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Microbiological and Chemical Quality of Cokelek Marketed in Elazığ

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In the present study, the microbiological and chemical quality of cokelek, a traditional dairy product produced in Elazığ and consumption of which plays an important role in nutrition of people of the region, were determined. A total of forty cokelek samples were collected from the marketplace in Elazığ.

The average numbers were found as 2.87x10^{8} cfu/g for total mesophilic aerobic bacteria, 8.53x10^{3} cfu/g for coliforms, 1.42x10^{3} cfu/g for *Staphylococcus-Micrococcus* microorganisms, 3.10x10^{7} cfu/g for yeast and molds, 1.5x10 cfu/g for Enterococcus spp., 2.97x10^{7} cfu/g for *Lactobacillus-Leuconostoc-Pediococcuss*, 1.03x10^{8} cfu/g for *Lactococcus* spp. *Escherichia coli* and *Staphylococcus aureus* were found in 39 (97.5 %) and 32 (92.5%) of the 40 cokelek samples, respectively. However, no *Salmonella* was detected in the samples.

The average values of the chemical parameters were 3.79 for pH, 1.25% for acidity (la.), 0.95 for water activity (aw), 1.38% for fat, 21.43% for dry substance, 6.94% for fat in dry substance,0.52 % for salt, 2.44% for salt in dry substance, 2.14% for ash and 17.91% for protein.

These results indicate that the cokelek samples analyzed contained microorganisms such as *Escherichia coli* and *Staphylococcus aureus* suggesting poor sanitation and hygienic practices during production. In addition, results of the chemical analysis revealed that level of dry substance and protein was relatively higher in cokelek samples.

Key Words: *Cokelek, Microbiological quality, chemical quality, traditional*

Elazığ'da Tüketime Sunulan Çökeleğin Mikrobiyolojik ve Kimyasal Kalitesi

Bu çalışmada, Elazığ Bölgesi'nde üretilen ve yöre insanının beslenmesinde önemli bir yeri bulunan çökeleğin mikrobiyolojik ve kimyasal kalitesi saptandı. Bu amaçla piyasadan temin edilen 40 adet çökelek örneği incelendi.

Örneklerde ortalama olarak toplam mezofilik aerob sayısı 2.87x10^{8} kob/g, koliform bakteri 8,53x10^{3} kob/g, *Staphylococcus-Micrococcus* 1.42x10^{3} kob/g, maya ve küf 3.10x10^{7} kob/g, *Enterococcus* spp.1,5x10 kob/g, *Lactobacillus-Leuconostoc-Pediococcus* 2.97x10^{7} kob/g, *Lactococcus* sayısı ise 1.03x10^{8} kob/g seviyelerinde tespit edildi. İncelenen 40 adet çökelek örneğinin 39'unda (%97.5) *Escherichia coli* ve 37'sinde (% 92.5) *Staphylococcus aureus* mikroorganizmalarını içerdiği belirlendi. Ancak, hiçbir örnekte *Salmonella* bakterisine rastlanmadı.

İncelenen örneklerdeki ortalama pH değeri 3.79, asitlik miktarı (la. cinsinden) % 1.25, su aktivitesi (aw) değeri 0.95, yağ % 1.38, kuru madde % 21.43, kuru madde de yağ % 6.94, tuz %0.52, kuru maddede tuz % 2.44, kül % 2.14 ve protein % 17.91 miktarlarında saptandı.

Sonuç olarak, incelenen çökelek örneklerinin *Escherichia coli* ve *Staphylococcus aureus* gibi mikroorganizmaları önemli oranlarda içerdiği, dolayısıyla ürünün yapımı sırasında hijyenik şartlara yeterince uyulmadığı ortaya çıkmaktadır. Yapılan kimyasal analiz sonucunda ise ürünün protein ve kuru madde miktarlarının oldukça yüksek olduğu belirlendi.

Anahtar Kelimeler: *Çökelek, Mikrobiyolojik, Kimyasal, Kalite, Geleneksel.*

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Introduction

Milk and dairy products play an essential role in human nutrition. It is recommended that dairy products to comprise at least 25% of the daily nutrition intake (1). Due to high processability of milk, there are a number of varieties of dairy products in the world. Some of the dairy products are well known in the world such as cheese and yogurt despite high diversity in their types. On the other hand, there are other dairy products which are not internationally known or recognized but within a definite society or region. Examples include khoa, shrikhand, burfi and rasogollas from India, lben, smen and jben from Morocco, quesilo from Chili. These ethnical products might be a primary way of dairy consumption for some people, particiularly for those living in rural areas. It is expected that a number of health risks may be involved in these products due to lack of sanitary conditions during production and marketing (2). In addition, there is no official microbiological criteria applied to these products. Cokelek is a popular traditional dairy product in Turkey (3).

