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## Some Properties of Fresh and Ripened Traditional Akcakatik Cheese

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**Abstract** Akcakatik cheese (yogurt cheese) is produced by drying strained yogurt with or without adding cloves or black cumin. The main objective of this study was to detect the properties of both fresh and ripened Akcakatik cheeses and to compare them. For this purpose the biogenic amine content, volatile flavor compounds, protein degradation level, chemical properties and some microbiological properties of 15 Akcakatik cheese samples were investigated. Titratable acidity, total dry matter, NaCl, total nitrogen, water soluble nitrogen, ripened index, histamine, diacetyl and acetaldehyde levels were found to be higher in ripened cheese samples than in fresh cheese samples. On the other hand, the clove and black cumin ratios were found to be higher in the fresh cheese samples. Sodium dodecyl sulphate polyacrylamide gel electropherograms of cheese samples showed that protein degradation was higher in ripened cheese samples than in fresh samples, as expected. The dominant Lactic acid bacteria (LAB) flora of Akcakatik cheese samples were found to be *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

**Keywords** akcakatik cheese (yogurt cheese), biogenic amine, chemical properties, microbiological properties, SDS-PAGE, volatile flavor compounds

## Introduction

Akcakatik cheese (yogurt cheese) is a traditional semi-hard cheese with a slightly sour taste that is made from strained yogurt produced with fatty cow's and/or goat's milk in the Western Mediterranean region of Turkey, especially in Burdur and Isparta provinces. It differs from other cheeses in that it is made from yogurt and produced by adding different spices. In appearance Akcakatik cheese is like Labneh or Labneh anbaris. However, it is harder than Labneh due to high levels of dry matter. Akcakatik cheese is consumed fresh or ripened. Fresh Akcakatik cheese is dried and matured in cloth bags to produce ripened Akcakatik cheese. Black cumin or clove-black cumin mixture may be added to the cheese to give flavor. These spices form a pleasing sharp taste and smell in the cheese (Kırdar, 2004; Simsek and Gun, 2009). In addition, the studies on clove and black cumin indicate

that these spices have antibacterial and antifungal activities (Burt and Reinders, 2003; Dorman and Deans, 2000; Pawar and Thaker, 2006; Ranasinghe et al., 2002; Viuda-Martos et al., 2007).

The production method used in all generically produced Akcakatik cheeses is as follows: cow's and/or goat's milk is used as the raw material. Skimmed milk is pasteurised at approximately 87°C and then cooled to fermentation temperature ( $42 \pm 2^\circ\text{C}$ ). Strained yogurt from the previous day is used as a starter. The fermentation procedure takes approximately 2.5-3 hours at  $42 \pm 2^\circ\text{C}$ . At the end of the fermentation, yogurt is kept at refrigerator temperature ( $+10^\circ\text{C}$ ) for one day. The next day, the yogurt is transferred to cloth bags for the production of strained yogurt. It is exposed to the straining procedure for 2-3 days until all the serum has dispersed. Then, salt and spices are added (cloves and black cumin are pestled). This mixture is transferred into cloth bags and pressed well. After the pressing procedure is complete, the cloth bags are stitched closed. The cheeses are kept in a cool place ( $8-10^\circ\text{C}$ ) for 3-4 months. The dried product is consumed in the winter months (Kirdar, 2004). Kirdar et al. (2017) analysed some chemical and microbiological characteristics of the product and found the content of Akcakatik cheese to be: dry matter (DM) content 71.38-85.02%, fat 21-34%, salt 3.96-9.28%, titratable acidity (TA) 1.36-2.95%, and yeast-mould content 1.99-3.70 log cfu/g. Simsek and Gun, (2009) reviewed some specifications and the free fatty acid composition of Akcakatik cheeses obtained from local markets and bazaars in Burdur province.

Although some specifications of Akcakatik cheeses were determined by these researchers, there is no information in the literature about whether the samples were taken from fresh or ripened Akcakatik cheeses. This research is the first reported comparison of some properties of fresh and ripened Akcakatik cheeses and the identification of LAB flora at the species level. The aim of this study was to detect the biogenic amine contents, volatile flavor compounds, protein degradation levels, chemical properties and some microbiological properties of fresh and ripened Akcakatik cheeses and to compare them.

## Materials and Method

### Cheese samples

In total, 15 Akcakatik cheese samples produced with cow's milk (approximately 600 g per sample) were analysed in this study. During the period June-September, seven fresh (not ripened) samples (named K1, K2, K3, K4, K5, K6, and K7) and eight ripened samples matured in cloth bags (named D1, D2, D3, D4, D5, D6, D7, and D8) for 2-4 months were collected from local markets in Burdur and Isparta provinces located in the Western Mediterranean region of Turkey. Cheese samples were transported and stored in the refrigerator at  $+4^\circ\text{C}$  until they were analysed.

