sample containing non-encapsulated T0 *L. reuteri* bacteria, the WHC reached 37.75% after 21 days of storage at 4°C. These results were consistent with those reported by (1). The WHC of yogurts made from sheep and buffalo milk is higher than that of yogurts made from cows and goats, due to the presence of a high percentage of total solids in the milk that enhances the firmness of the yogurt, because the protein content in yogurt jelly plays a major role in building the strength of the protein network. Unlike cow and goat milk, sheep and buffalo milk have higher levels of protein, which increases the ability to bind WHC water and prevents its leakage from within the bond of the protein matrix [35], this was consistent with [36], who stated that the higher the WHC value, the less whey excretion in the yogurt.

The highest percentage of STS was 83.27% in the Tc sample after 21 days of storage at 4°C; however, the lowest STS was in the encapsulated bacteria, which amounted to 68.3% and 61.85% in samples T1% and T3%, respectively. This supports our previous results on water binding ability due to high solids. These results differ from those of [1], who indicated that the STS of yogurt made from sheep's milk reached 71. This difference may be due to the addition of encapsulating bacteria to the yogurt, which increased the strength of gel formation and the cohesion of protein bonds and bridges, and reduced whey exudation. The difference in the chemical composition of the types of milk sources is responsible for the large percentage of variation in the STS of the types of yogurts, especially the percentage of protein responsible for increasing cross-linking in the protein network. Cross-linking in the protein network and water cross-linking increases, which reduces whey separation, which was consistent with what was found by [37]; this supports our results due to the high protein content of the encapsulated probiotic bacteria added to the yogurt. Therefore, (1) found that the amount of separated whey was less for each of the curd samples (sheep, buffalo) compared with the curd samples (goats, cows) because of the high total solids content in both sheep and buffalo milk [38]. Reducing STS is an important advantage of yogurt [39].

The viability and stability of *L. reuteri*, whether free or microencapsulated, were evaluated under simulated stomach conditions. Results revealed a rapid decline in ME % for free cells, whereas

encapsulated cells with whey proteins and gum Arabic displayed higher stability (Figure 1). The ME % of free cells decreased from 100.00% to 58.96% after an exposure period of 240 minutes. In the case of microencapsulation with whey proteins and gum Arabic decreased from 100.00% to 82.11%. For probiotic bacteria to provide the desired effects, cell survival in the stomach and intestinal environments is essential. The findings of this investigation are consistent with those of Frakolaki et al. [17], who demonstrated that the use of polymers in probiotic bacteria microencapsulation contributes to the protection and maintenance of the intended viability of probiotic bacteria in acidic environments. However, to express probiotic activity as best as possible, these bacteria need to survive the circumstances of the gastrointestinal tract (GI) in large quantities, reaching a count of 10^6 – 10^7 CFU/g or mL at the end of the product's shelf life. The bacterial cells are microencapsulated with components of proteins and polysaccharides that increase their stability and capacity to survive in low pH settings. [18].

Table 2. The chemical and physical properties of yogurt added to encapsulated bacteria during different storage periods.

Tests	Samples	Duration periods			
		1 day	7 day	14 day	21 day
Total acidity (%)	T _C	0.84	0.93	0.98	1.11
	T _f	0.87	0.94	0.96	1.07
	T ₁ %	0.94	0.97	1.15	1.21
	T ₃ %	0.98	1.13	1.19	1.28
pH	T _C	4.61	4.59	4.53	4.47
	T _f	4.61	4.55	4.50	4.46
	T ₁ %	4.58	4.52	4.48	4.45
	T ₃ %	4.51	4.48	4.44	4.41
WHC (%)	T _C	43.31	42.85	38.95	33.17
	T _f	45.45	43.23	40.16	37.75
	T ₁ %	47.90	44.45	45.70	42.21
	T ₃ %	58.40	57.2	56.75	55.15
STS (%)	T _C	71.45	73.06	75.50	83.27
	T _f	68.21	70.50	72.39	75.55
	T ₁ %	63.65	65.11	66.76	68.30
	T ₃ %	60.35	61.15	62.18	63.50

