Emirates Journal of Food and Agriculture. 2023. 35(5): 452-457
doi: 10.9755/ejfa.2023.v35.i5.3094
http://www.ejfa.me/

RESEARCH ARTICLE

Microbiological quality of probiotic added traditional Çamur cheese

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ABSTRACT

Çamur cheese is a traditional fermented dairy product produced in the Tire district of İzmir, Turkey. In this study, the microbiological quality of cheese samples purchased from local markets was determined. The Çamur cheeses with and without the addition of *Bifidobacterium animalis* ssp. *lactis* were produced in the laboratory. All purchased (PC), lab-produced (LPC) and lab-produced-functional cheese (LPFC) samples were stored at 4°C for 30 days which were then analyzed periodically to determine the microbiological profile during refrigeration. *Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter freundi* were detected in the PC samples, and total aerobic mesophilic bacteria (TAMB) counts were higher than 5 log CFU g^{-1} during the first 5 days. However, TMAB counts were lower in LPC samples until the end of Day30. LAB counts were >7 log CFU g^{-1} for LPFC samples until Day 20. In conclusion, adding probiotic bacteria led to longer shelf life and safer products thus making the traditional Çamur cheese more functional.

Keywords: Functional food; Microbial quality; Probiotic bacteria; Shelf life; Traditional cheese.

INTRODUCTION

Cheese is a fermented dairy product with countless forms and flavors produced all over the world subject to different cultures and resources. Most cheese types are generally well known in their respective regions of production, but unfortunately are not well known globally or even at the national level. The fact that these cheese varieties produced in small scale or by family businesses are not produced according to a certain standard coupled with the lack of suitable conditions in their production results in cheeses of different quality and structure. In regions with local cheese production, the interest in local cheese varieties has increased due to their excessive consumption, and some researches have been carried out to determine the production methods for carrying them over to the industrial level.

Çamur cheese produced from curd, a whey-based product, is locally produced, and consumed in the district of Tire in Izmir, Turkey. It is generally consumed by spreading on bread.It was made from sheep and goat milk in the past, but today it is generally made from cow's milk. However, the processing of this traditional cheese does not have a certain standard. It is estimated that Çamur cheese has been produced at an average 25 tons per year at 10 dairy farms in the Tire district (Ak and Nergiz, 1998). The whey, a byproduct of white cheese or İzmir Tulum cheese produced in the houses by traditional methods, is collected in another container after which milk can be added to it, optionally. After the milk and whey mixture is heated up to the boiling temperature, the cheese clot collected on the surface is removed and is left to drain. Following the addition of 2-3% salt to the cheese, it is left to rest after being mixed and afterwards is offered for consumption. While adding salt in production, it is optionally kneaded by adding cream/butter to the cheese clot. The cheese produced from whey curd has high protein, moisture, lactose and low salt content as well as high pH values and can easily be spoiled by microorganisms due to contamination from the environment. For this reason, curd cheeses have a limited shelf life due to the growth of psychrophilic bacteria, yeast, mold and Enterobacteriaceae (Gonzalez-Fandos etal., 2000; Papaioannou et al., 2007).

Nowadays, studies showing that foods affect health positively have also changed the nutritional habits of people and health-

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Received: 05 August 2022; **Accepted:** 24 April 2023

supporting foods have begun to attract attention. In this regard, functional foods, especially those containing probiotics have gained increased popularity (Linares et al., 2017). The word *probiotic* is an expression which is etymologically formed by combining the Latin words "pro" and "bios", meaning "for living". Probiotics are defined as living microorganisms with beneficial effects on the host when taken in sufficient amounts. The health benefits of these microorganisms are not yet fully known but have been consumed for years as dairy products. However, *Bifidobacterium* sp. and *Lactobacillus* sp., which are lactic acid bacteria species, have been proposed as probiotic-type species (Ljungh and Wadström, 2006). Probiotic microorganisms are obtained from intestinal flora of healthy people or non-human intestinal flora (Balthazar et al. 2018). Probiotic bacteria must attach to the intestinal epithelium and maintain a certain amount of viability (10^6-10^7 CFU/g) during gastrointestinal digestion in order to provide significant benefits to human health. The increase in the popularity of probiotic bacteria resulted in the production of probiotic added and health-friendly products (Ranadheera et al., 2018). Probiotics have been added to many products such as milk, ice cream, chocolate, cereal-based foods, kefir, yoghurt and cheese (Cruz et al., 2009; Kumar et al., 2015). Cheese has several advantages as a carrier for probiotics due to its physico-chemical structure compared to other fermented milk products (Castro et al., 2015). For this reason, the inoculation with *Lactobacillus* and *Bifidobacterium* species in different types of cheese has been widely investigated (Vinderola et al., 2000; Van de Casteele et al., 2006; Karimi et al., 2011; Ganesan et al., 2014). Studies mainly focused on maintaining probiotic viability during storage, and the survival of probiotic bacteria in the cheese matrix while passing through the gastrointestinal tract after consumption (Pitino et al., 2012). Therefore, even though the use of cheese as a probiotic food carrier offers potential advantages, industrial scale development requires knowledge of all technological steps involved in traditional processes (Blaiotta et al., 2017).

