

The effects of ripening on some physicochemical and microbiological characteristics of Çökelek cheeses: A market survey of fresh and skin-ripened Çökelek

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Abstract

Çökelek is a type of cheese produced via heating of skimmed buttermilk containing low- or no-fat. It is an important healthy dairy product being a cheap and good source of animal protein with low calorie. The present study evaluated some of the characteristics of fresh and skin-ripened Çökelek cheeses available in the Turkish market including compositional, microbiological, electrophoretic and colourimetric properties. The higher contents of total solids, salt and ash in the skin-ripened Çökelek were found to be the main compositional differences. None of the Çökelek samples contained *Escherichia coli*, coliforms, and fecal coliforms while the yeast and mould counts were 7.3 and 8.2 log cfu/g for fresh and ripened Çökelek samples, respectively. In terms of nitrogen fractions, there was no big difference determined between fresh and ripened samples suggesting that ripening did not affect proteolysis rates of Çökelek considerably. The ripened samples had lower brightness values (L^*) while redness (a^*) and yellowness (b^*) values were higher compared to the fresh ones.

Keywords: colour, Çökelek cheese, microbiological characteristics, proteolysis, ripening

Introduction

Çökelek is a whitish amorphous cheese with a crumbly texture and characteristic odor. Although it is commonly produced using traditional methods in small scale dairy plants it is described as one of the five main cheeses of Turkey (Kamber, 2007). Together with whey cheese (lor), it is a holder of annual 23.7 kilotons of a volume and approximately 30 million United States dollar (USD) of a value of production in Turkey (Tuik, 2015). Çökelek cheese contains both casein and whey proteins in significant amounts but almost no fat; e.g. Öksüztepe et al. (2007) reported 1.38% fat

and 17.91% protein content for Çökelek samples collected in Elazığ. Therefore, it is not only a relatively cheap product but also a good source of animal proteins with low-calorie. However, its composition varies depending on the raw material, production method and consumption types, fresh or ripened. It is mostly consumed at breakfast either plain or sweetened with grape molasses or jam. It is also used as a stuffing for patties and sometimes it is added to salads.

Given that it is sometimes called “yoghurt pieces” Çökelek is commonly made by boiling and straining of yoghurt (Simsek and Sagdic, 2010; Simsek and Sagdic, 2012). However, it can also be obtained from a variety of raw materials including milk, buttermilk or a mixture of whey and yoghurt depending on the region. Butter, milk, salt and a variety of herbs can be added at different stages of production to enhance the flavour of Çökelek. Either boiling the skimmed buttermilk or acidification of milk (by spontaneous lactic acid fermentation or by adding lemon juice) is required for curdling via coagulation of milk proteins depending on the raw material used (Figure 1). Due to these regional differences, variations in the raw materials and production methods, Çökelek is known under different names such as Tomas, Keş, Kurut, Ekşimik, Sürk, Minci, etc. (Kirdar, 2005; Hayaloglu et al., 2007; Kamber, 2007; Dervisoglu et al., 2009; Tarakçi et al., 2010). In the city of Malatya (Turkey), Çökelek is produced via heating of buttermilk, a by-product of churning yoghurt into butter, and Çökelek is obtained after drainage of the curd with a special cloth. Sometimes it is ripened in skin bags, pots or plastic drums to offer consumers a dry and slightly bitter taste while extending its shelf life (Tarakçi et al., 2003; Kirdar, 2005). Therefore, the aim of this study was to present traditional manufacturing procedure of Çökelek cheese, as well as compositional, microbial and proteolytic characteristics of fresh and ripened Çökelek.