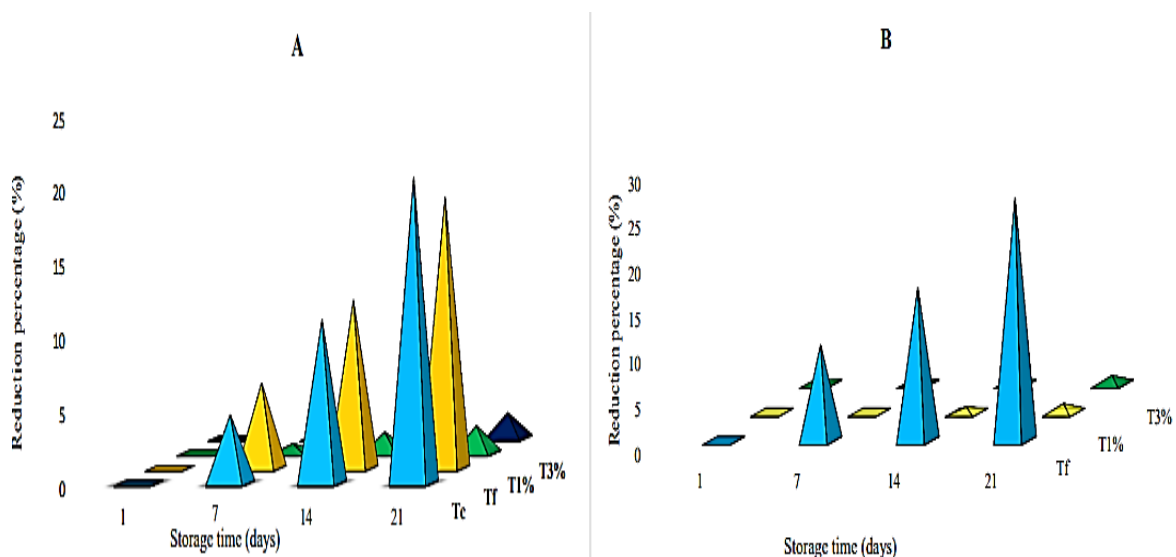
3.3. Microbial Tests for Yogurt

The results of Table (3) showed the logarithm of the live counts for lactic acid bacteria and the numbers of encapsulated and unencapsulated *L. reuteri* bacteria, coliform bacteria, and Staphylococci bacteria in samples of yogurt made from sheep's milk and during the period of refrigerated storage. On the first day of storage, the logarithm of the numbers of lactic acid bacteria reached 8.44, 9.55, 9.08, and 9.13 CFU/g for samples T_C, T_f, T₁%, and T₃%, respectively; however, the logarithm of the number of *L. reuteri* bacteria was 8.22, 8.00, and 8.13 CFU/gm for samples T_f, T₁%, and T₃%, respectively. No growth of coliform bacteria or *Staphylococci* species was observed. A decrease in the numbers of lactic acid bacteria and unencapsulated *L. reuteri* bacteria during periods of refrigerated storage. The viable counts of encapsulated *L. reuteri* bacteria in samples T₁% and T₃% showed a modest drop, but the decrease increased with increasing storage duration. The logarithm of their numbers on the last day of storage reached 7.92 and 8.05 CFU/g, respectively, whereas the logarithm of the viable counts of *L. reuteri* bacteria in the yogurt sample containing the non-encapsulated bacteria T_f was 6.02 CFU/g. No growth of coliform bacteria and *Staphylococci* species appeared at the end of the storage period. The low temperature and high acidity during the storage period harmed the number of viable cells of starter bacteria used in yogurt production. It is noted that samples T₁% and T₃% maintained the number of bacteria thanks to the careful packaging of these bacteria, which was able to preserve them in the amount required to be available in food as recommended by the Food and Agriculture Organization and the World Health Organization [6]. Stated that the recommended level of probiotic microorganisms in carrier foods ranges from (10^6 – 10^7 CFU/gm or ml) to obtain health benefits for the host.