The aim of the present study was to detect the microbiological quality of traditional Tire Çamur cheese obtained from local dairy farms; to determine the existing microflora and shelf life of cheese samples that are both purchased and produced in the laboratory; to improve the functional properties of Çamur cheese by adding probiotic bacteria and modifying the production method; and to prolong the shelf life by improving the microbial quality.

MATERIALS

In this study, purchased (PC), lab-produced (LPC) traditional Çamur cheese and lab-produced-functional cheese (LPFC) samples were used as materials. 1000 g of samples were purchased twice from four different dairy producers in Tire-Ödemiş region in the İzmir province of Turkey. On the other hand, Çamur cheese was produced under laboratory conditions with traditional methods and functional Çamur cheese was produced in the laboratory with the addition of *Bifidobacterium animalis* ssp. *Lactis*. These lab-productions were also repeated twice. All the samples were portioned into 100 g sterile bags as which were kept at -18°C for maximum 2 months until the analyses.

Çamur cheese production

Çamur cheese is traditionally produced from whey. In this study, whey was also produced in the laboratory as raw material of LPC and LPFC samples. The production methods (Fig. 1) of whey and Çamur cheese (Fig. 2) were both based on traditional methods.

Preparation of probiotic culture and inoculation of the cheese

In this study *Bifidobacterium animalis* ssp *lactis* B94 (Maflor, Mamsel İlaç San. Tic. A.Ş, $5x10^9$ CFU/g) used as probiotic culture for the production of functional Çamur cheese. 10 mg of culture was inoculated in 10 ml of Man, Ragosa and Sharpe Broth (MRSB, Lab M, LAB093, UK) under aseptic conditions which were then incubated (Binder, BD53, Germany) at 30°C for 3 days in anaerobic jars. The purity of the incubated cultures were controlled after which 10 ml of the culture was centrifugated (Hettich EBA 21, RD2901, Germany) for 5 minutes to discard the MRSB. The supernatant was discarded, and cell pellets were suspended with 0.1% sterile

