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Symbiotic microencapsulation to enhance *Bifidobacterium longum* and *Lactobacillus paracasei* survival in goat cheese

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Abstract

In the study, the microencapsulation method was applied in the production of white cheese from goat milk to prevent the loss of viability of *Lactobacillus paracasei* and *Bifidobacterium longum* due to environmental factors during their storage period. For this purpose, the cheese was obtained using three types of microcapsules - symbiotic microcapsules containing probiotic bacteria and probiotic + fructooligosaccharide, probiotic + inulin. In addition, probiotics, fructooligosaccharide and inulin have been added to cheese in free form, and a total of 7 different cheeses have been produced with the control sample. In the microencapsulation, 1% sodium alginate was used as coating material and the extrusion method was applied. The cheese samples have been stored at +4 ^{0}C for 180 days. It has been determined that the viability of *L. paracasei* and *B.longum* has been preserved with the microencapsulation technique, while the viability of probiotic bacteria is preserved at a higher level in cheese samples obtained with the addition of prebiotics to microencapsules. It has been concluded that probiotic microorganisms are available above the minimum therapeutic effect value in grams of cheese samples containing microcapsules and these cheese could be considered as probiotic.

Keywords: symbiotic microcapsules; *Bifidobacterium longum*; *Lactobacillus casei*; goat cheese; viability.

Practical Application: Microencapsulation technique used in our study significantly increases the viability levels of probiotic microorganisms in goat cheese.

1 Introduction

The importance given to goat milk and its products, which are distinguished technologically from cow and sheep milk and are economically more valuable because of their different taste, aroma and quality, has been recently increased. Goat milk is mostly used in cheese production among dairy products (Ranadheera et al., 2018, 2019; Herman-Lara et al., 2019; Lucatto et al., 2020). Probiotic microorganisms are considered as functional food components that have positive effects on human health (Guarner, 2017). Prebiotics, which are defined as non-digestible food ingredients, which positively affect human health by promoting the development and activity of beneficial bacteria in the large intestine, have been the source of many studies on the development of new fermented dairy products in the dairy industry. As a result of the symbiotic products produced by using probiotics and prebiotics together, the beneficial effects of probiotics on human health are supported by prebiotics. If probiotic microorganisms are consumed together with prebiotics, they can stay viable longer and provide more benefits for metabolism (Sanders et al., 2018). Although the studies conducted on the physiological effects of probiotic microorganisms have determined that the dose required for the preventive effect against diarrhea, lactose intolerance and colon cancer is 10^9–10^{10} cfu/g, the International Dairy Federation (IDF) has reported this rate as a minimum of 10^7 cfu/g live cells and has stated that foods defined as probiotic should contain at least the specified ratio of live bacteria (International Dairy Federation, 2008; Guarner, 2017).

In recent years, the microencapsulation method has been applied in order to increase the viability of probiotic microorganisms and to prevent the product from being affected by the compounds formed during the storage process (Rezaei et al., 2019; Menezes et al., 2019; Pivetta et al., 2020). Microencapsulation is defined as "[...] the coating of solid, liquid or gaseous materials as micro particles with a protein or carbohydrate-based material or both selected as coating material" (Čakarević et al., 2020). In recent studies, symbiotic microcapsules have been studied by adding prebiotics to the coating material during microencapsulation in order to increase probiotic viability and success has been achieved in this field. For this purpose, the studies have widely used prebiotics such as fructooligosaccharide, inulin, Hi-maize starch, isomaltooligosaccharides (Shori et al., 2018). In the light

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of these information, the study has aimed to obtain Symbiotic microcapsules by adding prebiotics (fructooligosaccharide and inulin) to the coating material, which encourage the viability and development of probiotic bacteria, and consequently to increase the viability of probiotic bacteria. Besides, with the addition of probiotic bacteria, microencapsulated probiotic bacteria and prebiotics to cheese in free form, the study has aimed to add microencapsulated symbiotic microcapsules to goat cheese separately and to compare the viability of cheese during production and storage.