Table 3. The logarithm of the number of bacteria in yogurt samples to which encapsulated bacteria were added and during different storage periods.

Tests	Samples	Duration periods			
		1 day	7 day	14 day	21 day
Total Lactic acid bacteria	T _C	8.44	8.05	7.51	6.68
	T _f	9.55	9.00	8.47	7.81
	T _{1%}	9.08	9.02	8.95	8.92
	T _{3%}	9.13	9.08	9.00	8.96
<i>L. reuteri</i>	T _C	-	-	-	-
	T _f	8.22	7.35	6.83	6.02
	T _{1%}	8.00	8.00	7.95	7.92
	T _{3%}	8.13	8.10	8.07	8.05
Total coliform bacteria	T _C	Nil	Nil	Nil	Nil
	T _f	Nil	Nil	Nil	Nil
	T _{1%}	Nil	Nil	Nil	Nil
	T _{3%}	Nil	Nil	Nil	Nil
<i>Staphylococci</i>	T _C	Nil	Nil	Nil	Nil
	T _f	Nil	Nil	Nil	Nil
	T _{1%}	Nil	Nil	Nil	Nil
	T _{3%}	Nil	Nil	Nil	Nil

Figure (1) shows the percentage reduction in the viable numbers of encapsulated and unencapsulated lactic acid bacteria and *L. reuteri* in yogurt samples during the refrigerated storage period. There was a significant decrease in the number of lactic acid bacteria in samples T_C and T_f. At the end of the storage period, it reached 20.58 and 18.21%, respectively, whereas it was 1.86 and 1.72 for the T_{1%} and T_{3%} samples, respectively. The percentage reduction of encapsulated and unencapsulated *L. reuteri* bacteria in yogurt samples after 21 days of refrigerated storage was 26.76%, 1.00%, and 0.98% for T_f, T_{1%}, and T_{3%} samples, respectively. The precise encapsulation of bacteria protects them from external conditions such as high acidity and low temperatures, which increases the viability of bacteria and maintains their living numbers. These results were consistent with many previous studies, which demonstrated the importance of the microencapsulation process of bacteria and therapeutic probiotics to maintain the number of bacteria within the required level in food products such as dairy products [40]. Encapsulated *L. reuteri* bacteria used in yogurt production with standard starter, which reduced the level of harmful cholesterol and the number of triglycerides [41].

**Figure 1.** Percentage reduction in the viable numbers of bacteria present in curd samples during the period of refrigerated storage, (A) lactic acid bacteria, (B) *L. reuteri* bacteria.

3.4. Sensory tests of yogurt

Figure (2) shows the results of the sensory evaluation of encapsulated and unencapsulated *L. reuteri* yogurt samples on the first and last days of refrigerated aging. The results showed that the sensory evaluation on the first day of storage showed a slight increase in the curd samples to which closed bacteria were added at concentrations of 1% and 3%. The reason may be due to the addition of *L. reuteri* bacteria to the starter bacteria, which led to the production of metabolic compounds, including flavor compounds, as a result of the decomposition of fats in milk, which gives more acceptance by consumers [41; 42]. Adding closed bacteria to curd samples increases the percentage of total solids, which improves the curd's texture and appearance. The materials used in packaging (whey proteins and Arabic gum) help bind water and reduce whey exudation. After 21 days of storage, no significant difference was observed in the characteristics of appearance, smell, taste, and general acceptability between the types of yogurt samples, these results agreed with [43] who showed that the use of encapsulated *Lactobacillus acidophilus* LA-5 bacteria in the production of yogurt, it did not affect the sensory qualities of the final product after 35 days of refrigerated storage, while [44] found that the use of encapsulated *Lacticaseibacillus paracasei* resulted in increased water binding capacity and improved rheological properties. Examination under an electron microscope showed fine pores in the texture, while these characteristics did not appear when using free (non-encapsulated) bacteria.