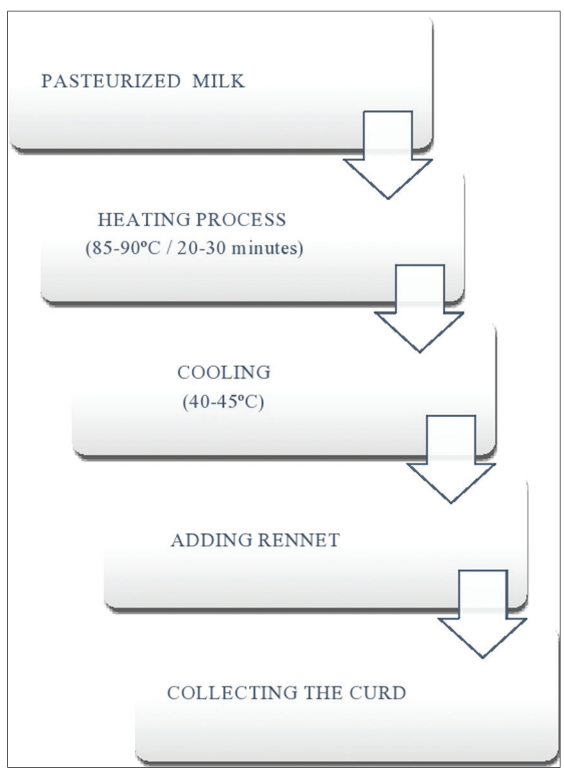

Fig 1. Whey production

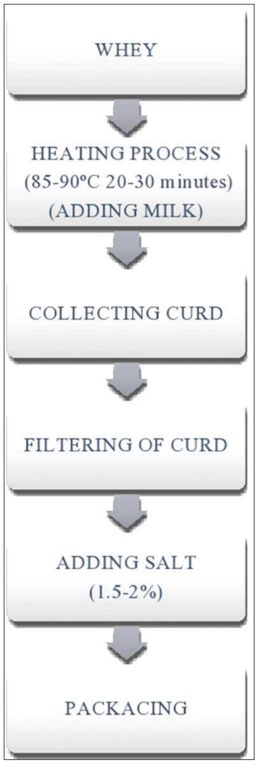

Fig 2. Camur cheese production

peptone water (PW, Merck, 1.07224, Germany) and this procedure was repeated twice. After the second washing process, the cell pellets were resuspended with 10 ml of 0.1% sterile PW, and this suspension was used for the inoculation of the cheese samples. 5 ml of homogenized suspension (probiotic culture) was added to 50 g of cheese sample, and the inoculated cheese samples were kept at room temperature for 2 hours in order to ensure the attachment of the culture.

METHODS

Microbiological analyses

In the first stage of this study, it was aimed to determine the initial microflora of the cheese samples purchased from local dairy producers. 10 g of sample and 90 ml of sterile 0.1% PW were homogenized for 270 seconds in the stomacher (IUL 707/470 Instruments, Spain). Decimal dilutions were prepared for enumeration. Total mesophilic aerobic bacteria (TMAB, FDA-BAM online, 2001a), total psychrophilic aerobic bacteria (TPAB, ISO 17410: 2001, 2001), total coliform bacteria (TCB, FDA-BAM online, 2013), fecal coliform bacteria (FCB, FDA-BAM online 2013), mold & yeast (M&Y, FDA-BAM online 2001) and lactic acid bacteria (LAB, ISO 15214: 1998, 1998) analyses were performed on the bases of standard methods. Additionally, the pathogen tests (*Salmonella* (ISO 6579, 2002), *Listeria monocytogenes* (ISO 11290, 1996), *Staphylococcus aureus* (ISO 6888, 2004) and *E. coli* (FDA-BAM online, 2013) in the Turkish Food Codex were applied for cheese standards.

In the second stage, traditional Çamur cheese production was carried out under laboratory conditions and the probiotic added -functional- Çamur cheese produced. All microbiological analyses applied to purchased (PC) samples were also applied to lab-produced (LPC) and lab-produced-functional cheese (LPFC) samples.

In the last stage, all cheese samples were stored at 4°C for 30 days in order to determine the shelf life, and TMAB, TCB, FCB, M&Y, LAB and *E. coli* analyses were performed at the beginning and on certain days (1, 3, 5, 10, 20 and 30th days) of storage.

Statistical analysis

Three replicate trials were conducted for each experiment. All results were analyzed using SPSS (Statistical 52 Package Program for the Social Sciences, Version 21, SPSS, Inc., Chicago, IL, USA) program. The results were given as mean ± standard deviation. The differences between the averages were evaluated using the Tukey test with a confidence interval of $p < 0.05$.

RESULTS AND DISCUSSION

The initial microflora of the cheese samples purchased from dairy producers was determined, and the results

Table 1: Results of microbiological analysis of purchased cheese samples (log CFU/g)

Tests (log cfu/g)	Dairy Producer A	Dairy Producer B	Dairy Producer C	Dairy Producer D
Total mesophilic aerobic bacteria	4.99±0.08^{ab}	5.69±0.21^{a}	4.49±0.56^{b}	4.56±0.56^{b}
Total psychrophilic aerobic bacteria	3.70±0.58^{a}	3.96±0.26^{a}	3.28±0.67^{a}	3.16±0.81^{a}
Lactic acid bacteria	4.54±0.06^{b}	5.30±0.10^{a}	3.46±0.15^{c}	4.51±0.13^{b}
Mold & yeast	4.04±0.04^{a}	3.35±0.10^{c}	3.87±0.09^{ab}	3.65±0.15^{b}
Total coliform bacteria	2.27±0.30^{a}	2.11±0.21^{a}	1.75±0.2^{a}	1.89±1.13^{a}
Fecal coliform bacteria	1.46±0.15^{a}	1.91±0.29^{a}	1.65±0.29^{a}	1.88±0.05^{a}
Staphylococcus aureus	ND	ND	ND	ND
Escherichia coli	ND	ND	ND	ND
Salmonella sp.	ND	ND	ND	ND
Listeria monocytogenes	ND	ND	ND	ND

Lowercase letters on the chart show the difference between columns for the same line. n=4 ± standart deviation; $P<0.05$. ND: Could not be detected.