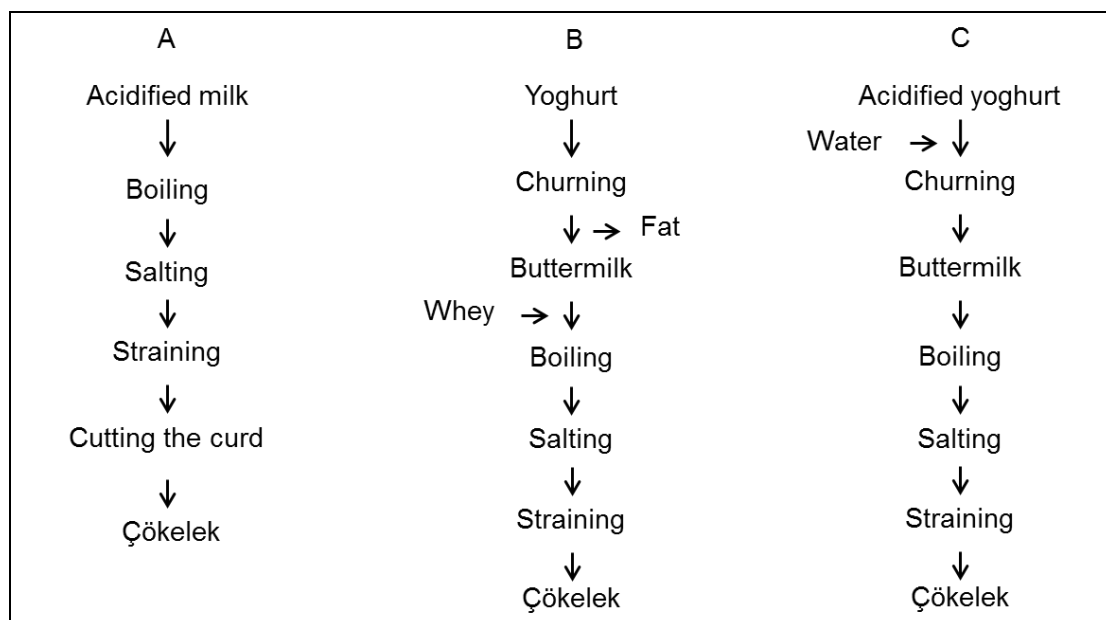


Figure 1. Çökelek production from (A): acidified milk, (B): yoghurt + whey and (C): acidified yoghurt, adapted from Kirdar (2005)

Materials and methods

Materials

A total of twenty samples, comprising ten fresh and ten skin-ripened Çökelek samples (250 g each), were randomly collected from local market places in the city of Malatya, Turkey in February 2016. All the samples were made from cow milk and skin-ripened ones were stored in skin bags at the time of purchase. However, the exact ages of the samples were not known but purchased relying on the seller's statements. The samples were transported to the laboratory in their original containers. After completing microbiological and colourimetric analysis, all samples were stored at -20 °C for further analysis.

Chemical and biochemical analysis

Total solid content was determined via a gravimetric method (International Dairy Federation, IDF, 2004). The ash contents of cheese samples were determined based on the weight difference of the sample before and after ignition in oven at 550 °C. Fat content was determined by the Gerber-van Gulik method (IDF, 1986) while salt content was determined according to the Mohr method by titration with AgNO₃. The titratable acidity (percentage of lactic acid) was determined by titrating filtered cheese slurries (10 g/100 mL) with 0.1 Normal NaOH, using phenolphthalein as the indicator (Kurt et al., 1996). To detect pH values, a pH meter (model Starter 3100; OHAUS, NJ, USA) probe was immersed into the cheese slurries and readings were recorded. Total nitrogen (TN) content of the cheeses was determined according to the Kjeldahl Method (IDF, 1993) using UDK-149 (VELP Scientifica, Italy) automatic distillation unit. Total proteins (%) were calculated by multiplying the nitrogen percent by the factor of 6.38. Protein fractions; water soluble nitrogen (WSN), 12% trichloroacetic acid-soluble nitrogen (TCA-SN) and 5% phosphotungstic acid-soluble (PTA-SN) nitrogen were determined according to Bütikofer et al. (1993) and all the nitrogen fractions were expressed as percentage of TN.

Urea-polyacrylamide gel electrophoresis (Urea-PAGE)

Urea-PAGE was performed according to the protocol given in Ardö and Polychroniadou (1999) with minor modifications. Samples (100 mg) were dissolved in 1 mL sample buffer and filtered. After addition of 25 µL of bromophenol blue (0.1%) and 50 µL of mercaptoethanol, samples were run in a vertical type electrophoresis system (SE600X Chroma Deluxe; Hoefer Inc., MA, USA) using a 1.5 mm thick double layered gel and applying 25 mA current (ELITE 300, Wealtec Corp., NV, USA) until the tracking dye front disappeared from the bottom of the gel slab. Gels were digitized by a scanner (Epson V750 Pro, Seiko Epson Corp., Japan).