Cokelek is produced by diluting yogurt at 1:5 ratio with potable water and churning for separation of milk fat. Fat is removed and used as cream. The remaining portion which is called ayran is boiled until precipitating. The resulting precipitate is placed into bags made from cheese cloths for removal of excess fluid and hanged for a period of time. The water content is reduced by keeping the cokelek under pressure for a short time (ca. 30 min). It is then removed into a large pot and kneaded by hand while adding salt at 1 or 2 % (w/w). Cokelek is consumed in a variety of ways including as a spread cheese in breakfast, as an ingredient of variety of recipes in Turkish kitchen (4, 5). Consumption of cokelek is more common in rural areas where animal protein intake may be limited compared to that in urban areas (6). Production of cokelek is more art than science. Most producers are small scale processing plants that might be practicing poor hygienic processing. Contradictory to its widespread in Turkey, studies with cokelek is limited. In a study carried out in the region of Van, eastern Anatolia, Turkey (7); twenty five samples of cokelek were analyzed. The results showed that the number of total mesophilic aerobic bacteria in samples was 9.8x106 cfu/g., the number of yeast and mold was at the level of 1.3x105 cfu/g. No coliform bacteria were detected. Chemical composition of the cokelek samples were 18.15 % for dry substance, 1.2 % for fat, 6.68 % for fat in dry substance, 8.04 % for protein, and 0.94 % for ash. Intrinsic factors of cokelek including water activity (aw), acidity, pH were 0.969, 1.92% (la) and 4.87, respectively. In a similar study carried out in a different region with herby- cokelek containing herb (5, 8), the dry substance was found as 22.07 %, moisture as 77.93 %, fat as 2.69 %, protein as 14.51 %, salt as 1.97 %, ash as 3.31 %, acidity (% la) as 1.93, the number of total mesophilic aerobic as 2.57x 107 cfu/g., the number of yeast-mold as 7.76x 106 cfu/g. and the number of coliform bacteria as 5.89x 102 cfu/g. All these studies indicate that microbiological and chemical qualities of cokelek vary from region to region. In general, microbiological quality of cokelek is poor despite high level of protein content.

The present study was intended to determine the microbiological and chemical quality of cokelek in Elazığ region.

Materials and Methods

A total of 40 Cokelek samples were collected from bazaars, shopping centers and supermarkets between August 2005 and October 2005 in Elazığ. Each sample (ca. 150-200g) was taken using aseptic techniques and transferred to the laboratory under cold chain (4±1°C) for analysis.

Microbiological analyses: Decimal dilutions (up to 10-6) of cokelek samples were prepared using sterile ¼ Ringer solution. The appropriate dilutions were pour-plated or surface plated on to appropriate media for enumeration of bacteria or yeast-mold (9, 10). The microbiological media and incubation conditions used for enumeration of microorganisms were Plate Count Agar (PCA) for mesophilic aerobic microorganisms (30±1°C, 72 hours) (9), Potato Dextrose Agar (PDA) (pH 3.5 by 10% tartaric acid) for yeast and mold (21±1°C 5 days) (11), Ragosa Acetate (AcA) agar (9, 12) for *Lactobacillus-Leuconostoc-Pediococcus* spp. (30±1 °C 5 days, bilayer plates), M17 agar for *Lactococcus* spp. (30±1 °C 48-72 h) (13), Mannitol Salt Agar (MSA) for *Staphylococcus-Micrococcus* spp. (37±1 °C 36-48 h), Thallous Acetate Tetrazolium Glucose Agar (TITA) for *Enterococcus* spp. (45±1 °C 48 h) (9, 14). The numbers of *Staphylococcus aureus* was determined by applying coagulase test on bright yellow halo colonies on Mannitol Salt Agar (11, 15, 16).