Table 2: Results of IMViC tests of purchased cheese samples

Purchased cheese samples (Dairy Producers)	Indol	Metil-red	Voges – Proskauer	Citrat	Result
A	-	-	+	+	*Klebsiella pneumonoiae*
B	±	-	+	+	*Enterobacter aerogenes*
C	-	+	-	+	*Citrobacter freundii*
D	-	-	+	+	*Klebsiella pneumonoiae*

of the enumeration tests of TMAB (between 5.69±0.21 and 4.49±0.56 log CFU/g), TPAB (between 3.16±0.81 and 3.96±0.26 log CFU/g), TCB (between 1.75±0.2 and 2.27±0.30 log CFU/g), FCB (between 1.91±0.29 to 1.46±0.15 log CFU/g), M&Y (3.35±0.10 and 4.04±0.04 log CFU/g) and LAB (3.46±0.15 and 5.30±0.10 log CFU/g) were presented in Table 1. *Staphylococcus aureus, E.coli, Salmonella* sp. and *L. monocytogenes* were not detected in PC samples (Table 1), and the microorganisms stated in Table 2 were determined as a result of IMViC tests.

Isleyici and Akyüz (2009) obtained 25 herbal cheese samples from markets in the Van province, which were treated for microbiological analysis. The number of TMAB was 7.82 ± 1.04 log CFU/g; TCB number was 2.23 ± 2.06 log CFU/g; *Staphylococcus* spp. was found as 3.93 ± 1.81 log CFU/g; lipolytic bacteria was 4.54 ± 1.14 log CFU/g; proteolytic bacteria was 6.05 ± 1.32 log CFU/g; M&Y number was 5.81 ± 1.39 log CFU/g; *Enterococcus* spp. was 2.31 ± 1.87 log CFU/g; *Lactococcus* spp. was 5.42 ± 2.39 log CFU/g; *Lactobacillus* spp., *Leuconostoc* spp. – *Pediococcus* spp. were 8.08 ± 0.83 log CFU/g. Yeniyol (2018), determined the TMAB, M&Y and TCB numbers as 7.36 log CFU/g, 6.86 log CFU/g and 1.76 log CFU/g respectively for civil cheese samples sold in the Ardahan province. A total of 110 Motal cheese samples were obtained from the markets from the Azerbaijan Karabakh region. The results of TMAB, TPAB, *Lactococcus* spp., *Lactobacillus* spp., coliform bacteria and yeast & mold analysis were determined as 3.94 log CFU/g, 4.75 log CFU/g, 5.69 log CFU/g, 4.40 log CFU/g, 2.76 log CFU/g and 2.61 log CFU/g, respectively. It was observed when the microbiological analysis results for traditional cheese samples produced using traditional methods were evaluated that the TMAB number of Tire Çamur cheese samples obtained from the markets (PC) is much lower compared with that of the most traditional cheese mentioned in the studies in literature. Also, M&Y test results are similar with the results of Sepet cheese (Ercan, 2009) and Motal cheese (Mammadova, 2018).

The TMAB and LAB counts of LPFC samples were 2.76 ± 0.17 log CFU/g and 7.24 ± 0.05 log CFU/g, respectively. TMAB number was 2.70±0.34 log CFU/g and LAB was 5.35 log CFU/g in LPC samples. M&Y, TCB, FCB, *S. aureus*, *Salmonella* sp. *L. monocytogenes* could not be detected in both LPFC and LPC samples (Table 3).

Meira et al. (2015), produced ricotta cheese from goat milk in a laboratory environment. They added *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *Lactis* to the cheese produced and examined the microbial loads of cheese samples during storage for 7 days. They determined the numbers of *Salmonella* spp., *S. aureus*, *L. monocytogenes* as <0.3 log CFU/g.

When the results were evaluated in terms of foodborne pathogens, these results are similar to our study.

In the next stage of the study, all cheese samples (PC, LPC and LPFC) were stored at 4°C for 30 days, which were then analyzed at the beginning of storage (day 0) and on certain periods of storage (Days 1, 3, 5, 10, 20 and 30). TMAB count results varied between 2.76±0.17 and 6.57±0.07 log CFU/g at the end of the storage. It should be noted based on the evaluation of the results that the PC samples were generally close to the spoilage limit (7 log CFU/g) at the end of 30 days of storage (Table 4). The difference between TMAB count results of LPC and LPFC samples at the beginning of the storage (day 0) was not significant ($p>0.05$), but it was significant from the first day of storage ($p<0.05$). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were added to curd cheese samples, and it was found that TMAB count results increased during storage for 55 days (Yalçın, 2016). Lioliou et al. (2001), stored the traditional Greek soft cheese (Manouri cheese) for 20 days and determined that the TMAB values ranged between 4.25 and 7.32 log CFU/g like as the results of our study.