2 Materials and methods

Goat milk cheese has been produced at Şemsi İgi Food product Ltd. Şti. (Pınarbaşı, Izmir, Turkey). *Lactococcuslactis* ssp. *lactis* + *Lactococcuslactis ssp. cremoris (*Mayasan-Sacco) as rennet and Lafti B22 *Bifidobacterium longum (B.longum)* and Lafti L26 *Lactobacillus paracasei (L.paracasei)* (DSM) as probiotic microorganisms (ratio in the mixture: 50%) have been used. Sodium alginate (1% solution) from natural polymers has been used in microencapsulation. In addition, 1% peptides (pancreatic digestible casein) have been added to promote the growth with fructooligosaccharide as a prebiotic into microcapsules, and 0.1 M $CaCl_2$ solution has been used as hardening agent.

2.1 Activation of cheese cultures

Cultures in lyophilised (freeze-dried) form transferred to MRS broth (Fluka Chemie GmbH, Buchs, Switzerland) under aseptic conditions have been reproduced at 37 °C and activated at least twice. Following the activation process, the cultures have been incubated at 37 °C for 1 night under anaerobic conditions within milk, which have been prepared from skimmed milk powder with 12% (w/v) dry matter and sterilized at 110 °C for 15 minutes. Then, they have been used in the cheese extractions. Before using the microorganisms in cheese production, the count of bacteria per mL of the cultures has been determined.

2.2 Preparation of probiotic microorganism cultures

In order to obtain probiotic bacteria in a certain number before microencapsulation and to prepare cell suspension, *B.longum* has been passaged to MRS broth containing 0.05% L-cysteine hydrochloride and incubated at 37 °C for 3 days under anaerobic conditions, and *L.paracasei* has been passaged to MRS broth (Fluka Chemie GmbH, Buchs, Switzerland) and incubated a 37 °C for 2 days under anaerobic conditions. This procedure has been performed 2 times. Following the incubation, the cultures centrifugated at 3000xg 4 °C' for 15 minutes have been washed in sterile peptone (0.1g/100g) solution or saline solution. The final concentration of probiotic microorganisms has been set to be a minimum of 10^9/mL.

2.3 Microencapsulation of probiotic microorganisms

Microcapsules have been prepared using the extrusion method in 3 different types. In this method, for Type 1 microcapsule extraction, 1% of probiotic culture mixture (*L. paracasei* + *B. longum*) concentrate has been pre-autoclaved (for 15 minutes at 121 °C) and mixed with 1% sodium alginate. For Type 2 microcapsule extraction, fructooligosaccharide (FOS) has been added to the Na-alginate + culture mixture. For Type 3 microcapsule extraction, inulin has been added to the Na-alginate + culture mixture. The most efficient FOS and inulin ratio, determined by Chen et al. (2005) was taken as reference, and the rate of prebiotics to be added was determined to be 2% in the preliminary trial. In addition, 1% digestible autoclaved pancreatic casein has been added to improve the development of probiotics. The resulting mixture has been slowly dropped into sterile 0.1 M $CaCl_2$ solution using injectors to obtain microcapsules. The distance between the recommended needle tip and the beaker where the $CaCl_2$ solution is found (10 cm) has been taken into consideration (Chen et al., 2006). After waiting for gelification process for 1 hour, the capsules were stored in a sterile 0.1% peptone solution at 4 °C.

2.4 Cheese production

In the cheese production process, goat milk was pasteurized at 75 °C for 15 seconds, cooled to 36 °C and divided into seven equal groups. In each group, 0.5% cheese culture, prepared from 0.01% $CaCl_2$ and sterilized skimmed milk powder and activated in milk was added. The cheese with SHPK code has been produced by adding the probiotic cultures (1%) (*L paracasei and B. longum*, 1/1) in cheese milk at 35-37 °C for the production of cheese containing probiotic culture in free form; The cheese with SHPK+F code has been produced by adding 2% FOS as well as the probiotic cultures for the production of prebiotic-added cheese;The cheese with SHPK+I code has been produced by adding 2% inulin as well as the probiotic cultures; The cheese with MKP code has been produced by adding only probiotic culture microencapsulated with alginate (1%);The cheese with MKP+F code has been produced by adding microcapsules containing FOSs as well as alginate as coating material, and the cheese with MKP+I code has been produced by adding microcapsules containing inulin as well as alginate as coating material. K-coded cheese is traditional cheese which is obtained with no probiotic culture additives.