Colour analysis

Colour measurements were performed using a colour analyzer (Minolta, Chromameter CR-400, Japan) and results were expressed based on CIE Scale where L^* , a^* and b^* corresponds to brightness, redness and yellowness, respectively.

Microbiological analysis

Under aseptic conditions, 10 g of sample was weighed into a sterilized container using a sterile spatula and homogenized with 90 mL buffered peptone water (LAB204; LABM Limited, UK). Serial decimal dilutions of the homogenates were prepared in the same solution and appropriate dilutions were plated on specific agar plates using the pour plate method. After incubation plates, carrying 30 to 300 colonies were taken into account for calculations.

Samples were plated on Violet Red Bile Agar (VRBA) (LAB031; Lab M Limited, UK) for enumeration of coliforms and fecal coliforms. Plates were incubated at 30 °C for 24 hours and 44 °C 24 hours for coliforms and fecal coliforms, respectively. All red/purple colonies (>0.5 mm in diameter) were counted following incubation. To detect yeast and molds, Potato Dextrose Agar (PDA) (LAB098; Lab M Limited, UK) adjusted to pH 3.5 via adding 14 mL of sterile tartaric acid (10%) into per L of autoclaved PDA medium, was used and plates were incubated at 25 °C for 5 days (Gerasi et al., 2003). The presence of *E. coli* was tested using *Harlequin*TM Tryptone Bile Glucuronide Agar (TBGA/TBX) (HAL003; Lab M Limited, UK) with the incubation norms of 44 °C for 24 hours. Plates were checked for appearance of blue/green colonies as an indicator of *E. coli* after incubation (Halkman, 2005).

Statistical analysis

All analyses were performed in duplicates and all results were given as descriptive statistics (minimum and maximum values, mean \pm standard deviation). To analyze the correlations between properties of Çökelek samples, either Pearson's correlation coefficients or Spearman's rank correlation coefficients were calculated for normally and non-normally distributed data, respectively (Table 1a, 1b). All correlation coefficients and corresponding significances were calculated using the Minitab statistical software (Minitab, 2013).

Results and discussion

Physicochemical characteristics

There were considerable compositional differences determined between fresh and skin-ripened Çökelek mainly due to the varying total solids content (Table 2). Ripening in skins allows evaporation of moisture through the pores and in this case, it caused an approximately 10% difference between the mean total solids of fresh and ripened Çökelek (23.9 vs. 34.07%). A large variety of total solids contents starting from 18.15% (Ağaoglu et al., 1997) up to 60.83% (Cardak, 2012) were found in different studies carried out up to the present for fresh and ripened Çökelek, respectively. Although the mean fat content (6.6%) in fresh Çökelek samples was

Table 1a. Correlations between characteristics of çökelek cheeses

Characteristics		pH	Fat	Acidity	Salt [§]	TS	Ash	Protein	WSN-TN	TCA-TN	PTA-TN [§]	L*	a*	b*
Fat	r	0.546*												
	P	0.013*												
Acidity	r	-0.174	-0.136											
	P	0.463	0.567											
Salt [§]	ρ	0.788	0.271	0.243										
	P	0	0.248	0.303										
TS	r	0.866	0.379	0.044	0.856									
	P	0	0.099	0.855	0									
Ash	r	0.756	0.329	0.107	0.873	0.872								
	P	0	0.157	0.654	0	0								
Protein	r	0.744	0.208	-0.01	0.768	0.843	0.577							
	P	0	0.379	0.967	0	0	0.008							

TS-total solids, [§]non-normally distributed data, P-P-value, r-Pearson's correlation coefficient, ρ -Spearman's rank correlation coefficient. Asterisks (*) represent significant differences ($P \leq 0.05$)

