

The Examination of the Microbiologic Quality in Örgü Cheese (Braided Cheese) Samples

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Summary

In this study, totally 105 Örgü Cheese samples were analyzed in terms of total mesophilic aerob bacteria, coliform bacteria, *Escherichia coli*, *Escherichia coli* 0157, *Staphylococcus-Micrococcus spp.*, *Lactobacillus spp.*, *Lactic streptococcus*, mould, yeast, *Salmonella spp.* and *Listeria spp.* The contamination rate of coliform bacteria, *E. coli*, *E. coli* 0157, *Staphylococcus-micrococcus spp.*, mould, yeast, and *Listeria spp.* were found 80.00%, 65.71%, 7.62%, 84.76%, 32.38%, 93.33%, and 2.86%, respectively. *Salmonella spp.* could not be determined in any of samples. The resistance to antibiotics of *E. coli* and *E. coli* 0157 were also examined. It was concluded that Örgü Cheese may be contaminated with some microorganisms during production or selling. These contaminations also may cause important public health risks.

Keywords: Örgü cheese, Microbiological properties, Quality, Antibiotic resistance

Örgü Peyniri Örneklerinde Mikrobiyolojik Kalitenin İncelenmesi

Özet

Bu çalışmada toplam 105 adet örgü peyniri örneği toplam mezofilik aerob bakteri, koliform bakteri, *Escherichia coli*, *Escherichia coli* 0157, *Staphylococcus-Micrococcus spp.*, *Lactobacillus spp.*, laktik streptokoklar, küf, maya, *Salmonella spp.* ve *Listeria spp.* mikroorganizmaları yönünden analiz edilmiştir. Analiz edilen örgü peynirlerinde koliform bakteri, *Escherichia coli*, *Escherichia coli* 0157, *Staphylococcus-Micrococcus spp.*, küf, maya ve *Listeria spp.* kontaminasyonu oranı sırasıyla % 80.00, % 65.71, % 7.62, % 84.76, % 32.38, % 93.33 ve % 2.86 düzeyinde bulunmuştur. Örneklerin hiçbirinde *Salmonella spp.* tespit edilememiştir. *E. coli* ve *E. coli* 0157'nin antibiyotiklere olan dirençleri ayrıca incelenmiştir. Örgü peynirlerinin üretim ve satış sırasında bazı mikroorganizmalar ile kontamine olabildiği sonucuna varılmıştır. Bu kontaminasyonlar da önemli halk sağlığı risklerine neden olabilir.

Anahtar sözcükler: Örgü peyniri, Mikrobiyolojik özellikler, Kalite, Antibiyotik direnci


INTRODUCTION


In Turkey, there are more than 130 types of cheese and its derivatives which differ in terms of regions and their production techniques. Örgü Cheese (Braided Cheese) is a special type of cheese that is specific to Southeastern Anatolia Region; it is produced using conventional methods primarily in Diyarbakır and in large parts of other cities such as Mardin, Siirt, Sanliurfa and Van provinces. Örgü Cheese constitutes the 60-65% of the cheese consumption in the region ¹. However, in

the recent years, Örgü Cheese has been produced in many production facilities located in Thrace, Eastern Anatolia, and Central Anatolia regions and is offered in the market all around Turkey ¹⁻³.

Especially, ewe's milk is preferred in the production of Örgü Cheese. Also goat's and cow's milk are used where ewe's milk is not available or available at scarce amounts. In the production of the Örgü Cheese with

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conventional methods, the curd, which is formed as a result of fermentation of milks at milking temperature, is drained and the obtained curd is left for fermentation. After the pH has fallen to approximately 5, scalding at 70-80°C in 3% saline water for 5-6 minutes is performed. Scalded and melted cheese is kneaded to give it an örgü shape. Örgü Cheese can either be consumed freshly or be offered for consumption after it has been ripened in brine solutions containing salt by 10-12%¹⁻⁴. Örgü Cheese, which is shaped as a braid, rich in terms of fat, homogenous and having elastic consistency, is accepted to be similar to the White Cheese (Feta Cheese) in terms of its composition, similar to the Circassian Cheese in terms of its taste, and similar to the Kashar, Abkhasian, and Maras Cheeses in terms of its production technology including the scalding operation in its manufacturing process¹.