### Chemical analysis

The percentage of titratable acidity, total dry matter (TS), fat and NaCl were determined according to Anonymous (1989). Titratable acidity of cheese samples was expressed as lactic acid percentage (LA%). Total nitrogen (TN) and water soluble nitrogen (WSN) contents were determined by the Kjeldahl method (Gripon et al., 1975). The ripening index (RI) was calculated from the ratio of soluble nitrogen to TN (Uraz and Simsek, 1998). The pH values of the cheeses were measured with a pH meter (WTW, Weilheim, Germany). The ratio of black cumin and clove was determined by modifying the Sagdic et al. (2003) method. The chemical analyses applied to the cheeses were repeated three times.

### Biogenic amine analysis

Sample preparation: 10 g Akcakatik cheese samples were treated with 25 mL of perchloric acid (Sigma-Aldrich 99%; 10 g/100 mL) in the stainless steel container of a Waring blender (8011 ES HGB2WTS3, Torrington, CT) for preparation of samples for biogenic amine analysis. Most of the fat was crystallised by cooling to 3°C and then the samples were centrifuged at 10,000 rpm for 10 min at 3°C (NF-1200R-Nuve-Turkey). The creamed layer was removed and the supernatant filtered. 5 mL of the filtrate was filtered with a sterile 0.45 µm syringe filter (Sartorius, Göttingen, Germany). Finally, this filtrate was diluted with distilled water to 50 mL and then 10 mL of this was injected into the column. The column was YMC-Pack ODS-AM C18 5 mm (250mm-4.6mm I.D.). The method given by Oner et al. (2006) was applied exactly without any modification.

Apparatus and chromatographic conditions: Shimadzu (Kyoto, Japan) system HPLC consisting of an LC 10 ADVP pump, DAD detector, autosampler [(SIL 10 AD VP (70 Vial Model Rack 7)] and oven (CTO 10 ACVP) was used. The mobile phase consisted of 30 mL acetonitrile/100 mL water at pH 8. The flow-rate was 1.1 mL/min.

Standards: Tyramine, 99% (Aldrich Chemical Co., T90344) Putrescine dihydrochloride, ≥98% (Sigma Chemical Co., P7505) Cadaverine dihydrochloride, >98% (Sigma Chemical Co., C8561) Histamine, ~97% (Sigma Chemical Co., H7125) β-phenylethylamine, 99% (Aldrich Chemical Co., 128945)

Standards Preparation: biogenic amine stock standard solutions at 1000 mg/L each by dissolving 100 mg of tyramine, 182.7 mg of putrescine dihydrochloride, 171.4 mg of cadaverine dihydrochloride, 96 mg of histamine, and 100 mg of phenylethylamine in separate 100-mL volumetric flasks. Bring each to volume with deionised water. Store stock solutions at 4°C and protect from light. Prepare working standard solutions for generating the calibration curve with an appropriate dilution of the stock solutions in 0.4 M perchloric acid. These solutions should be prepared fresh weekly and stored at 4°C when not in use.

The calibration curves for the biogenic amines are linear having  $r^2$  as follows: tryptamine  $r^2=0.9997$ , phenethylamine  $r^2=0.9998$ , putrescine  $r^2=0.9999$ , cadaverine  $r^2=0.9999$ , histamine  $r^2=0.9998$ , and tyramine  $r^2=0.9999$ .

### Volatile flavor compounds analysis

Volatile flavor compounds analysis was carried out by gas chromatography (Perkin Elmer Auto System XL) using an automatic head-space sampler (Turbo Matrix 16, Perkin Elmer). Two gram cheese samples from each treatment group were transferred into headspace vials (6 mL) and heated at 85°C for 5 min. Headspace vials were automatically injected on a CP WAX column (30m.x 0.32 mm I.D.x 1.2 mm film thickness; Perkin Elmer). The temperature was maintained at 35°C for 5 min. The flame-ionisation detector was set at 240°C and the injector at 180°C. Helium was used as the carrier gas (25 psi). Operating conditions were: the oven temperature was held at 5°C for 2 min and the temperature was increased in increments up to 240°C with a total cycle time of 20 min. Components were identified depending on their retention times by comparing with the retention times of corresponding standards. All samples were analysed in triplicate. Temperatures of transfer line, needle and vial oven of the HS method were adjusted to 120, 90 and 85°C, respectively. Injection, pressurise, withdrawal and thermostat times of the HS method were 0.08, 0.08, 0.5 and 5 min, respectively (Ulberth, 1991).