Table 1b. Correlations between characteristics of çökelek cheeses

Characteristics		pH	Fat	Acidity	Salt [§]	TS	Ash	Protein	WSN-TN	TCA-TN	PTA-TN [§]	L*	a*	b*
WSN-TN	r	-0.166	-0.057	0.209	-0.146	-0.291	-0.233	-0.177						
	P	0.483	0.811	0.377	0.538	0.214	0.323	0.454						
TCA-TN	r	0.079	0.134	0.164	0.017	-0.93	-0.043	-0.09	0.843					
	P	0.741	0.574	0.491	0.945	0.696	0.858	0.705	0					
PTA-TN [§]	ρ	-0.03	0.156	0.532*	0.106	-0.057	0.122	-0.217	0.588	0.62				
	P	0.9	0.511	0.016*	0.655	0.811	0.609	0.359	0.006	0.004				
L*	r	-0.894	-0.324	0.227	-0.745	-0.764	-0.651	-0.645	0.171	-0.042	0.108			
	P	0	0.164	0.335	0	0	0.002	0.002	0.472	0.86	0.65			
a*	r	0.486*	-0.021	-0.145	0.45*	0.43	0.44	0.276	-0.034	0.232	0.16	-0.548*		
	P	0.03*	0.93	0.543	0.047*	0.058	0.052	0.24	0.888	0.324	0.501	0.012*		
b*	r	0.72	0.362	-0.044	0.714	0.712	0.496*	0.752	-0.136	0.045	-0.013	-0.647	0.026	
	P	0	0.117	0.853	0	0	0.026*	0	0.567	0.849	0.957	0.002	0.912	
Yeast-mold counts	r	0.826	0.43	-0.065	0.809	0.754	0.838	0.515*	-0.212	-0.021	0.023	-0.769	0.602	0.455*
	P	0	0.058	0.784	0	0	0	0.02*	0.369	0.929	0.925	0	0.005	0.044*

TS-total solids, [§]-non-normally distributed data, P-P-value, r-Pearson's correlation coefficient, ρ -Spearman's rank correlation coefficient. Asterisks (*) represent significant differences ($P \leq 0.05$).

found to be higher than that of skin-ripened ones (6.3%), the high standard deviations should also be considered. The fat contents determined by Ağaoglu et al. (1997) (6.68%) and Öksüztepe et al. (2007) (6.94%) are similar to the fat contents of fresh Çökelek cheeses analyzed in this study while the fat contents in the studies by Keven et al. (1998) (13.19%), Tarakçı et al. (2003) (22.08%), Cardak (2012) (24.87%) and Kavaz et al. (2012) (41.31%) were evidently higher. The high fat contents found in these studies can be explained by either different production methods as in the Darende Dumas Çökelek where cream, yoghurt or milk are added into the Çökelek at different stages of production (Tarakçı et al., 2003) or variations in the starting raw materials as in the Kavaz et al. (2012) where milk was used. Besides, the fat content remained in the buttermilk is not consistent due to the non-standardized production methods.

The mean salt and ash contents were higher in skin-ripened samples (Table 2). The higher contents of salt and ash in skin-ripened Çökelek are primarily a result of the salt addition for long-time preservation purposes. The mean protein contents of skin-ripened Çökelek samples are higher than that of all the published studies concerning Çökelek (Ağaoglu et al., 1997; Keven et al., 1998; Tarakçı et al., 2003; Akkurt et al., 2005; Öksüztepe et al., 2007; Cardak, 2012; Kavaz et al., 2012).

Acidity value was found not to be significantly different between fresh (0.58%) and skin-ripened (0.55%) Çökelek samples. The mean acidity values were found to be similar to the values found by Cardak (2012) (0.49%) while Simsek and Sagdic (2012) (2.13-2.45%), Ağaoglu et al. (1997) (1.92%) and Öksüztepe et al. (2010) (≈ 1.5 -2%) found higher acidity values. The pH values for skin-ripened Çökelek samples (4.26-5.28) were considerably higher than that of the fresh ones (3.65-4.15) (Table 2). Given that Çökelek is mostly produced from yoghurt in the selected region, it is reasonable to expect a pH around 4.6, the pH of yoghurt. Also, it should be considered that yoghurts which are too sour for consumption are processed into Çökelek cheese which may explain the lower pH values obtained for the fresh Çökelek. Based on the relationship between pH values and ripeness of Çökelek samples determined in this study, it is plausible to say that the samples collected for the studies by Cardak (2012) and Öksüztepe et al. (2007) might be fresh (pH= 3.79 and 3.83, respectively) and the ones analyzed in the studies by Ağaoglu et al. (1997) and Keven et al. (1998) might be ripened (pH= 4.87 and 4.97, respectively). Kavaz et al. (2012) recorded the pH values starting from the 1st day of production (4.53) to the 21st day of storage (4.48) while pH values went down from 3.9 to 3.6 after 35 days of storage in the study by Öksüztepe et al. (2010). Therefore, it can be inferred that there is a trend of decrease during the storage, in contrary to our findings. However, the details of Çökelek production methods have to be known to make a rational judgment.