Following the addition of probiotics and prebiotics, the milk has been added with rennet, coagulated and pressured. The cheese clots have been kept under pressure for approximately 90 minutes. After releasing the pressure and cutting, they were kept in 15% (w/v) pasteurized brine for approximately 180 minutes. The cheese samples have been subjected to pre-ripening in the cases until the pH value is approximately 5.0-5.1, and have been packaged under vacuum in 0.5 kg units. The containers have been subjected to ripening for 180 days at 4 ± 1 °C. The microbiological analyzes have been performed to examine the viability of probiotic bacteria at days 1, 15, 30, 45, 60, 75, 90, 120, 150 and 180.

2.5 Determination of the position of microcapsules in the cheese matrix

The microcapsules obtained in the research were added to the milk before the fermentation process, and their positions in the cheese matrix have been determined on the first day and in certain periods of the ripening period. The cheese samples cut

in the size of 4-5 mm^3have been kept in 2.8% w/v glutaraldehyde solution for 1 day and washed with distilled water, and this procedure has been repeated 10 times. Following 2 additional fixation procedures for 30 and 60 minutes, the samples have been kept in 20%, 40%, 60%, 80%, 95% and 100% ethanol solution for at least 2 hours and oil has been extracted from them using chloroform solution at intervals of 15 minutes, and the cheese samples have been stored at -18 °C until analyzed (Sipahioğlu et al., 1999). The positions of the samples in the cheese matrix have been determined using Hitachi TV 1000 Table Top Microscope (Japan).

2.6 Probiotic microorganisms and cheese culture count

The *B.longum* count was measured in NPNL-MRS agar medium containing nalidixic acid, paromycin sulfate, neomycin sulfate and lithium chloride as inhibitory agents. After placing on the petri plates, the NPNL mixture, sterilized using a 0.45 µm single-use sterile filter (Sartorius Minisart® - Sartorius AG, Goettingen, Germany), in an amount of 20% (volume/volume) in the final composition of the medium has been added to sterile MRS agar (Merck KGaA, Darmstadt, Germany) medium. The petri plates have been incubated in anaerobic jars (Merck KGaA, Darmstadt, Germany) for 72 hours at 37 °C. The anaerobic medium was ensured using Anaerocult® A (Merck KGaA, Darmstadt, Germany) anaerobic kits. The *L. paracasei* count has been performed using the MRS agar (Merck KGaA, Darmstadt, Germany) growth medium (Gardiner et al., 2002). The samples have been incubated at 37 °C for 72 hours under anaerobic conditions.The Lactococcus (used as a starter culture) count has been performed using M 17 agar (Merck KGaA, Darmstadt, Germany) medium. The samples have been incubated at 37 °C for 48 hours.

2.7 Statistical analysis

Cheese samples were studied with two replications and three parallels, and the analysis of variance (ANOVA) was performed. The data were analyzed using SPSS version 15. Data from ANOVA were considered significant at $p < 0.05$ level based on the Duncan's multiple comparison test.

3 Results and discussion

3.1 The appearance of probiotic microcapsules and symbiotic microcapsules obtained by extrusion method in cheese mass

In our study, there was no difference between the culture type and microencapsulation method in terms of matrix structure of the cheese produced. Although the observed matrix structure showed slight differences during the storage period depending on the saline ratio, storage temperature and the water ratio in the mass, the mass maintained the matrix structure in which the casein micelles and its other components are being bound (Fangmeier et al., 2019). In cheese samples to which probiotic bacteria have been added in free form, it has been determined that they attach to the outer surfaces of the matrix, and microcapsules containing probiotic bacteria are capsular in the matrix structure (Figure 1). Similar results have been obtained in the studies by Madureira et al. (2005) and Rama et al. (2020).

3.2 Viability of Lactobacillus paracasei used as probiotic in cheese during the ripening period

The change of viability of *L. paracasei* in probiotic culture, prebiotic + probiotic culture, microencapsulated probiotic culture and goat cheese produced using symbiotic microcapsules during the ripening period has been given in Table 1.