LAB count results for the PC samples varied between 4.21 and 6.30 log CFU/g after 30 days of storage, while those for the LPC and LPFC samples ranged between 4.98±0.36 and 5.73±0.31 log CFU/g (Table 5). It was observed when the LAB count results of the LPFC samples were examined that there was no sharp decrease during the first 20 days of storage and that the LAB number was above 7 log CFU/g and remained relatively stable. *Bifidobacter bifidum*, *Bifidobacter longum, Lactobacillus acidophilus* and *Lactobacilus casei* were added to Argentina Fresco cheese in different combinations, and cheese samples were analyzed on days 0 (at the beginning), 30 and 60 of storage. It was found that count results of *Lactobacillus acidophilus* and *Bifidobacterium*

Table 3: Results of microbiological analysis of lab-produced cheese and lab-produced-functional cheese samples (log CFU/g)

Tests (log cfu/g)	Lab-produced-functional cheese samples	Lab-produced cheese samples
Total mesophilic aerobic bacteria	2.76±0.17[a]	2.70±0.34[a]
Lactic acid bacteria	7.24±0.05[b]	5.35±0.25[c]
Mold & yeast	<1.00[d]	<1.00[d]
Total coliform bacteria	<1.00[d]	<1.00[d]
Fecal coliform bacteria	<1.00[d]	<1.00[d]
Staphylococcus aureus	ND	ND
E. coli	ND	ND
Salmonella sp.	ND	ND
Listeria monocytogenes	ND	ND

Lowercase letters on the chart show the difference between columns for the same line. n=4 ± standart deviation; $P<0.05$. ND: Could not be detected.

Table 4: Total mesophilic aerobic bacteria count results of all Çamur cheese samples during storage (log CFU/g)

Days of the storage	Purchased cheese samples				Lab-produced cheese samples	Lab-produced-functional cheese samples
	Dairy Producer A	*Dairy Producer B*	*Dairy Producer C*	*Dairy Producer D*		
0	4.99±0.08[abE]	5.69±0.21[aD]	4.49±0.56[bC]	4.56±0.56[bD]	2.70±0.34[cC]	2.76±0.17[cE]
1	5.08±0.01[bE]	6.12±0.11[aC]	4.79±0.05[cC]	4.71±0.05[cD]	3.19±0.11[dBC]	5.11±0.03[bD]
3	5.57±0.09[abD]	6.52±0.15[aB]	4.85±0.02[bcC]	4.90±0.02[bBC]	3.90±0.83[cB]	5.29±0.09[bD]
5	5.79±0.01[bC]	6.18±0.07[aC]	6.21±0.09[aC]	5.38±0.03[cB]	3.91±0.08[dB]	6.28±0.12[aB]
10	6.60±0.04[aB]	6.16±0.07[aC]	6.48±0.08[aB]	5.41±0.10[bB]	4.09±0.08[cB]	6.47±0.07[aAB]
20	7.04±0.07[bA]	7.32±0.06[aA]	6.75±0.08[cA]	6.58±0.09[cA]	5.23±0.06[dA]	6.57±0.07[cA]
30	7.15±0.04[bA]	7.44±0.04[aA]	7.12±0.04[bA]	K	5.35±0.01[dA]	5.80±0.09[cC]

K: The relevant sample was removed from the analysis because it was contaminated during the storage phase. The difference between the samples for the same day is shown in lower case, and for the same example, the difference between days is shown in capital letter. n=4 ± standard deviation; $P<0.05$

Table 5: Lactic acid bacteria count results of all Çamur cheese samples during storage (log CFU/g)