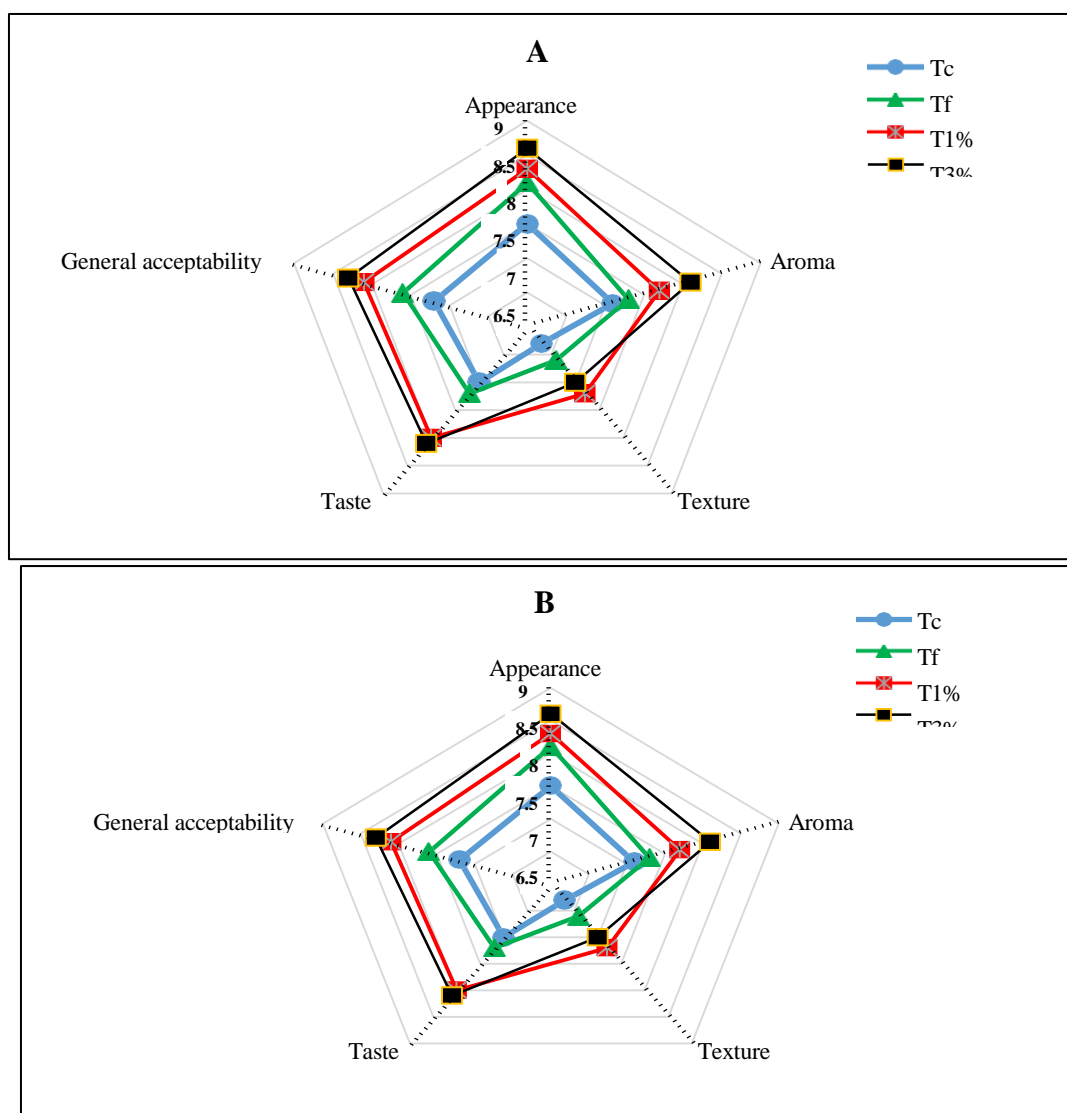


Figure 2. Sensory evaluation of yogurt samples containing encapsulated and unencapsulated *L. reuteri* bacteria, (A) on the first day of storage and (B) after 21 days of refrigerated storage.