Örgü Cheeses that are offered for consumption in Diyarbakir province are generally produced by conventional methods in enterprises without modern technology and under relatively low hygienic conditions; their compositions and quality vary depending on the experiences and working conditions of the masters performing the production. Because this non-standard production style is excessive, products with different compositions and quality are offered for sale. In the research studies conducted on the composition of the Örgü Cheese, it has been reported that total dry matter is 42.70-54.64%, fat is 14.72-17.84%, protein is 15.83-21.69%, ash is 6.43-8.01%, salt is 5.45-6.03%, and titrable acidity (% lactic acid) is 0.34-0.80²⁻⁵.

In this study it is aimed at determining the microbiological characteristics of brined Örgü Cheeses that are offered for consumption in Diyarbakir and evaluating the probable public health risks. Antibiotic resistances of *E. coli* and *E. coli* O157 microorganisms that are detected in the Örgü Cheese samples are also tested.

MATERIAL and METHODS

Collection of the Sample

In Diyarbakir province, 105 Örgü Cheese samples that had been sold without packaging were collected from several markets and delicatessens into sterile sample collection bags at 250 g amounts. The analyses were started immediately after the samples were brought to the laboratory under cold conservation conditions (4°C).

Microbiological Analyses

In the process of microbiologic analyses of Örgü Cheese samples, 10 g sample taken under aseptic

conditions was homogenized using 90 ml of 0.1% peptone water. Preparing decimal dilutions, the appropriate medium was inoculated by standard analysis methods. For the count of total mesophilic aerobic bacteria (TMAB), Plate Count Agar was inoculated using pour plate method and reproduced colonies were counted after 48 h of incubation at 37°C⁶. Violet Red Bile Agar (Oxoid CM107) was used for the purposes of isolating coliform bacteria. Red violet colored colonies of 2-3 mm diameter were counted after 24 hours of incubation at 37°C which was realized after inoculation with double layer pour plate inoculation method⁷. TBX agar (Oxoid CM945) was used for the purposes of *E. coli* isolation. After 4 h of incubation at 30°C and 18 h of incubation at 44°C green colored colonies were evaluated. Serologic tests were applied to all *E. coli* strains using Oxoid Dryspot *E. coli* O157 (DRO120M) test kit⁸. In order to count *Staphylococcus-Micrococcus spp.*, 48 h of incubation at 37°C was applied in the egg yolk telluride emulsion added Baird Parker Agar Base (Oxoid CM275 + SR54) medium⁷. Man-Rogosa Sharpe Agar (Oxoid CM361) was used in the counting process of lactic acid bacteria and after 48 hours of incubation at 30°C, gray-white colonies of 0.5-2.5 mm diameter were evaluated⁷. M17 Agar (Oxoid CM785) was used in the counting process of lactic streptococci and they were evaluated after 48 h of incubation at 37°C⁸. Potato Dextrose Agar (Oxoid CM139) was used in the counting process of mould and yeast and the counts were realized after 5 days of incubation at 25°C which started after inoculation by spread plate method⁶.

For the purposes of *Salmonella spp.* pre-enrichment, 25 g Örgü Cheese sample was incubated for 24 h at 35°C by adding buffered peptone water (Merck 1.07228). Taking 1 ml of pre-enrichment liquid, this amount was kept in Tetrathionate Broth (Oxoid CM0029) and Selenite Cystine Broth (Oxoid CM0699) at 35°C for 24 h and as selective media, XLD agar (Oxoid CM0469) and Salmonella Shigella Agar (Oxoid CM0099) were inoculated. Biochemical and serologic tests were applied to the typical colonies that reproduced in the selective agar media after 24 h at 35°C. In the isolation process of *Listeria spp.*, by adding 225 ml of Buffered Listeria Selective Enrichment Broth (Oxoid CM897) into 25 g cheese sample, the test material was incubated for 48 h at 30°C. Oxford Listeria Selective Supplement (Oxoid SR0140) added Listeria Selective Agar Base (Oxford) (Oxoid CM0856) medium was inoculated as a selective medium and after 48 h of incubation at 35°C black-haloed, small, brown-black, and swollen colonies were assessed to be under suspicion of being *Listeria spp.* Gram staining, motility, catalase, oxidase, urease, esculin, voges-proskauer, methyl red, sugar, hemolysis, and

CAMP tests were also applied to these colonies ^{8,9,10}.