Days of the storage	Purchased cheese samples				Lab-produced cheese samples	Lab-produced-functional cheese samples
	Dairy Producer A	*Dairy Producer B*	*Dairy Producer C*	*Dairy Producer D*		
0	4.54±0.06[cE]	5.30±0.10[bD]	4.05±0.15[dC]	4.51±0.13[cC]	5.35±0.25[bBC]	7.24±0.05[aAB]
1	4.89±0.04[cD]	5.34±0.31[bD]	4.21±0.04[dBC]	4.86±0.03[cB]	5.61±0.04[bB]	7.36±0.20[aAB]
3	6.43±0.07[bB]	5.79±0.10[cC]	4.25±0.07[dAB]	5.85±0.15[cA]	6.12±0.02[bA]	7.45±0.05[aAB]
5	5.88±0.13[dC]	6.64±0.10[bAB]	4.39±0.04[eAB]	6.18±0.18[cA]	6.49±0.08[bA]	7.56±0.14[aA]
10	6.90±0.02[bA]	6.91±0.01[bA]	4.85±0.07[cA]	6.14±0.22[cA]	6.61±0.12[aA]	7.64±0.03[aA]
20	6.78±0.08[aA]	6.83±0.04[aA]	4.44±0.07[cB]	5.23±0.35[bB]	5.26±0.08[bBC]	7.09±0.05[aB]
30	6.30±0.09[aB]	6.27±0.04[abB]	4.21±0.05[dBC]	K	4.98±0.36[cC]	5.73±0.31[bC]

K: The relevant sample was removed from the analysis because it was contaminated during the storage phase. The difference between the samples for the same day is shown in lower case, and for the same example, the difference between days is shown in capital letter. n=4 ± standard deviation; **P**<0.05.

Table 6: Mold & yeast count results of all Çamur cheese samples during storage (log CFU/g)

Days of the storage	Purchased cheese samples				Lab-produced cheese samples	Lab-produced-functional cheese samples
	Dairy Producer A	*Dairy Producer B*	*Dairy Producer C*	*Dairy Producer D*		
0	4.04±0.04[aF]	3.35±0.10[cE]	3.87±0.09[abE]	3.65±0.15[bD]	<1.00	<1.00
1	4.22±0.02[bE]	5.35±0.31[aBCD]	4.15±0.06[bD]	4.36±0.20[bD]	<1.00	<1.00
3	4.34±0.05[cE]	5.05±0.02[FbD]	4.25±0.07[cD]	5.84±0.15[aC]	<1.00	<1.00
5	4.54±0.07[dD]	5.20±0.01[bCD]	4.82±0.08[cC]	6.06±0.11[aAB]	<1.00	<1.00
10	4.73±0.07[dC]	5.46±0.04[bABC]	5.03±0.05[cB]	6.14±0.22[aAB]	<1.00	<1.00
20	6.58±0.03[aB]	5.52±0.06[bAB]	5.18±0.03[bAB]	6.82±0.76[aA]	4.25±1.06[cB]	<1.00
30	6.85±0.03[aA]	5.60±0.06[bA]	5.24±0.06[cA]	K	4.86±0.25[dA]	<1.00

K: The relevant sample was removed from the analysis because it was contaminated during the storage phase. In the schedule, the difference between the samples for the same day is shown in lower case, and for the same example, the difference between days is shown in capital letter. n = 4 ± standard deviation; $p<0.05$.

species were more than 6 log CFU/g (Vinderola et al., 2009). Buriti et al. (2005), added *Lactobacillus acidophilus* La-5 and *Bifidobacter animalis* B-12 to Minas cheese, and they found that the bacteria survived (6 log CFU/g) during storage. Verruck, et al. (2015) added *Bifidobacter animalis* Bb-12 to Minas fresco cheese obtained from buffalo milk after which they stored it for a period of 30 days. LAB counts were between 8.15 log CFU/g and 8.30 log CFU/g

Table 7: Total coliform bacteria count results of all Çamur cheese samples during storage (log CFU/g)

Days of the storage	Purchased cheese samples				Lab-produced cheese samples	Lab-produced-functional cheese samples
	Dairy Producer A	*Dairy Producer B*	*Dairy Producer C*	*Dairy Producer D*		
0	1.46±0.15^{aA}	1.91±0.29^{aA}	1.65±0.29^{aA}	1.88±0.05^{aA}	<1.00	<1.00
1	1.54±0.06^{aA}	1.96±0.34^{aA}	1.74±0.30^{aA}	2.09±0.60^{aA}	<1.00	<1.00
3	1.47±0.07^{aA}	1.64±0.23^{aA}	1.86±0.20^{aA}	1.88±0.51^{aA}	<1.00	<1.00
5	1.67±0.12^{aA}	1.75±0.09^{aA}	2.03±0.15^{aA}	1.86±0.20^{aA}	<1.00	<1.00
10	1.85±0.54^{aA}	1.77±0.34^{aA}	1.78±0.11^{aA}	1.99±0.46^{aA}	<1.00	<1.00
20	2.01±0.16^{aA}	2.15±0.77^{aA}	1.85±0.50^{aA}	1.75±1.25^{aA}	<1.00	<1.00
30	1.61±0.15^{aA}	2.00±0.03^{aA}	1.94±0.53^{aA}	K	<1.00	<1.00