Conclusions

Yogurt is a fermented milk product eaten in many countries. Using encapsulated bacteria as starters in the production of fermented milk improved the physicochemical, microbial, and sensory characteristics of the yogurt product. Cold storage did not affect the characteristics of the final product, which used encapsulated bacteria as a starter in its production; it increased the percentage of total solids and reduced whey exudation. The ability to bind water also increased. Bacterial viability increased during storage periods.

Funding: This research received no external funding.

Conflicts of Interest: The authors state that there is no conflict of interest.

References

- [1] Al-Bedrani, D.I.J., ALKaisy Q. H. , Rah A. K. , Saadi A. M. (2023). Evaluation of milk source on physicochemical, texture, rheological, and sensory properties of yogurts. *Journal of Applied and Natural Science*, 15(1): 128-136. <https://doi.org/10.31018/jans.v15i1.4269> .
- [2] Tropcheva, R., Georgieva, R., Paskov, V., Karsheva, M., and Danova, S. (2014). Sensory properties of Bulgarian yogurts, supplemented with lactobacilli as probiotic adjuncts. *Journal of Texture Studies*. 45(3):187- 194. <https://doi.org/10.1111/jtxs.12065>.
- [3] Cansu, D. (2017). Utilization of whey powder in the encapsulation of *Lactobacillus acidophilus* by spray drying for the production of probiotic yogurt. Thesis of the master, The graduate school of natural and applied sciences of/Middle East Technical University, Türkiye: pp143. <http://etd.lib.metu.edu.tr/upload/12621310/index.pdf> .
- [4] Visioli F., and Strata A. (2014). Milk, dairy products, and their functional effects in humans: a narrative review of recent evidence. *Advances in Nutrition*. 5(2): 131-143. Doi: 10.3945/an.113.005025.
- [5] Costa M.F, Pimentel T.C., Guimaraes J.T., Balthazar C.F., Rocha R.S., Cavalcanti R.N., Esmerino, E.A., Freitas, M.Q., Raices, R. S. L., Silva, M.C., and Cruz, A.G. (2019). Impact of prebiotics on the rheological characteristics and volatile compounds of Greek yogurt. *LWT – Food Science and Technology*. 105: 371-376. <https://doi.org/10.1016/j.lwt.2019.02.007>.
- [6] FAO&WHO (2006). Probiotics in Food. pp. 413-26. Available at: <http://www.fao.org/3/a0512e/a0512e.pdf>.
- [7] Morelli L, Capurso L(2012). FAO/WHO guidelines on probiotics: 10 years later. *J Clin Gastroenterol*.(46)Suppl:S1-2. doi:10.1097/MCG.0b013e318269fdd5 .
- [8] Gottel, M, Cires MJ, Carvallo C, Vega N, Ramirez MA, Morales P, Rivas P, Astudillo F, Navarrete P, Dubos C, et al. (2014). Probiotic screening and safety evaluation of *Lactobacillus* strains from plants, artisanal goat cheese, human stools, and breast milk. *J. Med Food*.17(4):487–95. DOI: 10.1089/jmf.2013.0030.
- [9] Sanders, M. E., & Marco, M. L. (2010). Food formats for effective delivery of probiotics. *Food Science and Technology*, 1, 65–85. DOI: 10.1146/annurev.food.080708.100743.
- [10] Balthazar C.F., Pimentel T.C., Ferrão L.L., Almada C.N., Santillo, Albenzio A. M., Mollakhalili N., Mortazavian A.M., Nascimento J.S., Silva M.C., Freitas, A.S. Sant’Ana M.Q., Granato D., Cruz A.G. (2017). Sheep Milk: Physicochemical Characteristics and Relevance for Functional Food Development. *Comprehensive Reviews in Food Science and Food Safety*; Vol(16), Issue2 ., Pages 247- 262. DOI: 10.1111/1541-4337.12250.
- [11] Balthazar CF, Silva HLA, Esmerino EA, Rocha RS, Moraes J, Carmo MAV, Azevedo L, Camps I, Abud YKD, Sant’Anna C,(2018). The addition of inulin and *Lactobacillus casei* 01 in sheep milk ice cream. *Food Chem*;246: 464-472. <https://doi.org/10.1016/j.foodchem.2017.12.002>.
- [12] Pisano MB, Deplano M, Fadda ME, Cosentino S. (2019). Microbiota of 9ardinian goat’s milk and preliminary characterization of prevalent LAB species for starter or adjunct cultures development. *Biomed Res Int*.;6131404. DOI: 10.1155/2019/6131404.
- [13] Niamah, A. K., Mohammed, A. A., & Alhelf, N. A. (2023). Antibacterial activity and identification of produced reuterin from local *Lactobacillus reuteri* LBIQ1 isolate. *Journal of microbiology, biotechnology, and food sciences*, 12(5), e4701-e4701. DOI:10.55251/jmbfs.4701.
- [14] Cansu, D. (2017). Utilization of whey powder in the encapsulation of *Lactobacillus acidophilus* by spray drying for the production of probiotic yogurt . Thesis of master, The graduate school of natural