Antibiotic Sensitivity Tests

Antibiotic sensitivity tests were applied to *E. coli* and *E. coli O157* bacteria that were isolated from the Örgü Cheese samples. Sensitivity tests of these bacteria to Cefazolin, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Ofloxacin, Oxacillin, Rifampin, Tetracycline, Trimethoprim + Sulfamethoxazole, and Vancomycin were conducted using “BD Phoenix™ 100 Automatic Microbiology Identification System” in accordance with the instructions of the manufacturer firm (BD Diagnostic Instrument Systems, Sparks, MD, USA).

Phoenix ID/AST Panel

“BD Phoenix™ 100 Automatic Microbiology Identification System” is a device which is designed for rapid identification (ID) of the bacteria and antimicrobial sensitivity tests (AST). In this system, 100 identification and antimicrobial sensitivity tests can be conducted. The ID part of the system contains a series of conventional, chromatogenic, and florogenic biochemical materials to determine the bacteria identification. Phoenix AST

method is a bouillon based micro-dilution test which is used for antimicrobial sensitivity measurement. In the ID part of the device, there are 45 shafts and 2 fluorescent control shafts which contain dried chemical substrates. In the AST part, the device has 84 shafts and reproduction control shafts which contain dried antimicrobial agents. The device operates by locating the prepared pure cultures in the device according to the manufacturer firm’s instructions and it performs the evaluations in full automatic system.

RESULTS

In this study, 105 Örgü Cheese samples were analyzed in terms of presence and contamination levels of TMAB, coliform bacteria, *E. coli*, *E. coli O157*, *Staphylococcus-Micrococcus spp.*, *Lactobacillus spp.*, lactic streptococci, mould, yeast, *Salmonella spp.*, and *Listeria spp.* For the Örgü Cheese, the microbial contamination rates were given in [Table 1](#), the presence of microorganisms was given in [Table 2](#), and the resistance of *E. coli* and *E. coli O157* strains to various antibiotics were given in [Table 3](#).

Table 1. Microbial Contamination Rates in the Örgü Cheese Samples (percent, %)

Tablo 1. Örgü peyniri örneklerinde mikrobiyel kontaminasyon oranları (yüzde, %)

Microorganism	Sample Number (n)	Contamination Rate	
		Positive (n)	%
Coliform bacteria	105	84	80.00
<i>E. coli</i>	105	69	65.71
<i>E. coli O157</i>	105	8	7.62
<i>Staphylococcus-Micrococcus spp.</i>	105	89	84.76
Mould	105	34	32.38
Yeast	105	98	93.33
<i>Salmonella spp.</i>	105	Non detectable	0.00
<i>Listeria spp.</i>	105	3	2.86

Table 2. Presence of microorganisms in the Örgü Cheese samples (cfu/g)

Tablo 2. Örgü peyniri örneklerindeki mikroorganizma varlığı

Microorganism	Sample Number (n)	Contamination Rate (cfu/g)		
		Minimum	Maximum	Average
TMAB	105	1.0x10 ⁶	2.4x10 ⁹	1.6x10 ⁸
Coliform bacteria	105	2.0x10 ¹	7.8x10 ⁵	3.6x10 ⁴
<i>E. coli</i>	105	1.0x10 ¹	4.9x10 ⁴	5.0x10 ³
<i>Staphylococcus-Micrococcus spp.</i>	105	2.0x10 ²	1.0x10 ⁷	1.4x10 ⁵
<i>Lactobacillus spp.</i>	105	1.0x10 ⁴	9.2x10 ⁷	3.4x10 ⁶
Lactic streptococci	105	1.0x10 ⁴	1.4x10 ⁸	3.7x10 ⁶
Mould	105	1.0x10 ²	2.6x10 ⁴	2.2x10 ³
Yeast	105	1.0x10 ²	3.7x10 ⁵	3.1x10 ⁴

Table 3. Some antibiotics resistance of detected *E. coli* and *E. coli O157* bacteria in the örgü cheese**Tablo 3.** Örgü peyniri örneklerinde tespit edilen *E. coli* and *E. coli O157* bakterilerinin bazı antibiyotiklere direnci

