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Research Article

INVESTIGATION OF THE MICROBIOLOGICAL QUALITY OF CHEESE SAMPLES OFFERED FOR SALE IN TIRE (IZMIR) REGION

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ABSTRACT: The purpose of this study was to investigate the basic chemical properties of the cheese varieties to be obtained from dairies in Tire by determining whether they comply with the Turkish Food Codex Cheese Communiqué (Communiqué No: 2015/6) in terms of their microbiological content In our study, the highest fat content was found in FP samples with 30.0% ±0.72, and the lowest fat rate was 5.0%±2.06 in LP samples. The highest protein ratio was found in 23.0±11.2in ÖP, and the lowest protein was found in 13.0±5.6 ÇP samples. The highest pH was found in 5.7±2.08 TİP samples, and the lowest pH was 4.8±1.08 in LP samples. Acidity (% in lactic acid) was found in LP samples with a value of 0.59±0.8, and the highest value was found in TİP samples with 1.26±0.8. The highest salt content was found in 3.85±6.03% OP samples. The highest water activity was found in TIP samples with 0.98±0.15. When we review the microbiological analysis results, we saw that Propionibacterium spp. highest count was found in OP samples with 3.0x102 cfu/gr.The highest Enterococcal count was found in OP samples with 4.6x104 cfu/g. The highest Yeast and Mold Count was found in WP samples as 2.6x102 cfu/g. The highest count of Pseudomonas was found in BP samples as 6.0x102 cfu/gr. While Staphylococcus aureus count was not found in any samples, it was found in CAP samples as 1.2x101 kob/g. From the results obtained in this study, we are of the opinion that infection and intoxication may occur from cheeses produced from raw milk and therefore may be a potential danger to public health.

Keywords: *Chese, Microbiology, Hygyene, Quality*

ÖZET: Yöresel ürünlerden Tire'ye özgü çamur peyniri, tulum peyniri, keçi peyniri ve koyun peynirinin mikrobiyolojik kalitesi değerlendirilecektir. Yapılacak olan bu çalışma ile Tire'de mandıralardan temin edilecek peynir çeşitlerinin temel kimyasal özellikleri belirlenerek mikrobiyolojik içerikleri açısından Türk Gıda Kodeksi Peynir Tebliği (Tebliğ No: 2015/ 6)'ne uygun olup olmadığı ortaya konulacaktır. Yaptığımız bu çalışmada en yüksek yağ oranı,%30,0 ±0,72 ile ÇP örneklerinde, en az ise,%30,0 ±0,72, en düşük yağ oranı ise, %5,0±2,06 LP örneklerinde bulunmuştur. En yüksek protein oranı ise,23,0±11,2, en düşük protein ise 13,0±5,6 ÇP örneklerinde bulunuştur. En yüksek pH ise 5,7±2,08 TİP örneklerinde, en düşük pH ise 4,8±1,08 LP örneklerinde bulunmuştur. Asitlik (%Laktik asit cinsinden) 0,59±0,8 değeri ile LP örneklerinde ve en yüksek ise 1,26±0,8 ile TİP örneklerinde bulunmuştur.% Kuru madde oranı en yüksek 71,15±2,5 ile ÖP örneklerinde, en az ise 54,48±1,05 TKP örneklerinde bulunmuştur. Tuz oranı en yüksek %3,85±6,03 OP örneklerinde, en az ise 1,05±1,12 ile LP örneklerinde bulunmuştur. Su aktivitesi en yüksek 0,98±0,15 ile TIP örneklerinde, en az ise 0,85±0,03 LP örneklerinde bulunmuştur. Mikrobiyolojik analiz sonuçlarını gözden geçirdiğimizde ise, Propionibacterium spp. Sayımı en yüksek 3,0x102 kob/gr ile OP örneklerinde bulunmuştur..Enterokok sayımı ise en yüksek 4,6x104kob/gr ile OP ve LP örneklerinde bulunmuştur. Maya ve Küf Sayımı ise en yüksek 2,6x102 kob/gr olarak ÇP örneklerinde bulunmuştur. Pseudomonas'ların Sayımı en yüksek i 6,0x102 kob/gr olarak BP örneklerinde bulunmuştur. Staphylococcus aureus Sayımı hiçbir örnekde bulunmazken1,2x101kob/gr olarak TKP örneklerinde bulunmuştur. Escherichia coli Sayımı ise en yüksek8,0x102 kob/gr olarak TKP örneklerinde bulunmuştur. Toplam Canlı Psikrofilik Bakteri Sayımı ise en yüksek 2,3x102 kob/gr olarak ÇP örneklerinde bulunmuştur. Toplam Canlı Mezofilik Bakteri Sayımı ise en yüksek4,2x109 kob/gr olarak TIP örneklerinde bulunmuştur. Toplam Canlı Termofilik Bakteri Sayımı ise hiçbir örnekde bulunmamıştır.