- and applied sciences of/Middle East Technical University, Türkiye: pp143. <http://etd.lib.metu.edu.tr/upload/12621310/index.pdf>.
- [15] Hernandez-Mendoza, A, Lopez-Hernandez A, Hill CG, Garcia HS.(2009). Bioconversion of linoleic acid to conjugated Linoleic acid by *Lactobacillus reuteri* under different growth conditions. *J. of Chemical Tech and Biotech* .84:180–185. <https://doi.org/10.1002/jctb.2021>.
 - [16] Visioli F., and Strata A. (2014). Milk, dairy products, and their functional effects in humans: a narrative review of recent evidence. *Advances in Nutrition*. 5(2): 131-143. doi: 10.3945/an.113.005025.
 - [17] Tamime, A. Y. & Robinson, R. K. (2007). Tammie and Robinson's yoghurt: science and technology. Tammie and Robinson's yoghurt: science and technology., (Ed.3). DOI:10.1533/9781845692612.
 - [18] Pspacova, I., Ahannach, S., Breynaert, A., Erreygers, I., Wittouck, S., Bron, P. A., ... & Lebeer, S. (2022). Spontaneous riboflavin-overproducing *Limosilactobacillus reuteri* for biofortification of fermented foods. *Frontiers in Nutrition*, 9, 916607. DOI: 10.3389/fnut.2022.916607.
 - [19] Niamah, A.K., T.G.S. Al-Sahlaney and A.J. Al-Manhel (2016). Gum Arabic Uses as Prebiotic in Yogurt Production and Study Effects on Physical, Chemical Properties and Survivability of Probiotic Bacteria During Cold Storage. *World Applied Sciences Journal* 34 (9): 1190-1196. DOI:10.5829/idosi.wasj.2016.34.9.184.
 - [20] Niamah, A. K., Al-Manhel, A. J., & Al-Sahlaney, S. TG (2018). Effect microencapsulation of *Saccharomyces boulardii* on viability of yeast in vitro and ice cream. *Carpathian Journal of Food Science and Technology*, 10(3), 111-118.
 - [21] Igbabul, B., Shember, J., & Amove, J. (2014). Physicochemical, microbiological, and sensory evaluation of yoghurt sold in Makurdi metropolis. *African Journal of Food Science and Technology*, 5(6), 129-135. DOI: 10.12691/ajfn-6-4-4.
 - [22] Merlin, JIA, Santos JS, Costa LG, Costa RG, Ludovico A, Rego FC, Santana EH(215). Sheep milk: physical-chemical characteristics and microbiological quality. *Arch Latinoam Nutr*. Vol;65(3):193-8. <https://www.alanrevista.org/ediciones/2015/3/art-9/>.
 - [23] Luigi, L., V. Lopreiato, F. Asroosh and A. Seidavi (2022). Physicochemical and Mineral Content of Milk from Talesh Buffalos, Sheep, Goats, and Cows, Saanen Goats, and Talesh-Mediterranean Buffalos: A Comparative Analysis. *Pakistan J. Zool.*, pp 1-8. DOI:10.17582/journal.pjz/20220121140148.