### Casein fractions analysis

Protein degradation levels of the seven fresh and eight ripened Akcakatik cheeses were evaluated by Sodium dodecyl

sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using a mini vertical gel system (E-C 120 mini vertical gel system E-C 250-90, EC Apparatus Corporation, USA) previously described by Laemmli (1970). Protein standards (Catalog number SDS7) were obtained from Sigma Chemical (St. Louis, MO) to identify degraded or breakdown products of protein molecules. Each sample was diluted with sample buffer to give a final concentration of 1 mg/ml protein. An acrylamide to bisacrylamide ratio of 30% w/v (29:1) was used and the acrylamide concentrations in the running and stacking gel were 12 and 5%, respectively. Samples were heated in boiling water for 3 min and 20 µl was applied to each well. Electrophoresis was performed at a constant 60 mA. Gels were stained with Coomassie Brilliant Blue R250 solution (Merck, Darmstadt, Germany). After the staining, gel was destained and preserved in 10% acetic acid solution. The developed gel pictures were used to quantify degradative products of casein fractions by an advanced computerised optical densitometer (UV Transilluminator 2000, Bio Rad, Italy) located at the Laboratory of Research Centre in Süleyman Demirel University, Isparta-Turkey. The final numerical values of each breakdown product of protein molecules were quantitatively analysed.

### Microbiological analysis

10 grams of each cheese sample were weighed and homogenised with 90 mL sterile saline solution (0.85% NaCl, w/v). Then 10-fold dilutions were made with the same solution. Total aerobic mesophilic bacteria (TAMB) were enumerated by spread on Plate Count Agar (Merck) incubated at 30°C for 72 h. LAB count was determined by spread on de Man, Rogosa and Sharpe Agar (Merck) containing 0.14% sorbic acid (w/v) incubated at 37°C for 48 h. Yeast-moulds were enumerated by spread on Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Merck) incubated at 25°C for five days. The coliform group bacteria count was determined by the pouring plate method. For the pour plate, a 1 mL sample was mixed with 15 mL melted Violet Red Bile Agar (VRB, Merck). VRB plates were incubated at 37°C for 48 h. *Escherichia coli* were enumerated by the double-layer plate method described by Cakir (1999). For double-layer plates, 1 mL sample was mixed with 15 mL melted Tryptic Soy Agar (TSA, Merck) and poured into sterile empty Petri plates. Then the plates were incubated at 35°C for 2 h. After the incubation, TSA plates were overlaid with 5 mL of VRB agar. Then the plates were incubated at 45.5°C for 24 h before counting.

### Phenotypic identification of LAB

For identification of LAB, isolates were randomly selected from MRS agar (Merck), M17 agar (Merck) and Neutral Red Chalk Lactose agar (NRCLA) (Harrigan and McCance, 1966). The isolates were tested by Gram staining, catalase and cultural tests growth in MRS broth (10°C, 15°C, 45°C and pH 9.6) and tolerance of different salt levels (4%, 6.5%, 8% or 10% NaCl) (Huggins, 1984; Holt et al., 1994). Gram positive, catalase-negative cocci and rods were selected as presumptive LAB and they were identified phenotypically based on the production of acids from saccharides and related compounds. The production of acids from saccharides and related compounds was investigated using API 50 CH (BioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions.

### Statistical analysis

The mean value and standard deviation were calculated from the data obtained from the three separate experiments. T-test was used to evaluate the significance levels of the data with SPSS/PC software (Version 15.0) for Windows (SPSS Inc., Chicago II, USA). The level of confidence required for significance was set at  $p < 0.05$  (Duzgunes et al., 1987).

## Results and Discussion

### Chemical characteristics of Akcakatik cheeses

The results of the chemical analyses applied to the samples (pH, titratable acidity, total dry matter, fat, NaCl, total nitrogen, water soluble nitrogen and clove and black cumin amounts) are given in Table 1. In this study, pH values of Akcakatik cheeses were determined between 3.68 and 4.29. The average of titratable acidity values of fresh and ripened Akcakatik cheeses was determined as  $2.30 \pm 0.62$  and  $2.67 \pm 0.31\%$ , respectively. There is no significant difference in the total dry matter of the samples between the groups. The average of total dry matter of fresh and ripened Akcakatik cheeses was found to be  $70.20 \pm 4.97$  and  $73.38 \pm 8.78\%$ , respectively. Fat and NaCl contents of cheese samples showed changes between 16.50 - 40% and 4.32 - 16.54%, respectively. Total nitrogen contents of the fresh and ripened cheeses were determined on average to be  $2.55 \pm 0.54$  and  $2.77 \pm 0.48\%$ , respectively. WSN values of fresh and ripened cheeses were detected on average as  $0.21 \pm 0.13$  and  $0.51 \pm 0.11\%$ , respectively. WSN levels were found to be high in all ripened cheese and two fresh cheese samples (K6 and K7). Ripening index of fresh and ripened cheeses was detected as  $7.85 \pm 3.41$  and  $18.35 \pm 1.75\%$ , respectively. The clove amounts of the cheeses were determined to be 0.06 g/ 10 g as the lowest, 0.47g/10 g as the highest and  $0.31 \pm 0.27\text{g}/10\text{ g}$  on average. The average of the clove and black cumin amounts in fresh cheese samples was found to be higher than ripened cheese samples. The black cumin amount of all cheese samples was determined to be lower when compared to the clove amount except for ripened cheese sample D2. The difference was determined as statistically significant in terms of titratable acidity (LA%), fat%, NaCl%, clove amounts (g/10 g), water soluble nitrogen% and ripening index% ( $p < 0.01$ ). Total nitrogen, water soluble nitrogen, and ripened index of ripened