Anahtar Kelimeler: *Peynir, Mikrobiiyoloji, Hijyen, Kalite*

INTRODUCTION

Milk and dairy products play an important role in human nutrition. It is recommended that at least 25% of the human diet should be milk or dairy products. Due to such a need for milk and dairy products, various types of dairy products (yoghurt, cheese, butter) are produced in the world. There are also dairy products that are not well known, but are known by a certain community or region. Khoa, Shrinkhand, Burfive Rasogolas in India; Iben, Smen, Jben in Morocco; Quesilo in Chile can be given as examples of these traditional foods [1]. Cheese has an important place in human nutrition because it is rich in nutrients. Cheese is a dairy product obtained by coagulating milk with rennin enzyme, and further separating the whey, shaping the curd, salting it, and consuming it either fresh or after ripening. By processing perishable milk into cheese, its moisture content is reduced. Thus, it is transformed into a product that has high nutritional value and can last longer without spoiling. In cheese making; Whole milk, cream, partially or completely skimmed milk, buttermilk, or a mixture of some or all of these are used as raw materials [2]. There are traditional cheeses specific to almost every region in our country and these vary during the construction phase. Therefore, the consumer's preference also changes [3,4].

The microbial load of raw milk used in cheese making has an important place in determining cheese quality [5]. As it is known, milk is a very good nutrient medium for the development of microorganisms due to its high nutritional content. Milk and dairy products have an important place in human nutrition due to the minerals and vitamins they contain. Microorganisms transmitted to milk from various sources easily reproduce in milk and dairy products [6,7]. The microbiological quality of cheese, which is consumed fondly all over the world is important and has hundreds of varieties. Microorganisms found in cheese will cause changes in the structure and taste of the cheese, and may cause food poisoning if undesirable microorganisms are found as a result of consumption. Compliance with hygienic rules at all stages of cheese production, from production to consumption, has an impact on the microbiological quality of the cheese. Türkoğlu et al detected coliform bacteria, aerobic-mesophilic bacteria, lactic acid bacteria, lipolytic-proteolytic microorganisms, and mold and yeast fungi in knitted cheese samples [8]. In a study examining the microbiological properties of homemade cheeses in the Muğla public market, aerobic-mesophilic bacteria, coliform group bacteria, E. coli, S. aureus, psychrophilic bacteria, proteolytic bacteria, yeast and mold fungi were detected in 26 cheese samples, while Salmonella species were not detected [9]. A detailed study conducted by Kamber and Şireli on the microbiological analysis of dairy product, which is popularly accepted as Turkish cheese in Hatay. In their study; aerobic mesophilic bacteria, lactobacilli, lactococci,

micrococci, Staphylococci, enterobacteria, coliform group bacteria and yeast-mold were isolated [10]. In another study on the microbiological quality of dolaz (tort) cheese, the traditional cheese of Isparta and its region, the total number of aerobic mesophilic bacteria was 5.41± 1.14 CFU/g yeast-mold count, 4.13±1.26CFU/g; the average number of psychrophilic bacteria was 3.24±1.19 CFU/g and Enterobacteriaceae was 1.50±0.68 CFU/g and Coliform and S. aureus number were determined to be <10 CFU/g in all samples [11].