 - [24] Madhusudan, N.C., Ramachandra C.T. and Mahesh K.G. (2020). A Comprehensive Review on Composition of Donkey Milk in Comparison to Human, Cow, Buffalo, Sheep, Goat, Camel and Horse Milk. *Mysore J. Agric. Sci.*, 54 (3): 42-50. <https://www.cabidigitallibrary.org/doi/full/10.5555/20203471484>.
 - [25] Dugo, P., Kumn, T., L.O.M., Chiofalo, B., Salimei, E., Fazio, A., Cotroneo, A. And Mondello, L. (2005). Determination of triacylglycerols in donkey milk by using high-performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry. *J. Sep. Sci.*, 28 (9-10): 1023 - 1030. DOI: 10.1002/jssc.200500025.
 - [26] Kalyankar, S.D., Khedkar, C.D., Patil, A.M. and Deosakar, S.S., (2016). Milk : Sources and Composition. In : Caballero, B., Finglas, P. and Toldra, F. (Ed.). *The Encyclopedia of Food and Health*, pp: 741 - 747. https://www.researchgate.net/publication/301216713_Milk_Sources_and_composition.
 - [27] Abodullahi, A., (2019). Camel Milk - A Review. *J. Animal Sci. & Livestock Prod.*, 3 (1:5): 13-18.
 - [28] Afzaal M, Khan AU, Saeed F, Ahmed A, Ahmad MH, Maan AA, Tufail T, Anjum FM, Hussain (2019). Functional exploration of free and encapsulated probiotic bacteria in yogurt and simulated gastrointestinal conditions. *Food Sci Nutr* ;7:3931– 3940. <https://doi.org/10.1002/fsn3.1254>.
 - [29] Domagala, J. (2009). Instrumental texture, syneresis and microstructure of yoghurts prepared from goat, cow and sheep milk. *International Journal of Food Properties*, 12(3), 605-615. <https://doi.org/10.1080/10942910801992934>.
 - [30] Erkaya, T. & Şengül, M. (2012). A Comparative Study on Some Quality Properties and Mineral Contents of Yoghurts Produced From Different Type of Milks. *Kafkas Universitesi Veteriner Fakültesi Dergisi*, 18(2). DOI:10.9775/kvfd.2011.5498
 - [31] Salaün, F., Mietton, B. & Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal*, 15 (2), 95-109. <https://doi.org/10.1016/j.idairyj.2004.06.007>.
 - [32] Urbach, G. (1995). Contribution of lactic acid bacteria to flavor compound formation in dairy products. *International Dairy Journal*, 5(8), 877-903. [https://doi.org/10.1016/0958-6946\(95\)00037-2](https://doi.org/10.1016/0958-6946(95)00037-2).
 - [33] Güler-Akın, M. B. & Akın, M. S. (2007). Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bioyogurt made from goat's milk. *Food Chemistry*, 100(2), 788-793. <https://doi.org/10.1016/j.foodchem.2005.10.038>.