**Table 1.** Chemical properties of fresh and ripened Akcakatik cheese samples

Samples	pH	Titratable acidity (LA%)*	Total dry matter (%)	Fat (%)*	NaCl (%)*	Total nitrogen (%)	Water soluble nitrogen (%)*	Ripening Index (%)*	Clove (g/10 g)*	Black cumin (g/10 g)
K1	4.09	2.04	67.86	26.33	11.28	2.14	0.13	5.95	0.38	0.14
K2	3.75	2.54	75.49	30.33	14.85	2.31	0.13	5.75	0.44	0.14
K3	3.77	2.07	77.55	37.00	4.32	1.96	0.07	3.80	1.17	0.05
K4	3.97	1.50	65.84	27.83	8.08	2.52	0.13	5.11	0.47	0.22
K5	3.88	2.61	71.26	40.00	10.34	2.28	0.24	10.75	0.39	0
K6	4.29	2.76	62.66	36.00	9.40	3.15	0.43	13.56	0.12	0.03
K7	3.68	2.56	70.73	34.83	13.53	3.53	0.35	9.99	0.12	0.01
Average	$3.92 \pm 0.21$	$2.30 \pm 0.62$	$70.20 \pm 4.97$	$33.18 \pm 5.08$	$10.26 \pm 3.33$	$2.55 \pm 0.54$	$0.21 \pm 0.13$	$7.85 \pm 3.41$	$0.44 \pm 0.33$	$0.08 \pm 0.08$
D1	3.76	1.94	61.68	20.50	16.54	2.10	0.41	19.74	0.21	0.15
D2	4.02	2.82	61.84	16.50	6.39	2.63	0.43	16.28	0.06	0.07
D3	4.04	2.61	64.28	27.50	13.72	2.00	0.32	16.27	0.12	0.07
D4	3.91	2.90	79.74	32.16	14.28	3.05	0.55	18.09	0.20	0.08
D5	3.89	2.61	76.84	22.66	11.46	2.80	0.49	17.33	0.12	0.02
D6	3.83	2.72	77.74	28.50	13.72	3.12	0.67	21.63	0.33	0.03
D7	3.69	2.96	82.63	40.00	11.28	3.40	0.63	18.41	0.15	0.02
D8	3.72	2.83	82.32	36.00	12.03	3.12	0.59	19.06	0.43	0.02
Average	$3.86 \pm 0.12$	$2.67 \pm 0.31$	$73.38 \pm 8.78$	$31.86 \pm 6.20$	$12.43 \pm 2.87$	$2.77 \pm 0.48$	$0.51 \pm 0.11$	$18.35 \pm 1.75$	$0.20 \pm 0.12$	$0.06 \pm 0.05$

K1-7: Fresh Akcakatik cheeses; D1-8: Ripened Akcakatik cheeses.

\*: Statistically significant different  $p < 0.05$ .

cheese samples showed that protein degradation was higher in ripened Akcakatik cheese samples than in fresh samples.

pH value was in conformity with the findings of Simsek and Gun (2009). These researchers reported that lactic acid and dry substance contents of Akcakatik cheeses were approximately 0.866 and 73.96%, respectively (Simsek and Gun, 2009). In addition, Kırdar (2004) detected the lactic acid and dry substance contents of Akcakatik cheeses at between 0.9-2.16 and 24.5-59.63%, respectively. NaCl results of this study were found to be higher than Simsek and Gun (2009) and similar to Kırdar (2004). Labneh contains 6.42-10.70% fat, 14.12-16.47% milk solids (non-fat), 20.54-24.61% TS, 1.07-1.33% minerals (ash) and a pH range of 3.67-4.05 (Salji et al., 1983). Guler and Uraz (2003) reported that the total nitrogen content of Tulum cheese was found to average 3.34% and its minimum and maximum values were 2.81 and 4.02%, respectively. Dinkci et al. (2012) indicated that the WSN value of Tulum cheese was 0.56% and the ripening index was 16.86%. Oner et al. (2003) reported that the WSN and ripening index of the Tulum cheeses were 0.98 and 26.51%, respectively. Renner (1983) indicated that the ripening index of a cheese is between 10-60%, depending on the cheese type, when its maturation has not completed. Laban zeer produced in Egypt and considered as a nutritious fermented dairy product was found to be rich in essential amino acids and total protein averaged 17.14% and 8.77% soluble N, respectively (Ibrahim et al., 1999). Chemical analysis results of Akcakatik cheese were lower than the results of Tulum, Labneh and Laban cheeses.