The cheese sample with the highest viability on average during 180-day ripening period of *L. paracasei* was MKP+F, followed by MKP+I, MKP, SHPK+F, SHPK+I and SHPK cheese samples. The statistical analysis has shown that the viability of *L. paracasei* during 180-day ripening period was insignificant ($p > 0.05$) in the SHPK, SHPK+F and SHPK+I samples, but the difference of these samples from the MKP, MKP+F and MKP+I samples was significant ($p < 0.05$).

The decrease in the count of probiotic bacteria in the cheese matrix during the ripening period has been obtained in similar studies as well. It has been stated that the main reason for this was associated with the increase in saline concentration in the cheese matrix during the ripening period (Castro et al., 2015; Evangelia et al., 2016; Moraes et al., 2018).

A study examining the viability of *L. paracasei* NFBC 338 strain in cheddar cheese during a 6-month ripening period has reported that the viability of the bacterium is at a high level and the relevant bacterium maintains its viability at the level of 10^8cfu/g during the ripening period (Gardiner et al., 2002).

In the MKP cheese samples containing microencapsulated *L. paracasei*, the count of *L. paracasei* has decreased from 10.36 $\log_{10}$ cfu/g at the beginning to 9.75 $\log_{10}$ cfu/g at the end of day 180. In the SHPK cheese samples added with *L. paracasei* in free form, the bacteria count has decreased to 6,59 $\log_{10}$ cfu/g at the end of 180-day ripening period, and in the MKP cheese samples containing microencapsulated *L.paracasei*, this count was higher. In addition, the count of *L.paracasei* in the MKP+F and MKP+I cheese samples at the end of ripening period was higher in MKP cheese samples. According to these data, it has been determined that the viability of probiotic bacteria is preserved at a higher level in cheese samples obtained with the addition of inulin and FOS to microencapsules. Another study on the effect of alginate concentration varying between 1% and 3% used in the production of probiotic microencapsules containing *L.acidophilus* and *L.casei* on the viability of the probiotic bacteria has reported that the alginate concentrations have no effect, but the viability of microencapsules with FOS, isomaltooligosaccharide and peptide together with *L. acidophilus* and *L.casei* could be highly preserved (Chen et al., 2005).

A study examining the viability of *W.paramesenteroides, L. paraplantarum, E. faecalis* and *L. plantarum* microencapsulated with arrowroot starch has determined that the microencapsulation technique had positive effects on the viability (Samedi & Charles, 2019). In the light of these data, our results are consistent with the literature (Chaudhary & Patel, 2019).