Table 2. Chemical characteristics of Çökelek cheeses

Properties	Fresh Çökelek (n=10) ^a			Skin-ripened Çökelek (n=10)		
	Min.	Max.	$\bar{X} \pm SD^b$	Min.	Max.	$\bar{X} \pm SD$
Total solids (%)	21.4	28.2	23.9±2.4	28.6	41	34.1±3.7
Protein-in-dry matter (%)	63.6	96.6	88.3±12.3	73.9	80.3	78.2±4
Fat (%)	0.5	3.3	1.6±0.8	1.5	3	2.1±0.6
Fat-in-dry matter (%)	2.3	13.1	6.6±3	3.7	8.9	6.3±1.9
Salt (%)	0.1	1.8	0.3±0.5	1.7	5.2	2.8±1.1
Salt-in-dry matter (%)	0.5	6.5	1.3±1.8	5.5	14.3	7.9±2.6
Ash (%)	0.3	4	1±1.1	1.9	5.6	3.1±1.2
Ash-in-dry matter (%)	0.8	14.1	4.1±3.9	6.3	15.2	8.9±2.7
pH	3.7	4.2	3.8±0.2	4.3	5.3	4.6±0.3
Acidity (LA%) ^c	0.4	0.8	0.6±0.2	0.4	0.9	0.6±0.1

^an-number of samples, ^b $\bar{X} \pm SD$ -mean±standard deviation, ^cLA %-acidity expressed as percentage of lactic acid (LA).

Nitrogen fractionation is an indicator of proteolysis and consequently ripening. The values for nitrogen fractions are presented in Table 3. The wide range of values determined for all nitrogen fractions are indicative of non-standard production and ripening processes. Ripened samples had a lower mean value (6.26%) for water soluble nitrogen fraction than that of fresh ones (7.83%). The mean trichloroacetic acid-soluble nitrogen (TCA-SN) (5.74 vs. 5.74%) and PTA-SN (1.04 vs. 1.12%) ratios for fresh and skin-ripened Çökelek were found to be similar. Basically, Table 3 reveals that the proteolysis rates are fairly low for all samples and there is no big difference between the proteolysis rates of fresh and skin-ripened Çökelek. The most likely reasons for the occurrence of this scenario are; no enzyme addition in the Çökelek production and the limitation of the proteolytic activities of the natural microbiota and indigenous enzymes due to the applied heat during production. Also, the heat-denatured whey proteins act as barriers and make the casein molecules inaccessible to the proteolytic enzymes (Barac et al., 2016).

Table 3. Nitrogen fractions of Çökelek cheeses

Properties	Fresh Çökelek (n=10) ^a			Skin-ripened Çökelek (n=10)		
	Min.	Max.	$\bar{X} \pm SD^b$	Min.	Max.	$\bar{X} \pm SD$
WSN ^c (% of TN ^d)	3.05	16.03	7.83±4.23	2.86	10.14	6.26±2.38
TCA-SN ^e (% of TN)	2.17	11.13	5.74±2.58	2.69	8.81	5.74±2.15
PTA-SN ^f (% of TN)	0.53	1.73	1.04±0.42	0.49	3.6	1.12±0.93

^an-number of samples, ^b $\bar{X} \pm SD$ -mean±standard deviation, ^cWSN-water soluble nitrogen, ^dTN-total nitrogen, ^eTCA-SN-12% trichloroacetic acid-soluble nitrogen, ^fPTA-SN-5% phosphotungstic acid-soluble nitrogen.