The presence of *Salmonella* was determined as previously described by Andrews and Hammack (17). Count of coliform group bacteria was determined on Violet Red Bile Agar (VRBA) (30±1°C 24 h). Presence of *Escherichia coli* was determined by applying IMVIC tests to the typical dark red colonies from (VRBA). Those resulting +, +, -, - from IMVIC tests at 44.5±0.2 °C and were evaluated as *Escherichia coli* (18, 19).

Chemical analyses: The pH values of samples were determined using a pH meter (EDT, GP 353) 25±1 °C (10). The values of water activity aw were measured using a water activity meter (TESTO-400) (20). The amounts of dry substance, ash and protein, fat and acidic rates were determined in accordance with the methods as defined in Association of Official Analytical Chemists (21). The obtained fat amount was formulized thus the fat amount in dry substance was counted on based of percent. The salt level was determined using Mohr method (21).

Statistical Analyses: SPSS statistical analysis program was used to determine the correlation between the numbers of microorganisms and salt or aw values of the cokelek samples (22).

Results and Discussion

The results of microbiological analysis of the cokelek samples are shown in Table 1 and 2. Chemical composition is presented in Table 3. The average number of total mesophilic aerobic microorganisms was found as 2.87x10^{8}cfu/g (2.0x10^5-2.36x10^8 cfu/g) (Table 1). These values were higher than those reported in previous reports (5, 7, 8). The total number of mesophilic aerobic microorganism was found > 1.0x10^5 cfu/g in all samples (100%) (Table 2). Although cokelek is considered as a cooked product, these results indicate a high level of environmental contamination during production and marketing, probably resulting from poor product handling. Insufficient cooking might have also been contributed to the high level of aerobic mesophilic bacteria in cokelek.

Table 1. Microbiological Findings of Cökelek (n=40).

Microorganism	Average (cfu/g)^{a} (x±Sx)	Minimum ^{a}	Maximum ^{a}
Total Mesophilic Aerob	2.87x10^{8}±2.50x10^{8}	2.00x10^{5}	2.36x10^{8}
Coliform	8.53x10^{3}□±1.73x10^{4}	2.00x10^{1}	9.50x10^{4}
Escherichia coli	1.09x10^{3}±2.73x10^{3}	<10	1.10x10^{4}
Staphylococcus-Micrococcus	1.42x10^{3}±2.10x10^{3}	1.00x10^{1}	9.00x10^{3}
Staphylococcus aureus	1.04x10^{2}±1.36x10^{2}	<10	7.00x10^{2}
Yeast and Mold	3.10x10^{7}±3.13x10^{7}	2.70x10^{4}	5.60x10^{7}
Salmonella	-^{b}	-	-
Enterococcus spp.	1.5x10^{1}±0.7x10	<10	3.00x10^{2}
Lactobacillus-Leuconostoc-Pediococcus	2.97x10^{7}±2.73x10^{7}	2.00x10^{5}	1.10x10^{8}
Lactococcus	1.03x10^{8}±1.17x10^{8}	1.15x10^{5}	2.54x10^{8}

a, cfu: colony forming unit b, (-) : Not detected

Table 2. Distribution of the Level of Microorganisms in Cökelek Samples.

Microorganism (cfu/g)	<1.0x10 cfu/g		1.0x10- 9.9x10 cfu/g		1.0x10^{2}- 9.9x10^{2} cfu/g		1.0x10^{3}- 9.9x10^{3} cfu/g		1.0x10^{4}- 9.9x10^{4} cfu/g		>1.0x10^{5} cfu/g	
	n	%	n	%	n	%	n	%	n	%	n	%
Total Mesophilic Aerob	-	-	-	-	-	-	-	-	-	-	40	100
Coliform	-	-	4	10	10	25	18	45	8	20	-	-
Escherichia coli	1	2.5	14	35	19	47.5	4	10	2	5	-	-
Staphylococcus.-Micrococus	-	-	6	15	21	52.5	13	32.5	-	-	-	-
Staphylococcus aureus	3	7.5	17	42.5	20	50	-	-	-	-	-	-
Yeast and Mold	-	-	-	-	-	-	-	-	6	15	34	85
Salmonella	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus spp.	4	10	36	90	-	-	-	-	-	-	-	-
Lactobacillus-Leuconostoc-Pediococcus	-	-	-	-	-	-	-	-	-	-	40	100
Lactococcus	-	-	-	-	-	-	-	-	-	-	40	100

Table 3. Results of the Chemical Analysis of Cökelek Samples (n=40).