- [34] Espírito-Santo, A. P., Lagazzo, A., Sousa, A. L. O. P., Perego, P., Converti, A. & Oliveira, M. N. (2013). Rheology, spontaneous whey separation, microstructure, and sensorial characteristics of probiotic yogurts enriched with passion fruit fiber. *Food Research International*, 50 (1), 224-231. <https://doi.org/10.1016/j.foodres.2012.09.012>.
- [35] Al-Bedrani, D. I., ALKaisy, Q. H. & Mohammed, Z. M. (2019). Physicochemical, rheological, and sensory properties of yogurt flavored with sweet orange (*Citrus sinensis*) marmalade. In *IOP Conference Series: Earth and Environmental Science* (Vol. 388, No. 1, p. 012052). DOI 10.1088/1755-1315/388/1/012052.
- [36] Kim, S. Y., Hyeonbin, O., Lee, P. & Kim, Y. S. (2020). The quality characteristics, antioxidant activity, and sensory evaluation of reduced-fat yogurt and nonfat yogurt supplemented with basil seed gum as a fat substitute. *Journal of Dairy Science*, 103(2), 1324-1336. <https://doi.org/10.3168/jds.2019-17117>.
- [37] Vital, A. C. P., Goto, P. A., Hanai, L. N., Gomes-da-Costa, S. M., de Abreu Filho, B. A., Nakamura, C. V., & Matumoto-Pintro, P. T. (2015). Microbiological, functional, and rheological properties of low-fat yogurt supplemented with *Pleurotus ostreatus* aqueous extract. *LWT-Food Science and Technology*, 64(2), 1028-1035. DOI:10.1016/j.lwt.2015.07.003.
- [38] Ibrahim, D., & Doosh, K. S. (2017). Physicochemical and sensorial properties of low energy yogurt produced by adding whey protein concentrate. *Iraq journal of agricultural research*, Volume 22, Issue 5, Pages 125-142..
- [39] Gilbert, A., Rioux, L. E., St-Gelais, D. & Turgeon, S. L. (2020). Characterization of syneresis phenomena in stirred acid milk gel using low frequency nuclear magnetic resonance on hydrogen and image analyses. *Food Hydrocolloids*, 106, 105907. <https://doi.org/10.1016/j.foodhyd.2020.105907>.
- [40] Niamah, A. K., Al-Sahlaney, S. T. G., Ibrahim, S. A., Verma, D. K., Thakur, M., Singh, S., & Utama, G. L. (2021). Electro-hydrodynamic processing for encapsulation of probiotics: A review on recent trends, technological development, challenges , and future prospect. *Food Bioscience*, 44, 101458. DOI:10.1016/j.fbio.2021.101458.
- [41] Jones, M. L., Martoni, C. J., Parent, M., & Prakash, S. (2012). Cholesterol-lowering efficacy of a microencapsulated bile salt hydrolase-active *Lactobacillus reuteri* NCIMB 30242 yogurt formulation in hypercholesterolaemic adults. *British Journal of Nutrition*, 107(10), 1505-1513. DOI: 10.1017/S0007114511004703.
- [42] Al-Sahlaney, S. T., & Niamah, A. K. (2022). Bacterial viability, antioxidant stability, antimutagenicity and sensory properties of onion types fermentation by using probiotic starter during storage. *Nutrition & Food Science*, 52(6), 901-916. <https://doi.org/10.1108/NFS-07-2021-0204>
- [43] Ribeiro, M. C. E., Chaves, K. S., Gebara, C., Infante, F. N., Grosso, C. R., & Gigante, M. L. (2014). Effect of microencapsulation of *Lactobacillus acidophilus* LA-5 on physicochemical, sensory and microbiological characteristics of stirred probiotic yoghurt. *Food Research International*, 66, 424-431. <https://doi.org/10.1016/j.foodres.2014.10.019>.
- [44] Li, H., Liu, T., Yang, J., Wang, R., Li, Y., Feng, Y., & Yu, J. (2021). Effect of a microencapsulated synbiotic product on microbiology, microstructure, textural and rheological properties of stirred yogurt. *Lwt*, 152, 112302. <https://doi.org/10.1016/j.lwt.2021.112302>.