### Biogenic amine levels of Akcakatik cheeses

Biogenic amine levels of fresh and ripened Akcakatik cheese samples are given in Table 2. Phenyletilamine, putrescine and cadaverine were not determined in any cheese samples. On the other hand, tryptamine was detected on average as  $0.03 \pm 0.01$  and  $0.02 \pm 0.00$  mg/kg, respectively. In general, histamine levels in ripened cheese samples were found to be

**Table 2.** Biogenic amine levels of fresh and ripened Akcakatik cheese samples

Samples	Biogenic Amines (mg/ kg)					
	Tryptamine	Phenyletilamine	Putrescine	Cadaverine	Histamine	Tyramine
K1	0.03	-	-	-	10.3	-
K2	0.03	-	-	-	7.06	-
K3	0.04	-	-	-	9.19	-
K4	0.03	-	-	-	5.17	-
K5	0.02	-	-	-	18.9	-
K6	0.03	-	-	-	7.85	-
K7	0.03	-	-	-	3.55	-
Avarage	$0.03 \pm 0.01$	-	-	-	$8.86 \pm 4.73$	-
D1	0.03	-	-	-	13.7	-
D2	0.02	-	-	-	5.80	-
D3	0.02	-	-	-	8.94	-
D4	0.02	-	-	-	8.55	0.05
D5	0.02	-	-	-	21.7	-
D6	0.02	-	-	-	23.2	0.03
D7	0.02	-	-	-	6.94	-
D8	0.02	-	-	-	44.6	0.03
Avarage	$0.02 \pm 0.00$	-	-	-	$16.64 \pm 12.45$	$0.013 \pm 0.01$

K1-7: Fresh Akcakatik cheeses; D1-8: Ripened Akcakatik cheeses.

higher than in fresh cheese samples. Maximum and minimum histamine levels in ripened cheese samples were detected as 5.80 and 44.6 mg/kg, respectively. In addition, tyramine was detected in only three of the ripened cheese samples (D4, D6 and D8) with an average of  $0.013 \pm 0.01$  mg/kg.

Butikofer et al. (1990) carried out studies on Gorgonzola, Emmental, Camembert, Roquefort and Feta cheeses and reported that Gorgonzola cheese was found to have the highest biogenic amine content with 4 mg/kg phenylethylamine, 69 mg/kg putrescine, 120 mg/kg cadaverine, 123 mg/kg histamine and 822 mg/kg tyramine. Durlu-Ozkaya et al. (2000) indicated that phenylethylamine was detected at the highest level (average 33.2 mg/100 g) among the biogenic amine contents in Tulum cheese. It is known that 70-1,000 mg histamine taken orally leads to clinical intoxication (Edwards and Sandine, 1981; Chang et al., 1985). The results of this study showed that histamine levels in fresh and ripened Akcakatik cheese samples were below the toxic level.

### Volatile flavor compounds of Akcakatik cheeses

As a result of analysis of Akcakatik cheese samples with GC/HS, aroma components detected at their highest amounts are given in Table 3. Volatile flavor compound analysis showed that acetaldehyde, diacetyl, ethanol and ethyl acetate are major carbonyl compounds in Akcakatik cheese samples. Acetaldehyde, diacetyl, ethanol and ethyl acetate in ripened cheese were detected on average as  $31.62 \pm 3.23$ ,  $73.42 \pm 57.24$ ,  $8.04 \pm 7.60$  and  $60.21 \pm 68.66$  ppb, respectively. According to statistical analysis, there is no statistically significant difference between the carbonyl compounds of the groups. 2-butanone and acetone were not detected. Carbonyl compounds of the Akcakatik cheese samples in this study were detected at lower levels than the results of Atamer et al. (2004) who analysed carbonyl components in the yogurts.

**Table 3.** Volatile flavor compounds of fresh and ripened Akcakatik cheese samples (ppb)

Samples	Carbonyl Compounds			
	Acetaldehyde	Diacetyl	Ethanol	Ethyl acetate
K1	27.67	134.84	14.48	385.85
K2	30.88	66.69	15.61	173.59
K3	32.98	32.23	0.27	2.94
K4	30.65	44.28	7.69	6.31
K5	29.91	27.29	15.85	4.15
K6	29.36	3.78	10.01	1.33
K7	32.30	13.43	1.62	13.43
Average	$30.53 \pm 1.78$	$46.07 \pm 44.17$	$9.36 \pm 6.49$	$83.94 \pm 147.16$
D1	28.50	60.48	20.44	60.48
D2	32.55	199.16	17.59	199.16
D3	27.95	73.51	13.22	73.51
D4	32.54	97.12	1.19	3.28
D5	28.95	57.99	7.20	80.56
D6	37.08	37.89	0.99	ND
D7	33.60	18.43	2.83	1.33
D8	28.68	29.84	13.29	3.47
Average	$31.62 \pm 3.23$	$73.42 \pm 57.24$	$8.04 \pm 7.60$	$60.21 \pm 68.66$

K1-7: Fresh Akcakatik cheeses; D1-8: Ripened Akcakatik cheeses.