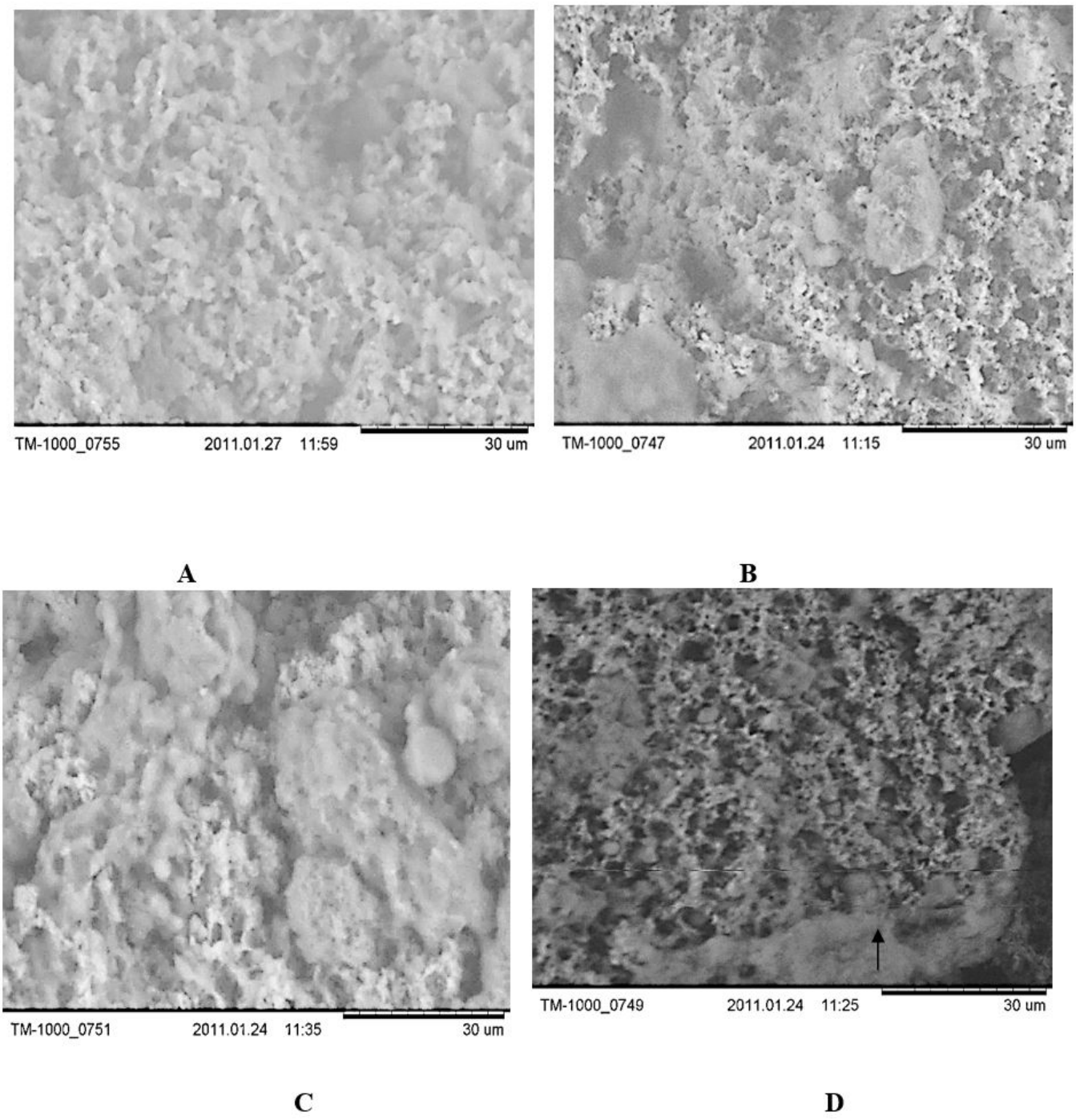

Figure 1. Appearance of some cheese matrices with Scanning Electron Microscope. (A) Appearance of probiotic bacteria in SHPK cheese sample; (B) Appearance of microcapsules in MKP cheese sample; (C) Appearance of microcapsules in MKP+F cheese sample; (D) Appearance of microcapsules in MKP+I cheese sample.

3.3 Viability of Bifidobacteriumlongum (B.longum) used as probiotic in cheese during the ripening period

The change of viability of *B.longum* in probiotic culture, prebiotic + probiotic culture, microencapsulated probiotic culture and goat cheese produced using symbiotic microcapsules during the ripening period has been given in Table 2. As can be seen in the Table 2, the cheese sample with the highest viability on average during 180-day ripening period of *B. longum* was MKP+F, and the lowest one was SHPK. The statistical analysis has shown that the viability of *B.longum* during 180-day ripening period was insignificant ($p > 0.05$) in the SHPK, SHPK+F and SHPK+-I samples, but the difference of these samples from the MKP, MKP+F and MKP+I samples was significant ($p < 0.05$). The effect of ripening time on cheese samples was found to be significant in the SHPK cheese sample after Day 75, in the

Table 1. Changes in the number of *Lactobacillus paracasei* in cheese samples during ripening log $_{10}$ (cfu/g).