K: The relevant sample was removed from the analysis because it was contaminated during the storage phase. The difference between the samples for the same day is shown in lower case, and for the same example, the difference between days is shown in capital letter. n=4 ± standard deviation; P<0.05

Table 8: Fecal coliform bacteria count results of all Çamur cheese samples during storage (log CFU/g)

Days	Purchased cheese samples				Lab-produced cheese samples	Lab-produced-functional cheese samples
	Dairy Producer A	*Dairy Producer B*	*Dairy Producer C*	*Dairy Producer D*		
0	2.09±1.17^{aA}	2.27±0.66^{aA}	2.30±0.83^{aA}	2.34±1.20^{aA}	<1.00	<1.00
1	2.44±0.13^{aA}	1.46±0.44^{bA}	2.12±0.46^{abA}	1.61±0.31^{abAB}	<1.00	<1.00
3	2.17±0.26^{aA}	2.68±0.61^{aA}	1.91±1.13^{aA}	1.91±1.13^{aA}	<1.00	<1.00
5	2.23±0.36^{aA}	1.79±0.84^{aA}	2.25±0.25^{aA}	2.46±0.51^{aA}	<1.00	<1.00
10	2.72±0.28^{aA}	1.91±0.64^{aA}	2.09±0.82^{aA}	2.27±0.28^{aA}	<1.00	<1.00
20	2.52±0.49^{aA}	2.23±0.73^{bA}	1.81±1.16^{cA}	2.43±0.45^{aA}	<1.00	<1.00
30	2.31±0.33^{aA}	1.49±0.94^{abA}	2.42±0.31^{aA}	K	<1.00	<1.00

K: The relevant sample was removed from the analysis because it was contaminated during the storage phase. The difference between the samples for the same day is shown in lower case, and for the same example, the difference between days is shown in capital letter. n = 4 ± standard deviation; P < 0.05

during storage. Albenzio et al. (2013), conducted studies on Scamorza cheese made from sheep milk; Fritzen-Freire et al (2010) and Scheller and O'Sullivan (2011) studied cheddar cheese, and they all obtained similar results in their studies. Bezerra et al. (2017), studied on *L. lactis* subsp. *Lactis* and *L. lactis* subsp. *cremoris*; *L. acidophilus; L. paracasei*; and *B. lactis* added cheese samples, and the samples were stored for 28 days. As a result, they found that probiotic cultures were above 6.5 log CFU/g on the first day and were 7 log CFU/g on the 28th day. Dantas et al. (2016), produced Minas Frescal cheese in 4 different formulations in their study and examined the viability of the culture during storage. It was found that *L. casei* remained in high amounts indicating the viability of the cheese regardless of storage period. Boylston et al. (2004), reported that the reason that *Bifidobacterium* species displayed high viability during storage was due to the pH value of the cheese, which is appropriate for these bacteria, as well as the quality of the lipid content of the milk used in cheese making, and our results were also confirmed this assertion

A significant difference was found between samples A, B, C and D (p <0.05) was observed when the M&Y count results of PC samples were evaluated at the beginning of storage (Day 0). However, M&Y could not be detected in the cheese samples (LPC and LPFC) produced in the laboratory during the first 10 days of storage. While LPFC samples were stable for M&Y counts until the end of storage, which is probably due to the protective effect of their probiotic culture, LPC samples were probably contaminated and could not be protected by their spontaneous microbial flora (Table 6). Papaioannou, Chouliara et al. (2007) determined M&Y counts between <2 – 3 log CFU/g in their study on Anthotryros cheese. Tsiotsias et al. (2002), determined the number of yeasts in Anthotyros cheese as 3.80 log CFU/g initially which increased during the storage period. Pintado et al. (2001), determined the yeast mold counts ranging from 3.55 to 6.04 log CFU/g in curd cheeses. When the results of current study were compared with the literature data, similar results were obtained with the literature. It is thought that the reason why the M&Y could not be detected in Çamur cheese with probiotic addition is due to the suppression of mold growth due to antifungal metabolites produced by lactic acid bacteria. Several low molecular weight antifungal metabolites produced by lactic acid bacteria have been reported to have the ability to suppress mold growth.