Antibiotic	<i>E. coli</i> (n=69)						<i>E. coli O157</i> (n=8)					
	S		R		I		S		R		I	
	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Amoxicillin-Clavulanate	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Ampicillin	53	76.81	16	23.19	-	-	7	87.50	1	12.50	-	-
Cefazolin	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Cefepime	59	85.51	-	-	10	14.49	6	75.00	-	-	2	25.00
Cefoperazone-Sulbactam	62	89.86	-	-	7	10.14	7	87.50	-	-	1	12.50
Cefotaxime	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Cefoxitin	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Ceftazidime	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Ciprofloxacin	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Gentamicin	69	100.00	-	-	-	-	8	100.00	-	-	-	-
İmipenem	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Levofloxacin	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Meropenem	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Piperacillin	55	79.71	-	-	14	20.29	8	100.00	-	-	-	-
Piperacillin-Tazobactam	69	100.00	-	-	-	-	8	100.00	-	-	-	-
Trimethoprim-Sulfamethoxazole	49	71.01	20	28.99	-	-	6	75.00	2	25.00	-	-

S: Sensitive; R: Resistance; I: Intermediate

DISCUSSION

With this study, samples of Örgü Cheese, which were offered for consumption in Diyarbakir, were analyzed in terms of the presence of various microorganisms and their counts. Akyuz et al.⁵, Ozdemir et al.², Aksu et al.³, and Turkoglu et al.⁴ have detected the count of TMAB in Örgü Cheeses as 1.6×10^6 cfu/g, 1.0×10^7 cfu/g, 3.9×10^5 cfu/g, and 6.89 log cfu/g respectively. In our study the count of TMAB was detected to be 1.6×10^8 cfu/g, which is higher than the results obtained by other researchers in their studies on the Örgü Cheese.

In this research, coliform bacteria contamination was detected to be 80% and the average count of coliform bacteria was 3.6×10^4 cfu/g. In their study, Aksu et al.³ have reported the count of coliform bacteria to be 3.2×10^2 cfu/g and the contamination level to be 78%. In other research studies conducted on Örgü Cheeses, the count of coliform bacteria was reported to be 9.5×10^1 cfu/g by Akyuz et al.⁵, 3.7×10^2 cfu/g by Ozdemir et al.², and 3.73 log cfu/g by Turkoglu et al.⁴. In this study of ours, *E. coli* and *E. coli O157* contamination were detected to be 65.71% and 7.62% respectively, where the count of *E. coli* was found to be 5.0×10^3 cfu/g. Aksu et al.³ reported *E. coli* contamination to be 34% and the count of *E. coli* to be 4.3×10 cfu/g. In several types of cheeses *E. coli O157:H7* level was detected to be 1% by Akkaya et al.¹¹ and Aslantas and Yildiz¹², 2% by Aksu et al.¹³, 3.2% by

D'Aubert et al.¹⁴ and 3.33% by Gonul¹⁵. The results that we have found are also higher than these values.

E. coli and *E. coli O157* strains that were detected in this study were analyzed in terms of their resistance to various types of antibiotics. All *E. coli* strains isolated from the Örgü Cheese samples were determined to be sensitive to amikacin, amoxicillin-clavulanate, cefazolin, cefotaxime, cefoxitin, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, and piperacillin-tazobactam. However, in addition to these antibiotics, *E. coli O157* strains were determined to be sensitive to piperacillin. In terms of resistance, 23.19% of *E. coli* and 12.50% of *E. coli O157* strains were detected to be resistant to ampicillin, and, their resistance to trimethoprim-sulfamethoxazole was detected to be 28.99% and 25.00% respectively. Strains of *E. coli* were detected to be semi-resistant to cefepime by 14.49%, to cefoperazone-sulbactam by 10.14%, and to piperacillin by 20.29%; however strains of *E. coli O157* were detected to be semi-resistant to cefepime by 25.00%, and to cefoperazone-sulbactam by 12.50%.