### Casein fractions of Akcakatik cheeses

The composition of  $\alpha$ ,  $\beta$  and  $\gamma$ -caseins formed from other peptides (%) are given in Table 4. These values were obtained by taking the average of the areas on the gel of two different gel bands. In general, the composition of the samples belonging to  $\alpha$ -casein in ripened Akcakatik cheese was detected as  $27.30 \pm 2.25\%$ .  $\beta$ -casein and  $\gamma$ -casein compositions were detected as  $29.61 \pm 0.75$  and  $43.43 \pm 2.94\%$ , respectively. When the findings were statistically reviewed, it was observed that the difference among the groups was significant ( $p < 0.01$ ). While no difference was detected between  $\alpha$  and  $\beta$  casein ratios of ripened and fresh cheese samples, the difference between data formed from  $\gamma$ -caseins peptides of ripened samples was detected as significant. However, an increase was observed in peptide compositions while  $\alpha$  and  $\beta$  casein compositions decreased in the samples during the ripening. The protein slabs obtained from SDS-PAGE showed that all of the Akcakatik cheeses were made from cow's milk.

**Table 4.**  $\alpha$ -casein,  $\beta$ -casein,  $\gamma$ -casein and peptide composition of Akcakatik cheese samples

Samples	$\alpha$ -casein (%)	$\beta$ -casein (%)	$\gamma$ -casein and peptides (%)*
K1	30.51	31.51	37.98
K2	31.79	33.24	34.97
K3	29.10	29.91	40.99
K4	31.50	32.36	36.14
K5	29.49	31.01	39.50
K6	29.21	30.98	39.81
K7	30.95	32.08	36.97
Average	$30.36 \pm 1.10$	$31.58 \pm 1.08$	$38.05 \pm 2.16$
D1	29.83	30.23	39.94
D2	28.82	30.02	41.16
D3	28.99	30.77	40.24
D4	28.40	29.77	41.83
D5	24.10	28.77	47.13
D6	24.97	28.70	46.11
D7	24.88	28.92	46.20
D8	28.41	29.69	44.90
Average	$27.30 \pm 2.25$	$29.61 \pm 0.75$	$43.43 \pm 2.94$

K1-7: Fresh Akcakatik cheeses; D1-8: Ripened Akcakatik cheeses.

\*: Statistically significant different ( $p < 0.05$ ).

Hassan El-Deeb (1988) detected the lowest  $\alpha$ -casein ratio as 40.1% in Roquefort cheese and the highest ratio as 55.3% in Kariesh cheese among nine commercial mature cheeses. Hernandez et al. (1988) reported that while the  $\alpha$ -casein ratio of soft goat cheeses was detected as 21.1% on the second day of maturation, this ratio decreased to 13.1% at the end of the 28<sup>th</sup> day. While  $\alpha$ -casein ratio was detected as 61.4% in the kulek cheeses on the first day, it was detected as 49.4% at the end of the 90<sup>th</sup> day (Dervisoglu and Yazici, 2001). While  $\beta$ -casein ratio is 29.9% in soft cheeses at the beginning of maturation, it decreases to 21.7% at the end of the 28<sup>th</sup> day (Hernandez et al., 1988). The decrease in casein compositions ( $\alpha$ - and  $\beta$ -casein), which is evidenced by the maturation reported by other researchers, is similar to the casein composition of Akcakatik cheese. Degradation of casein is probably the most important biochemical event during the ripening of most cheese varieties, with a major impact on flavor and texture (Fox, 1989). In this study,  $\gamma$ -caseins and peptides showed an

increase at the maturation of Akcakatik cheese. As  $\gamma$ -caseins are fragmentation products of  $\beta$  caseins they increase depending on fragmentation of the  $\beta$  caseins during maturation. It was shown that:  $\beta_a$  contains  $\gamma_1$ -casein [ $\beta$ -casein (29–209)] and the correlated peptide [ $\beta$ -casein (30–209)];  $\beta_b$  contains  $\gamma_2$ -casein [ $\beta$ -casein (106–209)] and  $\gamma_3$ -casein [ $\beta$ -casein (108–209)] (Mayer, 1996; Giaschi et al., 2001),

### Microbiological characteristics of Akcakatik cheeses

Microbiological analysis results of Akcakatik cheese samples are given in Table 5. Microbiological analysis results were not found to have statistically significant differences ( $p>0.05$ ) between fresh and ripened cheeses. The results of microbiological analysis showed that TAMB counts of fresh and ripened cheese samples ranged from 7.21 to 8.13 and from 7.12 to 8.52 log cfu/g, respectively. When compared with our results, lower TAMB counts were reported by Kırdar et al. (2017) for ripened Akcakatik cheese samples collected from Burdur province in Turkey. On the other hand, some researchers reported a high count of TAMB in traditional Turkish cheeses such as Urfa (Yetişmeyen and Yıldız, 2003), Coklek (Onganer and Kirbag, 2009) and Aho (Temiz and Kılıç, 2016), as confirmed in this study. Yetişmeyen and Yıldız (2003) found that the average TAMB number of Urfa cheese samples is  $1.0 \times 10^9$  cfu/g. Onganer and Kirbag (2009) found that the average count of TAMB in Coklek cheese samples was determined as  $8.49 \pm 0.79$  log cfu/g. Similarly, Temiz and Kılıç (2016) reported that the TAMB count of Aho cheese, a traditional Turkish cheese made from cow's milk, ranged from 6.20 log cfu/g to 7.44 log cfu/g, and the highest value was determined as 7.58 log cfu/g. Bacterial contamination of raw milk usually originates from the environment, udders and milking equipment (Rysha et al., 2014). The high TAMB number in both fresh and ripened Akcakatik cheeses is worrying for consumer health because pathogen bacteria can be found within the number assessed as TAMB.