Parameter	Average (X±Sx)	Minimum	Maximum
pH	3.79±0.107	3.57	3.98
Acidity (l.a %)	1.25±0.214	0.68	1.68
Water activity (a_w)	0.95±0.020	0.90	0.98
Dry substance (%)	21.43±4.270	17.50	25.05
Fat (%)	1.38±0.432	0.60	2.00
Fat in dry substance (%)	6.94±3.390	2.57	16.00
Salt (%)	0.52±0.154	0.21	0.76
Salt in dry substance (%)	2.44±0.581	1.21	3.27
Ash (%)	2.14±0.861	0.65	4.00
Protein (%)	17.91±4.115	10.32	25.92

The average number of coliforms was 8.53x10^{3} cfu/g (2.0x10^{1} - 9.50x10^{4} cfu/g) (Table 1). The number of coliform bacterium was found between 1.0x10 cfu/g and 9.9x10^{3} cfu/g in 32 samples (80%), between 1.0x10^{4} and 9.9x10^{4} cfu/g in 8 samples (20%) (Table 2). Unlike mesophilic aerobic bacteria, numbers of coliforms were higher than those previously reported (5, 7, 8). In the present study, a moderate correlation (r = - 0.40) was found between the levels of coliforms and salt (NaCl). This result can be explained by the differences in production of cokelek due to strong locality of the product. Our results also showed that *Escherichia coli* was present in 39 (97.5%) of 40 samples of cokelek. Altogether, these results indicate that particularly the sanitary process (cleaning + disinfection) has not been practiced sufficiently in the processing plants. As known, *Escherichia coli* and coliform habitats in the intestines of human and animals and the existence of such bacterium in product indicates contamination directly or indirectly with fecal material (23, 24).

The average number of *Staphylococcus-Micrococcus* microorganism in the samples was 1.42x10^{3} cfu/g (1.0x10^{1} - 9.0x10^{3} cfu/g) (Table 1). Statistical analysis indicated a weak correlation (r = 0,29) between the numbers of *Staphylococcus-Micrococcus* and NaCl level. This result confirms that *Staphylococcus-Micrococcus* are the halotolerant microorganisms. The numbers of *Staphylococcus-Micrococcus* microorganisms were at levels between 1.0x10^{2} and 9.9 x 10^{2} cfu/g in 21 samples (52.5 %) (Table 2). *Staphylococcus aureus* was present in 37 of cokelek samples (92.5%) and the number of such bacterium was found at least <10 cfu/g, at most 7.0x10^{2} cfu/g and 1.04x10^{2}±1.36x10^{2} cfu/g in samples (Table 1, 2). Such microorganisms are of human and animal origin. *Micrococcus* bacterium plays an important role in causing food spoilage (23, 24).

The average number of yeast and mold was 3.10 x 10^7 cfu/g (2.7 x 10^4 - 5.60 x 10^7 cfu/g) (Table 1). These results are higher than the values reported by other researchers (5, 7, 8, 25) for cokelek (i.e. 6.24x10^5-7.76x10^6 cfu/g). Numbers of yeast and mold was distributed as 1.0x10^4 - 9.9x10^4 cfu/g in 6 samples (15%) while greater than 1.0x10^5 cfu/g in 34 samples (85%) (Table 2). A moderate correlation (r =0.43) between the number of yeast and mold and the level of dry substance was found. A weak correlation was also found between the values of pH and water activity (r=0.39, r=0.33). It was suggested that there was no homogenous distribution among the samples on basis of numbers of yeast and mold. Such occurrence is possible for the products produced in small scale sites and sold at outlets places. No *Salmonella* was detected in samples of cokelek.

Enterococcus spp. was found as 1.5x10^1 cfu/g (<10 cfu/g-3.0x10^2 cfu/g) in cokelek samples. It was seen that these microorganisms were <1.0x10^1 cfu/g in 4 samples (10%) where as they were present between 1.0x10^1 and 9.9x10^1 cfu/g in 36 samples (90 %) (Table 1, 2). As the result of statistical analysis, it was found that there was a weak correlation between *Enterococcus* spp. and salt level (r = 0.21). Some species included in this group (*Streptoccocus liquefaciens, Streptoccocus faecium, Streptoccocus zymogenes*) can grow in the environment where pH is 9.6 and salt level is 6.5% (19).