In this research, average counts of TMAB, coliform bacteria and *E. coli* in the analyzed Örgü Cheeses were found to be higher than the similar studies. It was considered to be originated from bad raw material, production conditions which were neither modern nor hygienic, unsuitable conditions storage, low salt concentration, non-hygienic equipment, and

contaminations induced by the environment and personnel.

Several researchers report *Staphylococcus aureus* borne intoxications arising from cheese consumption¹⁶. The Örgü Cheeses analyzed in our study were also analyzed in terms of *Staphylococcus-Micrococcus spp.* to give an idea about their hygienic quality. In this research, the contamination level of *Staphylococcus-Micrococcus spp.* in the Örgü Cheeses was found to be 84.76% and their count was found to be 1.4×10^5 cfu/g. These results were higher than both the result of Ozdemir et al.² which was 2.2×10^1 cfu/g and the result of Aksu et al.³ which was 1.0×10^3 cfu/g. The fact that the *Staphylococcus-Micrococcus spp.* count was high is assessed as the exposure of the Örgü Cheese to environmental and personnel related contaminations in the production and sale stages.

Lactic acid bacteria constitute the dominant flora of the cheese¹⁷. Average *Lactobacillus spp.* and lactic streptococci counts in the Örgü Cheeses offered in Diyarbakir for consumption were detected to be 3.4×10^6 cfu/g and 3.7×10^6 cfu/g, respectively. However Turkoglu et al.⁴ and Ozdemir et al.² were detected the average count of lactic bacteria to be 6.78 log cfu/g and 1.0×10^7 cfu/g, respectively. The results we have found are lower than those of these researchers.

Since moulds can develop in a very wide range of pH, water activity and temperatures, they develop in cheese easily and cause appearance, odor, and taste deformations. Hence, they are analyzed by many researchers in various cheeses^{18,19}. On the other hand, yeasts lower the quality of the cheese by forming pores and they cause the deformation which is called early swelling. Therefore they are not desired to be present in the cheeses²⁰. High mould and yeast counts are reported to be caused by not following the hygienic rules in the period from the production to the marketing of the cheese²¹. In this research, mould and yeast contamination in the Örgü Cheese samples were detected to be 32.38% and 93.33%, respectively. In their studies, Akyuz et al.⁵, Ozdemir et al.², Aksu et al.³, and Turkoglu et al.⁴ reported the count of mould-yeast in the Örgü Cheeses as 1.7×10^5 cfu/g, 1.0×10^5 cfu/g, 4.9×10^4 cfu/g, and 5.45 log cfu/g, respectively. In our study, the average mould count was detected to be 2.2×10^3 cfu/g and the average yeast count was detected to be 3.1×10^4 cfu/g. These results are lower than those results of other researchers. In the studies regarding mould and yeast counts, different results have been reported in terms of *Lactobacillus spp.* It is possible that this situation is originated from the production techniques, ripening period, and differences in conservation and sale conditions.

In this research, the counts of TMAB, coliform, *E. coli*, mould, yeast, and presence of *Listeria spp.* in the analyzed Örgü Cheeses are higher than the maximum allowable values provided in the Turkish Food Codex Microbiological Criteria Notification²².

The number of research studies conducted on *Listeria spp.* and *Salmonella spp.* contaminations in Örgü Cheeses was limited. It has been reported that 29 of 60 mass intoxication events observed in the Europe after 1980 as a result of milk and dairy products consumption were originated from *Salmonella spp.* and 10 of these events were originated from *Listeria*²³. *Listeria monocytogenes* was deemed responsible for 25 of 55 mass intoxication events in the United States of America and an intoxication case that caused the death of 48 people as a result of cheese consumption in Mexico^{24,25}. In this research, although *Listeria spp.* contamination was detected to be 2.86%, *Salmonella spp.* were not detected in any of the samples.

Which is one of basic food items as cheese and raw milk is a good environment for the development of various microorganisms²⁶. With this research determined that the Örgü Cheeses offered to the market for consumption in Diyarbakir were low quality and contaminated with pathogen. These contaminations also may cause important public health risks. We concluded that standardization of the Örgü Cheese production, the use of high quality raw materials, production in modern enterprises and hygienic conditions, conservation in cold, prevention of without package sales, good and hygienic sale applications, and vacuumed packaging or packaging under modified atmosphere will be effective in prevention of the probable dangers in terms of public health.

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