Days/ Samples	SHPK	SHPK+F	SHPK+I	MKP	MKP+F	MKP+I
1	9.30 ± 0.09 a^A	9.34 ± 1.13 a^A	9.32 ± 0.47 a^A	10.36 ± 0.05 b^A	10.46 ± 1.10 c^A	10.45 ± 2.21 c^A
15	9.26 ± 0.14 a^A	9.31 ± 1.45 a^A	9.30 ± 0.26 a^A	10.34 ± 0.02 b^A	10.43 ± 1.23 c^A	10.44 ± 1.87 c^A
30	9.17 ± 0.12 a^A	9.26 ± 1.20 a^A	9.27 ± 0.86 a^A	10.32 ± 0.12 b^A	10.39 ± 1.45 c^A	10.39 ± 1.96 c^A
45	9.09 ± 0.06 a^A	9.23 ± 1.71 a^A	9.23 ± 0.41 a^A	10.31 ± 0.14 b^A	10.37 ± 1.86 c^A	10.37 ± 0.56 c^A
60	9.05 ± 0.03 a^A	9.18 ± 1.33 a^A	9.19 ± 0.44 a^A	10.34 ± 0.44 b^A	10.351.74 c^A	10.35 ± 0.78 c^A
75	8.91 ± 0.01 a^A	9.03 ± 1.46 a^A	9.04 ± 0.70 a^A	10.17 ± 0.57 b^A	10.32 ± 1.31 c^A	10.32 ± 0.26 c^A
90	8.78 ± 0.08 a^B	8.53 ± 1.52 a^B	8.51 ± 0.39 a^B	10.03 ± 0.66 b^A	10.23 ± 1.42 c^A	10.22 ± 1.68 c^A
120	8.53 ± 0.11 a^B	8.51 ± 1.86 a^B	8.51 ± 0.82 a^B	10.03 ± 0.41 b^A	10.23 ± 0.13 c^A	10.23 ± 1.52 c^A
150	7.45 ± 0.22 a^C	7.63 ± 1.55 a^C	7.60 ± 0.77 a^C	9.90 ± 0.33 b^B	10.07 ± 0.47 c^B	10.07 ± 0.23 c^B
180	6.59 ± 0.47 a^C	6.68 ± 1.63 a^C	6.63 ± 0.33 a^C	9.75 ± 0.12 b^B	9.90 ± 0.45 c^B	9.90 ± 1.25 c^B

^{a,b,c}The differences between the values in the same row are statistically significant ($p < 0.05$). ^{A,B,C}The differences between the values in the same column are statistically significant ($p < 0.05$).

Table 2. Changes in the number of *Bifidobacterium longum* in cheese samples during ripening $\log_{10}$ (cfu/g).

Days/ Samples	SHPK	SHPK+F	SHPK+I	MKP	MKP+F	MKP+I
1	9.29 ± 0.41 a^A	9.32 ± 0.20 a^A	9.32 ± 1.12 a^A	10.37 ± 0.04 b^A	10.45 ± 1.12 b^A	10.44 ± 0.08 b^A
15	9.25 ± 0.02 a^A	9.31 ± 0.18 a^A	9.30 ± 1.45 a^A	10.34 ± 0.01 b^A	10.43 ± 1.08 c^A	10.39 ± 0.01 c^A
30	9.14 ± 0.06 a^A	9.26 ± 0.43 a^A	9.25 ± 1.23 a^A	10.31 ± 0.14 b^A	10.39 ± 1.04 c^A	10.38 ± 0.02 c^A
45	9.08 ± 0.08 a^A	9.20 ± 0.73 a^A	9.19 ± 1.18 a^A	10.30 ± 0.19 b^A	10.37 ± 1.13 c^A	10.36 ± 0.08 c^A
60	9.02 ± 0.11 a^A	9.16 ± 0.13 a^A	9.15 ± 1.14 a^A	10.27 ± 0.24 b^A	10.34 ± 1.07 c^A	10.30 ± 0.11 c^A
75	8.90 ± 0.26 a^A	9.03 ± 0.40 a^A	9.00 ± 0.08 a^A	10.14 ± 0.25 b^A	10.31 ± 0.08 c^A	10.30 ± 0.14 c^A
90	8.46 ± 0.45 a^{AB}	8.78 ± 0.09 a^A	8.78 ± 1.16 a^A	10.04 ± 0.12 b^A	10.03 ± 0.07 c^A	10.03 ± 0.25 c^A
120	8.52 ± 0.06 a^A	8.54 ± 0.05 a^A	8.52 ± 0.01 a^A	10.02 ± 0.20 b^A	10.21 ± 1.25 c^A	10.20 ± 0.17 c^A
150	7.39 ± 0.04 a^B	7.62 ± 0.07 a^B	7.61 ± 0.09 a^B	9.89 ± 0.10 b^B	10.05 ± 2.34 c^B	10.04 ± 0.47 c^B
180	6.58 ± 0.10 a^C	6.68 ± 0.17 a^C	6.67 ± 1.23 a^C	9.75 ± 0.04 a^B	9.90 ± 1.56 a^B	9.90 ± 0.56 a^B

^{a,b,c}The differences between the values in the same row are statistically significant ($p < 0.05$). ^{A,B,C}The differences between the values in the same column are statistically significant ($p < 0.05$).