MATERIALS AND METHODS

In this, 8 pieces of Mud cheese (ÇP), 3 pieces of Tulum cheese (5 pieces of cow cheese and 3 pieces of sheep cheese), 3 pieces of full-fat ripened white cheese (from sheep and goat milk) (BP), 3 pieces of full-fat braided cheese (ÖP), 3 sweet curd cheese (LP) samples and a total of 25 cheese samples were analyzed (Table 1).

Inserted Table1.

Table 1. Types and quantities of cCheese collected from Tire used

Type of Cheese		Total
Mod Cheese (ÇP)		8
Tulum Cheese	Ripened Cow Cheese (TİP)	5
	Ripened Sheep Cheese (TKP)	3
Full fat rippened White Cheese (From sheep and goat milk (BP)		3
Full fat braided cheese (ÖP)		3
Sweet curd cheese (LP)		3
Total		25

CHEMICAL ANALYSIS

pH Determination

Since the pH of cheese samples will vary from piece to piece, these samples were thoroughly ground or crushed to make them homogeneous. An amount of the homogenized sample was taken and placed in a small container that could easily contact the electrode of the digital pH meter as described previously[12].

Determination of Acidity

A 100 g piece cut from the cheese sample was crushed or grated in a mortar or crushed into small particles by passing it through a suitable machine. Approximately 10 g of cheese sample was weighed in a 250 mL conical flask with an accuracy of 0.1 mg. A volume of 3-5 mL of distilled water was added and mixed with a glass drumstick to turn it into a slurry. The glass drumstick was washed into the conical flask with 20 – 30 mL of distilled water. Afterward, 2-3 drops of phenolphthalein indicator solution were added and titrated with 0.1 NaOH solution until a light pink color was obtained. The total milliequivalent number of acids in 100 g of sample was calculated and the result was given in terms of lactic acid.

% Acidity (as Lactic acid) = V x N x f x 0.090 x 100 / m

V: NaOH solution spent in titration, mL
m: Amount of test sample used in titration, g
N: Normality of NaOH.
f : Factor of NaOH
0.090: Milliequivalent gram of lactic acid

Determination of Fat

The fat content of cheese samples was determined using a butyrometer using the Gerber method as previously described. Briefly, 3 grams of sample was weighed. Afterward, 10 mL of sulfuric acid was added to the butyrometer, followed by adding 3 mL of distilled water and incubated at 60°C[13].

Determination of Salt

The widely used Mohr method was used to determine salt present in the samples [14.]

Determination of Water Activity

Water activity (aw) value was measured using the Testo-650 device, a portable hygrometer device [15].

Dry matter:

Moisture analysis in cheese was performed according to the method of AOAC Association of Official Analytical Chemists International) in accordance with 926.08 [16.]

MICROBIOLOGICAL CONTENT ANALYSIS

To determine the importance of microorganisms for food safety and possible microbial content of cheese samples that may play role in the fermentation and ripening stages, the following microorganisms will be counted:

For the determination of *Lactobacilli* and *Lactococci*, MRS (de Man, Rogosa, Sharpe) (Merck) and M17 agar (Merck) will be used, respectively. Incubation of the Petri dishes will be carried out for 48-72 hours under anaerobic conditions at 37°C and 24 hours under aerobic conditions at 37°C, respectively.

Enumeration of Propionibacterium spp.

Propionibacterium spp. YEL (Yeast Extract Lactate) agar will be used in the counting and the cultivated petri dishes will be incubated under anaerobic conditions at 30°C for 7 days. YEL Agar media (Trypticase 10 g; yeast extract 10 g; 60% Na-lactate 10 ml; KH_2PO_4 2.5 g; $MnSO_4$ 5 mg; Agar 15 g) was prepared and the pH was adjusted to approximately 7.0±02.