**Table 5.** Microbiological analysis results (log cfu/g) of Akcakatik cheese samples

Samples	TAMB	LAB	Yeast-moulds	Coliform*	E. coli*
K1	8.13	7.89	7.71	<1.00	<1.00
K2	7.79	7.69	7.14	<1.00	<1.00
K3	7.77	7.26	7.55	<1.00	<1.00
K4	7.73	7.09	7.61	<1.00	<1.00
K5	7.72	6.66	6.54	2.00	<1.00
K6	7.27	7.24	5.55	<1.00	<1.00
K7	7.21	7.16	6.72	<1.00	<1.00
Average	$7.66 \pm 0.30$	$7.28 \pm 0.38$	$6.98 \pm 0.73$	-	-
D1	8.36	8.28	7.48	<1.00	<1.00
D2	8.21	8.02	7.63	<1.00	<1.00
D3	8.50	8.23	8.11	<1.00	<1.00
D4	7.12	6.64	6.88	<1.00	<1.00
D5	7.15	6.82	6.85	<1.00	<1.00
D6	7.92	7.83	7.01	<1.00	<1.00
D7	8.52	8.15	8.20	<1.00	<1.00
D8	8.32	7.90	8.04	<1.00	<1.00
Average	$8.02 \pm 0.55$	$7.74 \pm 0.61$	$7.53 \pm 0.54$	-	-

K1-7: Fresh Akcakatik cheeses; D1-8: Ripened Akcakatik cheeses; TAMB: Total aerobic mesophilic bacteria; LAB: Lactic acid bacteria.

\*: In calculating the average of <1 log cfu/g value has been recognized as 0.

In fresh and ripened Akcakatik cheeses, the average numbers of LAB were detected as  $7.28 \pm 0.38$  and  $7.74 \pm 0.61$  log cfu/g, respectively. LAB play an important role in the production of a variety of dairy products probably through three major biochemical conservations of milk components glycolysis, proteolysis and lipolysis, so contributing to their typical taste and flavor (Leroy and De Vuyst, 2004; Steele et al., 2013; Chen et al., 2017).

Yeast-mould count of fresh and ripened cheese samples ranged from 5.55 to 7.71 and from 6.85 to 8.20 log cfu/g, respectively. These results showed that ripened cheese samples had higher yeast-mould counts than fresh cheese samples. Conversely to our results, yeast-mould count was determined on average to be  $2.04 \pm 0.19$  log cfu/g by Kırdar et al. (2017) for ripened Akcakatik cheese samples. Our results showed that all fresh and ripened Akcakatik cheese samples had higher yeast-mould counts than allowed in the Turkish Food Codex. According to the Turkish Food Codex, a maximum count of 100 cfu/g of yeast-moulds is allowed in cheese and yogurt (Anonymous, 2010). However, high yeast-mould counts were detected in other types of traditional Turkish cheeses such as Urfa (Yetişmeyen and Yıldız, 2003), Carra (Aygun et al., 2005), Cokelek (Onganer and Kirbag, 2009), and Aho (Temiz and Kılıç, 2016), as confirmed in this study.

Coliform group bacteria were detected only in sample K5 (2.00 log cfu/g). In the other samples, coliform group bacteria were found below the level of 1.00 log cfu/g. *Escherichia coli* was not detected in all samples, as confirmed by Kırdar et al. (2017). The Turkish Food Codex suggests that dairy products should contain no more than 100 cfu/g coliform bacteria, and no *E. coli* (Anonymous, 2010). Both fresh and ripened Akcakatik cheese samples were found to be legal according to the Turkish Food Codex. Conversely to our results, *E. coli* was detected in some traditional Turkish cheeses such as Orgu (Turkoglu et al., 2003), Golot (Tuncturk and Ozdemir, 2005), Cokelek (Onganer and Kirbag, 2009) and Beyaz (Sener and Cakici, 2013).