SHPK+F, SHPK+-I samples after Day 90 and in the MKP, MKP+F, MKP+I samples after Day 150 ($p < 0.05$).

The count of *B.longum* in the MKP+F cheese samples was higher than that in the MKP+I samples, but this difference was statistically insignificant ($p > 0.05$). In these samples, it has been concluded that the count of live probiotic bacteria is much higher than the minimum therapeutic effect level (10^7cfu/g) and that these cheese can be considered as probiotics. Our study has concluded that the viability of *B.longum* could be preserved with the microencapsulation method and the MKP and MKP+F cheese samples can be considered as probiotic cheese. Environmental factors such as pH change, dissolved oxygen concentration, salt penetration and concentration in pickled white cheese create an inhibitory effect on classical probiotic bacterial strains, and therefore, the related product may rapidly lose its probiotic character (Özer et al., 2009). The study by Silva et al. (2018) showed that symbiotic microencapsulation provided greater viability of *L. acidophilus* during the storage in yogurt, as well as providing functional characteristics of yogurt with added of alginate-gelatin-FOS microbeads. One of these studies conducted by Amine et al. (2014) has used encapsulated *B. longum* strain in the production of cheddar cheese. The study has determined that a good survival with 2 $\log_{10}$ cfu/mL reduction after 21 days, as compared to droplet extrusion encapsulated *B. longum* and free cells with 3 and 4 $\log_{10}$ cfu/mL reductions respectively.

The study by Chen et al. (2005) has examined the microencapsulation technique and the viability of probiotic bacteria such as *L. acidophilus*, *L.casei*, *B. longum*, *B. bifidum*. The study has determined that the count of *B.longum* and *B.bifidum* in free form was 8.11 $\log_{10}$ cfu/g at the beginning and decreased to 5.89 $\log_{10}$ cfu/g at the end of Day 16. It has also determined that the count of microencapsulated *B.longum* and *B. bifidum* was 8.01 $\log_{10}$ cfu/g on day 1 and 7.90 $\log_{10}$ cfu/g at the end of storage period.

4 Changes in the total count of starter bacteria in cheese during ripening period

The changes in the count of Lactococci in the composition of the starter culture used in the production of goat cheeses produced using probiotic culture, prebiotic + probiotic culture, microencapsulated probiotic culture and symbiotic microcapsule in our study are collectively given in Table 3.

It has been determined that the count of starter cultures at the end of 180-day ripening period in the SHPK, SHPK+F, SHPK+I, MKP, MKP+F, MKP+I and K cheese samples decreased by 4 $\log_{10}$ from 10^9 cfu/g to 10^5cfu/g. Significant decreases in the count of starter cultures have been observed in all cheese samples during the 180-day ripening period due to reasons such as salt transition from pickled to cheese mass and acidity development during the ripening period.

Table 3. Changes in the number of starter culture in cheese samples during ripening $\log_{10}$ (cfu/g).