Enumeration of Enterococci

Kanamycin Aesculin Azide Agar (Merck) was used for the counting of Enterococci. The incubation of the petri dish was carried out under aerobic conditions at 37° C for 24-48 hours. Black colonies formed on petri dishes at the end of incubation was counted as Enterococci [17,18.]

Enumeration of Yeast and Mold

Yeast Glucose Chloramphenicol Agar (Merck) was used for yeast and mold counting of the samples and the counts was made by incubating the petri dishes at 25°C for 3-5 days. At the end of the incubation, petri dishes containing between 30-300 colonies was evaluated [19.]

Enumeration of *Pseudomonas* spp

Counting of *Pseudomonas* was performed using Cetrimite Agar (Merck) to which 15 mg.L-1 concentration of nalidixic acid was added in order to suppress other foreign flora (Merck, 1.06219). Petri dishes was incubated at 35°C for 48-72 hours. After incubation, blue-green pigmented colonies was counted [20.]

Enumeration of *Staphylococcus aureus*

Baird Parker Agar (Merck) petri dishes containing egg yolk tellurite emulsion planted at the appropriate dilution for *Staphylococcus aureus* counting was incubated under aerobic conditions at 37°C for 24-48 hours. At the end of the incubation, the petri dishes was kept at

+4°C for 24 hours and counting will be carried out according to the results of the lecithinase activities of the colonies [21,22].

Enumeration of *Escherichia coli*

Sorbitol-MacConkey Agar (Oxoid) was used as a selective solid medium for *Escherichia coli* enumeration, and cultivation was done under aerobic conditions. At the end of 24 hours of aerobic incubation at 37°C, *E. coli* O157:H7 and other bacteria that cannot use sorbitol form colorless colonies, while sorbitol positive ones form red colonies due to neutral rejection, which is a pH indicator. The number of *E. coli* O157:H7 will be given by counting such colonies formed after incubation [23.]

Statistical Analysis

Microbiological data will be converted to log10 cfu/g. According to the results of normality analysis, it will be determined that the data meets the parametric test assumptions. Independent Samples t-test will be applied to compare the microbiological data and chemical analysis values of two groups, openly sold and vacuum packaged. In the evaluation, statistical significance will be accepted as $p \leq 0.05$. Data presented will be given as mean ± standard error. Statistical Analysis System (SAS) package program (Version 8, 1999, SAS Institute Inc., Cary, NC, USA) was used for statistical evaluation of the study (Statistical Analysis System (SAS) version 8.0. SAS Institute. Cary, North Caroline, USA, 1999).

RESULTS

The chemical analysis results of the analyzed samples are given in Table 2. The Table 3 shows the microbiological analysis results obtained from cheese samples.

Table 2: Chemical analysis results obtained from cheese samples

Type of Cheese	Chemical composition * (Average ± SH)						
	Oil (% at least in dry matter)	Protein (g)	pH	Asitlik (%g laktik asit cinsinden)	Dry Matter (%)	Salt (%g)	Water Activity (aw)
ÇP	30,0±0,72	13,0±5,6	4,9±1,5	0,79±0,09	67,86±2,93	1,10±3,5	0,90±0,06
TİP	26,5±3,04	21,9±2,5	5,7±2,08	1,26±0,8	58,6±2,48	2,50±2,02	0,98±0,15
TKP	28,0±6,44	22,5±4,04	5,6±2,05	1,11±0,06	54,48±1,05	2,40±1,12	0,93±0,12
BP	18,0±0,8	18,5±2,8	5,3±0,5	0,93±0,04	69,05±4,02	1,30±2,02	0,94±0,08
ÖP	21,0±4,72	23,0±11,2	5,1±1,04	0,85±0,01	71,15±2,50	3,85±6,03	0,90±0,15
LP	5,0±2,06	14,5±5,02	4,8±1,08	0,59±0,8	70,3±0,39	1,05±1,12	0,85±0,03