It was observed when CB count results were evaluated that there are no significant differences between A, B, C and D samples during storage(p> 0.05). CB count was below the detectable limits during storage in the LPC and LPFC samples (Table 7). When the FCB count results were examined, no significant difference was observed between the PC samples at the beginning of storage (day 0) (p> 0.05).

However, FCB counts of PC samples were associated with lack of hygiene (Table 8). Lioliou et al. (2001), stored the traditional Greek Manouri cheese for 20 days and analyzed the surface microflora of the cheese. They determined the number of CB on days 0, 5, 10, and 20 of storage in spring which were determined as 2.28 log CFU/g, 3.45 log CFU/g, 4.39 CFU/g, 5.34 log CFU/g, respectively. On the same sampling periods of storage in summer the count results of CB were determined as 3.06 log CFU/g, 5.12 log CFU/g, 6.59 log CFU/g, 8.32 log CFU/g, respectively. As a result of the study, when the FCB and CB counts during storage were compared with the literature, it was determined that the literature results were higher than the Çamur cheese count results. This situation can be associated with the fact that the studies in the literature concentrate more on cheeses consumed after ripening and the increase in the number of these bacteria during ripening.

CONCLUSION

The microbiological analyzes were carried out on Çamur cheese samples obtained from Tire dairies (CP) during the present study. Coliform, fecal coliform and *E.coli* analyzes were evaluated to determine the microbial safety of the samples in addition to compliance with the Turkish Food Codex Microbiology Criteria. Accordingly, it was observed that the analysis results were higher than the values specified in the Microbiology Criteria, which is an indication of poor hygienic conditions in these enterprises with local production conditions. Moreover, it has been observed that the results regarding the CP samples obtained from dairies differ from each other, and this is due to local factors, the variety of raw materials and the recipe followed by the producer, as is the case with many traditional products that do not have a standard production method. In the next stage of the study, cheese samples (LPC) were produced by following traditional methods under laboratory conditions which were then compared with CP samples by performing a shelf-life study. According to the TMAB count results accepted as a criterion of spoilage, almost all CP samples reached the level of 6 log CFU/g in the first 5 days of storage, and it was determined that the average shelf life could be at most 5-7 days. It was observed that the TMAB results of LPC samples did not reach the level of 6 log CFU/g until the 30th day, and it was determined that the biggest problem that shortens the shelf life is inappropriate production conditions. From this point of view, it was observed that the shelf life of Tire mud cheese may be increased up to 20 days if produced under appropriate conditions which was considered as important data for literature considering that no shelf-life study has been conducted before for Tire mud cheese. It was predicted based on the study data that a much longer shelf life could be achieved by trying different packaging techniques in the further stages. In another part of the study, *Bifidobacterium animalis* ssp *lactis* B94 was added to provide functional properties, increase food safety and extend the shelf life of mud cheese produced using traditional methods under laboratory conditions (LPFC). The fact that *Bifidobacterium animalis* ssp *lactis* B94 survived at 7 log CFU/g until the 20th day of storage in LPFC samples, and even on the 30th day, the counting results close to 6 log CFU/g and it showed that this LPFC samples preserved its functional quality for a long time. For this reason, it is thought that LPFC samples will have a beneficial effect on health by showing probiotic effects if consumed within 20 days after production. While LPC samples became moldy on the 20th day of storage, no mold growth was observed in LPFC samples during storage and the M&Y count was found to be under the detectable level. For this reason, it has been observed that the probiotic addition not only gives a functional effect, but also extends the shelf life of the product by contributing to food safety. Adding functional additives to the cheese is considered as the most effective solution for extending the shelf life of whey cheeses which is a general problem. As a result, bringing this kind of traditional products to the industry will contribute to both the local people and the national economy. These traditional cheeses are produced by small scale firms and still do not have a standard production method, but they are consumed in large quantities by the locals. Standardizing the production methods of traditional cheeses and bringing them to the industry with functional effects through modifications in production methods such as the addition of probiotic bacteria is of great importance for the reliability and preference of traditional foods.

ACKNOWLEDGMENT

The financial support of Tokat Gaziosmanpaşa University Research Foundation (No:2019/23) is gratefully acknowledged.

Authors' contributions

Şeniz Karabiyikli Çiçek: Research hypothesis, methodology and experimental procedures, analysis, data collection, statistical analysis, manuscript writing.

Sümeyya Erdoğmuş: Research hypothesis, methodology and experimental procedures, analysis, data collection.

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