Days/Samples	SHPK	SHPK+F	SHPK+I	MKP	MKP+F	MKP+I	K
1	9.3 ± 0.88 a^A	9.33 ± 0.11 a^A	9.32 ± 0.09 a^A	9.48 ± 0.91 ab^A	9.56 ± 1.04 ab^A	9.51 ± 0.03 ab^A	9.47 ± 0.06 a^A
15	9.22 ± 0.12 a^A	9.31 ± 0.53 a^A	7.30 ± 0.02 a^A	9.32 ± 0.07 b^A	9.54 ± 0.03 a^A	9.53 ± 0.09 a^A	9.61 ± 0.42 a^A
30	8.79 ± 0.08 a^B	9.12 ± 0.33 a^A	9.11 ± 0.15 a^A	9.16 ± 0.05 b^A	9.48 ± 0.09 a^A	9.51 ± 0.07 a^A	9.55 ± 0.63 a^A
45	8.74 ± 0.02 a^B	8.87 ± 0.24ab^B	8.87 ± 0.46 ab^B	9.02 ± 1.12 a^A	9.39 ± 0.07 a^A	9.36 ± 0.19 a^A	9.41 ± 0.40 a^A
60	8.68 ± 0.14 a^B	8.82 ± 0.47 b^B	8.81 ± 0.22 b^B	9.28 ± 1.09 a^A	9.29 ± 1.16 a^A	9.26 ± 1.24 a^A	9.37 ± 0.51 a^A
75	8.45 ± 0.36 a^B	8.67 ± 0.86 a^B	8.67 ± 0.55 a^B	8.80 ± 1.06 b^B	8.81 ± 0.08 a^B	8.80 ± 1.10 a^B	8.40 ± 0.60 a^B
90	7.67 ± 0.87 a^C	7.70 ± 0.33 a^C	7.70 ± 0.72 a^C	7.69 ± 0.23 a^B	7.68 ± 0.03 a^B	7.68 ± 0.05 a^B	8.68 ± 0.89 a^B
120	7.41 ± 0.54 a^C	7.40 ± 0.29 a^C	7.37 ± 0.49 a^C	7.35 ± 0.31 a^B	7.40 ± 0.19 a^B	7.39 ± 0.87 a^B	7.45 ± 0.71 a^B
150	6.61 ± 0.41 a^C	6.62 ± 0.21 a^C	6.61 ± 0.24 a^C	6.53 ± 0.27 b^C	6.54 ± 0.57 b^C	6.53 ± 0.53 b^C	6.63 ± 0.43 a^C
180	5.60 ± 0.12 a^D	5.61 ± 0.17ab^D	5.60 ± 0.83 ab^D	5.48 ± 1.23 b^C	5.51 ± 0.63 b^C	5.51 ± 0.21 b^C	5.62 ± 0.54 a^C

^{a,b,c}The differences between the values in the same row are statistically significant ($p < 0.05$). ^{A,B,C}The differences between the values in the same column are statistically significant ($p < 0.05$).

The analyzes made in our research have shown that there were small differences in all cheese samples due to vaccination, inoculation rate and a decrease was observed at the end of 180-day ripening period. The analysis of variance has revealed that the difference between the samples was statistically insignificant ($p > 0.05$) and the use of probiotic bacteria as a support culture in cheese production had no effect on the development of the starter Lactococci in the cheese matrix. In addition, the effect of the ripening process on starter bacteria was significant ($p < 0.05$).

5 Conclusion

Successful results have been obtained in the protection of probiotic microorganisms in foods against the negative effects of environmental factors by microencapsulation method. As a result of this study carried out with the idea of benefiting from this technology, which is successfully applied in different dairy products, in the production of pickled white goat cheese. it has been determined that the viability of *L. paracasei* and *B. longum* is preserved with the microencapsulation technique, and the viability of probiotic bacteria is preserved at a higher level in cheese samples obtained with the addition of prebiotics to microencapsules.

In conclusion, the viability of probiotic microorganisms when used in cheese samples in free form was found to be 1-1.5 logarithmic units lower than that when they were encapsulated. This case continued throughout the entire ripening period in our study and was clearly revealed at the end of the storage process, especially in cheese produced using probiotic cultures. The result that the microencapsulation technique used in our study significantly increases the viability levels of probiotic microorganisms has been proven by many researchers (Özer et al., 2009; Liu et al., 2017; Ningtyas et al., 2019; Siang et al., 2019).

Considering that probiotic microorganisms are available above the minimum therapeutic effect value in grams (10^9cfu) of cheese samples containing microcapsules, the MKP, MKP+I and MKP+F cheese samples produced by us in our study can be considered as "Probiotic Goat Cheese" or "Functional Goat Cheese" based on the international standards. Based on this, it is considered that consuming an amount of 100 g or more per day from the cheese samples obtained in our study may be sufficient to demonstrate the probiotic effect.

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