* Each analysis was performed in two parallels and the arithmetic means were written
SH: Standard error values

Table 3: Microbiological analysis results obtained from cheese samples

Microbiological Analysis	Types of Cheese Averages **					
	ÇP	**TİP**	**TKP**	**BP**	**ÖP**	**LP**
Enumeration of Propionibacterium spp.	$1,1x10^2$	$2,3x10^1$	$4,1x10^1$	$8,4x10^1$	$3,0x10^2$	0
Enumeration of Enterococci	$4,6x10^4$	$1,9x10^2$	$8,3x10^3$	$4,5x10^2$	$1,2\ x10^3$	$4,6x10^4$
Enumeration of Yeast and Mold	$2,6x10^2$	$9,4x10^1$	$2,5x10^2$	$1,7x10^2$	$1,1x10^1$	$1,6x10^2$
Enumeration of Pseudomonas spp	$5,6x10^1$	$1,2x10^2$	$1,7x10^1$	$6,0x10^2$	$2,5x10^1$	$4,4x10^1$
Enumeration of Staphylococcus aureus	0	0	$1,2x10^1$	0	0	0
Enumeration of *Escherichia coli*	$1,0x10^1$	$3,4x10^1$	$8,0x10^2$	$1,5x10^1$	$4,1x10^1$	$1,0x10^1$
Total Psychyrophilic Bacteria Count[a]	$2,3x10^2$	$5,5x10^1$	$4,0x10^1$	$5,2x10^2$	$2,5x10^1$	$1,7x10^1$
Total Mesophilic Bacteria Count[b]	$2,2x10^8$	$4,2x10^9$	$1,1x10^9$	$4,64x10^8$	$1,0x10^8$	$3,5x10^7$
Total Thermophilic Bacteria Count[c]	0	0	0	0	0	0

*The result was made and evaluated in accordance with the Turkish Food Codex cheese communiqué

**: Each analysis was made in two parallels and the arithmetic averages were written.

a: Incubated at 15±1 °C.

b: Incubated at 37±1 °C

In our study, the highest fat content was found in FP samples with 30.0% ±0.72, and the lowest fat rate was 5.0%±2.06 in LP samples. (Table2). The highest protein ratio was found in 23.0±11.2in ÖP, and the lowest protein was found in 13.0±5.6 ÇP samples (Table 2). The highest pH was found in 5.7±2.08 TİP samples, and the lowest pH was 4.8±1.08 in LP samples. When the pH in cheese samples is less than 5, conditions are provided for the growth and development of microorganisms that spoil the cheese, and if it is greater than 5.3, conditions are provided for the development of pathogenic microorganisms. The pH range in which each group of microorganisms thrive is different. Therefore, it is important that the pH of cheese samples varies. Acidity (in terms of lactic acid) was found in LP samples with a value of 0.59±0.8, and the highest value was found in TİP samples with 1.26±0.8.%. The dry matter ratio was highest in TP samples with 71.15±2.5, and at least 54.48±1.05 was found in CAP samples. Large differences were observed in the dry matter amounts in the samples. While the amount of dry matter is compatible with the findings of Şen (1991) [24.] in the cheeses obtained from raw and heated milk at 56°C, as well as the findings of Akşam et al., [25] and Yöney [26],, it is different from the results of Demirci [27] which showed low result. The difference between the sample is due to the nature of the milk, the brine concentration used and the determined ripening time. The highest salt content was found in OP samples with 3.85±6.03%, and the lowest was found in LP samples with 1.05±1.12%. *Öksüztepe et al.* found the salt content in dry matter to be at least 3.14%, at most 6.69% and on average 4.30% [28]. However, the values obtained in this study are quite low. The highest water activity was found in TIP samples with 0.98±0.15, and the lowest was found in LP samples with 0.85±0.03. The aw value decreases as a result of water loss in cheese due to ripening time and the increase in the amount of salt added to the cheese [29]. The high aw values of Çamur cheese samples in this study may be due to the short storage period of the products. However, differences in production techniques may cause different aw values in mud cheese samples. When we observed the microbiological analysis results, we found out that Propionibacterium spp. showed highest count in OP samples with $3.0x10^2$ cfu/g (Table 3). In addition, propionic acid bacteria can be used in hard and semi-hard cheeses due to their protective properties and contribution to ripening. These bacteria and their lysate preparations, are used as preservatives in products such as yoghurt, processed cheese and fresh cheese. The highest enterococcal count was found in OP and LP samples with 4.6x104 cfu/g (Table 3). Possible sources of enterococci found in milk and cheese:

They can be listed as udders of dairy cows, human and animal feces, contaminated water, milking equipment, transport and storage tanks [30,31]. The highest Yeast and Mold Count was found in WP samples as 2.6x102 cfu/g. Keleş and Atasever (1996) [32]. determined the average number of yeast/mold as $3.50x10^6$/g . Yeast and molds can easily grow at the pH of the cheese and tolerate the salt content and anaerobic environment in the cheese quite well[33]. The highest Pseudomonas Count value was found in BP samples as 6.0x102 cfu/g. In a study investigating the microbiological quality of Divle tulum cheese, *Pseudomonas* sp. was detected in 38 samples out of 50 cheese samples. The number was determined as 3.60 log10 cfu/g [34].

While *Staphylococcus aureus* count was not found in any samples, it was found in CAP samples as $1.2x10^1$ cfu/g (Table 3). High numbers of *Staphylococcus* indicate that hygiene and sanitation procedures are not sufficient. It also indicates that the heat treatment was not done properly. The highest *Escherichia coli* count was found in CAP samples as 8.0x102 cfu/g (Table 3). *E. coli*, found in the intestinal flora of humans and animals, is generally considered a harmless bacteria. Some pathogenic species can contaminate food, and in epidemics that have increased in recent years, it has been determined that hundreds of thousands of people are affected and hundreds of people die every year. *E.coli* bacteria causes food poisoning due to its presence in human and animal feces [35.]

The highest Total Viable Psychrophilic Bacteria Count was found in WP samples as $2.3x10^2$ cfu/g (Table 3). As a result of the growth of psychrophile microorganisms in cheeses, it causes disorders such as rancid taste, bitterness and color change [36]. The presence of significant levels of psychrophilic microorganisms in half of the cheese samples examined suggests that flavor defects may occur due to the enzymes such as lipase and protease produced by these microorganisms. The highest Total Live Mesophilic Bacteria Count was found in TIP samples as 4.2x109 cfu/g (Table 3). As a result of microbiological analysis of divle tulum cheese, Morul and İşleyici (2012) found the average aerobic mesophilic number in 50 samples to be 6.78±1.42 log10 cfu/g. The high number of total aerobic mesophilic bacteria used to determine the microbial load suggests that other microorganism groups will also be high[37]. Total Viable Thermophilic Bacteria Count was not found in any sample.

CONCLUSION

In this study, which was conducted to determine the microbiological status of traditional homemade cheese samples sold in Tire district markets, it was determined that the microorganism load contained in the cheese samples was quite high. As a result, it was determined that the microbiological quality of the cheeses offered for consumption in Tire public markets did not comply with the declared standards. We are of the opinion that due to the high microbiological load in the cheese samples analyzed, infection and intoxication may occur in cheeses produced from raw milk offered for consumption and therefore may be a potential danger to public health.

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Conflıct of ınterest

The authors declare that there is no conflict of interest.

Authors' contrıbutıon

NCG and DK wrote original draft. NCG and DK wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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Data avaılabılıty All datasets generated or analyzed during this study are included in the manuscript.

Ethıcs statement Not applicable.

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