Electrophoretograms of the Çökelek cheeses were compared while no attempt was made to quantify the levels of caseins by densitometric scanning (Figure 2). In general, skin-ripened Çökelek samples presented stronger protein bands mainly due to the difference between the contents of total solids. β -casein was found to be more intact than α_s -casein in all Çökelek samples, unlike in the study by Kavaz et al. (2012) where they found the rate of hydrolysis of these fractions were similar due to the short storage period. There was no specific pattern of protein degradation detected in the skin-ripened Çökelek samples. Although some of the skin-ripened samples (S6, S8, S9) had an apparently high number of protein fractions, some of these fractions could also be observed in some of the fresh ones (F1, F6, F9). Electrophoresis could be used as an indicator of ripening period for cheeses since it shows the proteolysis patterns based on the cleavage of specific protein fractions, especially caseins, during the storage period. Therefore, the inconsistent degradation rates determined in the electrophoretograms suggest uncontrolled ripening processes and possible adulterations such as mixing fresh Çökelek into ripened ones or labeling fresh Çökelek as ripened to charge more money from the customers.

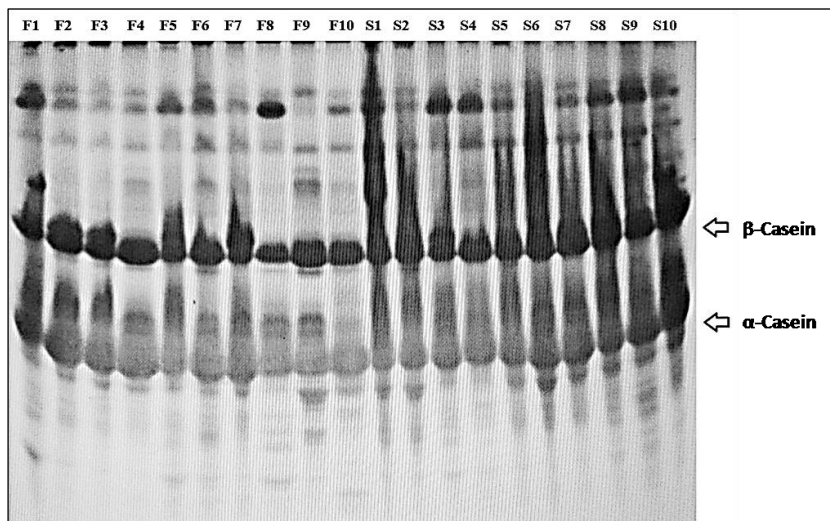


Figure 2. Electrophoretograms for Çökelek cheeses (F1-F10: Fresh Çökelek samples, S1-S10: Skin-ripened Çökelek samples)

Fresh and skin-ripened Çökelek cheeses possessed distinct colours that could even be differentiated with the eye (Figure 3). Skin ripened samples had higher mean yellowness value (7.89) than the fresh ones (5.19). This could be explained biochemical reactions, especially Maillard, triggered by temperature, oxygen and light exposure during the ripening periods. When fresh and skin-ripened Çökelek samples were compared, the principle colour difference was found to be in L^* value corresponding to brightness (Table 4). The white colour of cheese is mainly a result of reflection of light by the casein micelles clustered together and as cheese ages this cluster becomes less intact and more soluble due to the proteolysis occur. The decrease in brightness value in ripened Çökelek can be explained with transformation of casein into a more soluble form as ripening proceeds (Johnson, 1999). The yellowness and redness values increase with ripening therewithal. The increase in redness values with ripening could be attributed to the products of biochemical reactions mentioned above during prolonged ripening period. Aloğlu et al. (2012) determined the L^* , a^* and b^* values of Minci cheese (a type of Çökelek) as 92.92-1.2 and 11.25, respectively. This difference might be arisen due the differences in the production methods and compositional differences, especially in protein and fat contents.