The average number of *Lactobacillus-Leuconostoc-Pediococcus* was found 2.97x10^7 cfu/g (2.0x10^5-1.10x10^8 cfu/g) (Table 1). This finding is different from that of Dumas cokelek in which numbers of the lactic acid bacteria was found as 1.50x10^6 cfu/g as reported by Tarakçı et al (5). This can happen possibly because of the poor quality of raw material used. It was observed that the numbers of *Lactobacillus-Leuconostoc-Pediococcu*s were >1.0x10^5 cfu/g in all samples (100 %) (Table 2). A weak correlation was found between *Lactobacillus-Leuconostoc-Pediococcus* and salt level and water activity value (r = 0.21 and r = 0.29). The number of *Lactococcus* spp. was found at least 1.15x10^5 cfu/g, at most 2.54x108 cfu/g and at average 1.03x10^8 cfu/g in samples (Table 1). It was observed that the number of *Lactococcus* spp. was >1.0x10^5 cfu/g in all samples (100 %) (Table 2). A very weak correlation was found among *Lactococcus* spp. and salt levels and water activity (r = -0.19 and r = - 0.07).

As for the chemical analysis, it was found that pH values were between 3.57 and 3.98, respectively. The average pH was 3.79±0.107 in samples (Table 3). This finding is lower than previously reported values as 4.87 from 25 samples of cokelek in Van (7). This can be explained by different quality of raw material used in production of cokelek. The average acidity was 1.25□0.214 % (la) (0.68 % - 1.68 %) (Table 3). These findings are lower than the values reported by other researchers (5, 7, 8). Such difference can occur due to the different raw material and the difference in production method.

The average water activity (a_w) was found as 0.95±0.020 in the samples of cokelek (Table 3) which was lower than previously reported (a_w = 0.969) from 25 samples (7). The fat level was found varying between 0.60 % and 2.00 % with and average value of 1.38±0.432 % in cokelek samples (Table 3). The average fat level was lower than 22.08 % that was found by Tarakçı et al (5) from 12 samples of Darende Dumas cokelek and the value of 2.69 % found by Küçüköner and Tarakçı (8) in cokelek contenting herb in Van. Such difference can occur due to the raw material content. The dry substance in cokelek samples and the fat ratio in dry substance were found as 21.43±4.270 % and 6.94±3.390 %, respectively in the samples (Table 3). These values were determined as lower than the findings reported by some researchers (5, 8, 25) and relatively higher than the findings of other researchers (7).

Salt increases the shelf life of the products such as cheese and cokelek as well as giving them their characteristic taste and affecting their consistency and yield. As seen in Table 3, the salt level is between 0.21% and 0.76% where as the salt in dry substance is between 1.21 % and 3.27 %. The relatively low level of salt found in cokelek samples in the present study can be explained by preferably low addition of salt in Elazığ region. It was found that the ash level was at least 0.65 %, at most 4 % and at average 2.14±0.861 % in cokelek samples (Table 3). This value was similar to 2.39 % found by Tarakçı et al (5) and higher than the samples value of 0.94% found by Ağaoğlu et al (7). The differences found between the present study and the previous literature can be due to cokelek samples produced in different ways.

The average protein level was found as 17.91 % (10.32 %-25.92 %). This finding was lower than 21.66 % reported by Tarakçı et al (5) for Darende Dumas cokelek. It was higher than the value of 8.04 % found by Ağaoğlu et al (7) in cokelek produced in Van and the samples value of 14.51 % found by Küçüköner and Tarakçı in cokelek containing various herbs (8). The discrepancy found between the present study and the previous literature is probably caused by the variations in productions of cokelek.

In conclusion, these results indicate that chemical composition of the cokelek may vary from region to region due to a number of reasons including differences in processing and quality of raw material. As a secondary product, nutritional composition of cokelek is still valuable for people of rural areas. Microbiological quality of cokelek, however, is rather poor and exhibits health risk to the consumers. Poor or weak correlations between microbiological findings and salt-a_w values indicate that these barriers are not sufficient for inhibiting the microbiological growth in cokelek. In addition to practicing Good Manufacturing Practices (GMP) during cokelek production examples of which include proper chilling and packaging, implementation of Hazard Analysis and Critical Points (HACCP) to the production is required for achieving the acceptable hygienic condition of cokelek.

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