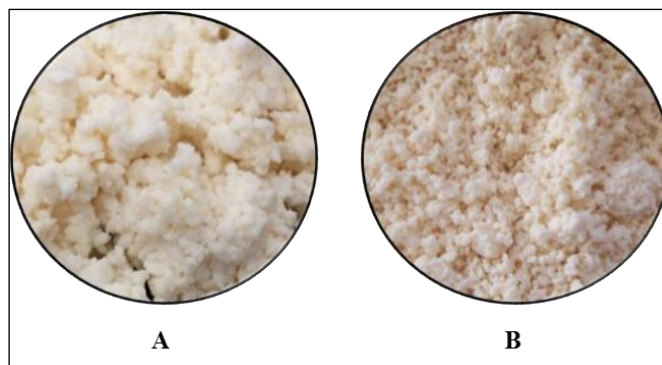


Figure 3. Physical appearances of fresh (A) and skin-ripened (B) Çökelek cheeses

Table 4. CIE colour values (L^* , a^* , and b^*) of Çökelek cheeses

Colour values ^c	Fresh Çökelek (n=10) ^a			Skin-ripened Çökelek (n=10)		
	Min.	Max.	$\bar{X} \pm SD^b$	Min.	Max.	$\bar{X} \pm SD$
L^*	92.24	95.45	93.93±0.88	85.14	91.82	89.73±2.12
a^*	2.29	3.06	2.67±0.27	2.585	3.615	3.09±0.34
b^*	3.515	7.55	5.19±1.29	5.355	11.715	7.89±1.77

^an-number of samples, ^b $\bar{X} \pm SD$ -mean±standard deviation, ^c L^* : 100=white, 0=black; a^* : +, red; -, green; b^* : +, yellow; -, blue.

Microbiological characteristics

None of the samples contained *E. coli*, coliform and fecal coliform bacteria (Table 5) contrary to the results by Öksüztepe et al. (2007). This indicates either there is no fecal contamination or the heat applied during production was high enough to destroy all coliforms. On the other hand, the yeast and mould counts varied between 6.41 and 7.89 log cfu/g for fresh, 7.99 and 8.51 log cfu/g for skin-ripened Çökelek samples. The yeast and mould counts were found similar to those reported by Tarakçi et al. (2003), Akkurt et al. (2005) and Öksüztepe et al. (2007) (1.09×10^8 , 7.75 log and 3.1×10^7 cfu/g, respectively) but higher than those reported by Öksüztepe et al. (2010), Onganer and Kirbag (2009) and Kavaz et al. (2012) (5.07, 6.67 and 5.62 log cfu/g). High yeast and mould counts are usually associated with inadequate hygiene conditions during production and storage. Also, the non-refrigerated open-air sale conditions in the farmer's markets give rise to the high yeast and mould counts determined in Çökelek cheeses.

Table 5. Microbiological analyses of Çökelek cheeses (log cfu/g)

Microorganisms	Fresh Çökelek (n=10) ^a			Skin-ripened Çökelek (n=10)		
	Min.	Max.	$\bar{X} \pm SD^b$	Min.	Max.	$\bar{X} \pm SD$
Yeast-Mould	6.41	7.89	7.26±0.53	7.99	8.51	8.13±0.16
Coliforms	-	-	-	-	-	-
Fecal coliforms	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-

^an-number of samples, ^b $\bar{X} \pm SD$ -mean±standard deviation.

Conclusions

The primary compositional differences between Çökelek samples were higher contents of total solids, salt, and ash found in the skin-ripened ones. This difference occurs mainly due to the moisture loss during ripening and salt addition for preservation purposes in skin-ripened ones. Values obtained from nitrogen fractions indicate that either protein degradations in Çökelek is limited and do not increase considerably with ripening or Çökelek cheeses sold as fresh are not fresh, in fact. Furthermore, no prominent differences were observed between the electrophoretograms of fresh and skin-ripened Çökelek samples. This is probably due to the Çökelek production method where no enzymes and starter cultures are used, and most of the natural microbiota and enzymes are destroyed by the heat application. However, a Çökelek production from the beginning and its ripening under controlled conditions are required in order to further understand the progress of proteolysis. Having no coliforms, fecal coliforms, and *E. coli* brings in a healthy and safe perspective to the Çökelek samples analyzed. On the other hand, the yeast and mould counts of the samples were found to be high indicating improper production, storage and marketing conditions. In order to increase the consumption of this traditional unique cheese, owing to its low-fat and high protein content, more hygienic and standardized production, storage and marketing conditions are required.

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