### Phenotypic identification of LAB

A total of 60 presumptive LAB were isolated from Akcakatik cheese samples. The isolates were found to be Gram positive and catalase negative cocci (55%, 33 strains) or rods (45%, 27 strains). Coccoid-shaped 16 and 17 isolates were selected on M17 agar and NRCLA plates, respectively. All rod-shaped isolates were isolated on MRS agar plates. All isolates failed to grow in MRS broth at 10°C, 15°C, pH 9.6 and presence of 4%, 6.5%, 8% or 10% NaCl. On the other hand, they were grown in MRS broth at 45°C. The saccharide fermentation test results showed that dominant LAB species isolated from Akcakatik cheese samples are *Streptococcus salivarius* subsp. *thermophilus* (55%) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (31.67%). These results are not surprising because Akcakatik cheese is produced from yogurt. Yogurt is a fermented dairy product produced by the protooperative action of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Tamime and Robinson, 1985; Erkus et al., 2014). In addition, we also isolated five *Lactobacillus acidophilus* (8.33%), two *Lactobacillus delbrueckii* subsp. *lactis* (3.33%) and one *Lactobacillus helveticus* (1.67%) strains. These species were only isolated from ripened Akcakatik samples. Similar to our results, Roushan et al. (2014) identified *Lactobacillus helveticus* (14%), *Lactobacillus acidophilus* (6%) and *Lactobacillus delbrueckii* subsp. *lactis* (2%) from Iranian traditional yogurts by phenotypic methods.

## Conclusion

The biogenic amine contents, volatile flavor compounds, protein degradation, chemical properties and some microbiological properties of 15 Akcakatik cheeses (seven fresh and eight ripened) were reviewed. There was no

significant difference ( $p>0.05$ ) among pH, total dry matter and total nitrogen levels of fresh and ripened Akcakatik cheese samples. However, there was a significant difference among titratable acidity, fat, NaCl, water soluble nitrogen and the ripening index levels of fresh and ripened cheese samples. The histamine levels in all cheese samples were detected to be below the toxic level. Volatile flavor compound analysis showed that acetaldehyde, diacetyl, ethanol and ethyl acetate are major carbonyl compounds. Protein degradation level in ripened Akcakatik cheese samples was found to be higher than in fresh cheese samples, as expected. Microbiological analysis results were not found to be statistically significantly different ( $p>0.05$ ) between fresh and ripened cheeses.

Akcakatik cheese is a semi-hard cheese with a slightly sour taste that is made from strained yogurt produced in the Mediterranean region of Turkey by traditional methods. When the results obtained were examined, it was seen that Akcakatik cheese does not have a standard production period. Standardisation of the milk used, production procedures applied, packing and maturation are required with the aim of developing product quality.

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## References

- Anonymous. 1989. Beyaz Peyniri Standardı TS 591. Türk Standartları Enstitüsü. Necatibey cad. No:112. Bakanlıklar Ankara (in Turkish).
- Anonymous. 2010. Türk Gıda Kodeksi (TGK) Mikrobiyolojik Kriterler Tebliğinde Değişiklik Yapılması Hakkında Tebliğ. 08/01/2010 tarih ve 27456 sayılı Resmi Gazete, Tebliğ No:2009/68, Ankara (in Turkish).
- Atamer M, Şenel E, Öztekin ŞA. 2004. Traditional products: Yayık tereyağ conventional way of manufacturing and its some properties. Proceed. Int. Dairy Symp. Recent Developments in Dairy Science and Technology, Isparta-Turkey, pp 149-152.
- Aygun O, Aslantas O, Oner S. 2005. A survey on the microbiological quality of Carra, a traditional Turkish cheese. J Food Eng 66:401-404.
- Burt SA, Reinders RD. 2003. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett. Appl Microbiol 36:162-167.
- Butikofer U, Fuchs D, Hurni D, Bosset JO. 1990. Beitrag zur bestimmung biogener amine in kase. Mitt Gebiete Lebensm Hgy 81:120-133.
- Cakir İ. 1999. Koliform grubu bakteriler ve E. coli. In: Gıda mikrobiyolojisi ve uygulamaları. Akcelik M, Aydar LY, Ayhan K, Cakir İ, Dogan HB, Gurgun V, Halkman AK, Kaleli D, Kuleasan H, Ozkaya DF, Tunail N, Tukul C (eds), Armoni Matbaacılık Ltd. Şti., Ankara, pp 215-222 (in Turkish).
- Chang SF, Ayres JW, Sandine E. 1985. Analysis of cheeses for histamine, tyramine, tyriptamine, histidine, tyrosine and tyriptophane. J Dairy Sci 68:2840-2846.
- Chen C, Zhao S, Hao G, Yu H, Tian H, Zhao G. 2017. Role of lactic acid bacteria on the yogurt flavor: A review. Int J Food Prop 20:S316-S330.
- Dervisoglu M, Yazici F. 2001. Ripening changes of Kulek cheese wooden and plastic containers. J Food Eng 48:243-249.
- Dinkci N, Unal G, Akalın A S, Varol S, Gonc S. 2012. Chemical and microbiological properties of Kargi Tulum Cheese (in Turkish). Ege Üniv. Ziraat Fak Derg. 49:287-292.
- Dorman HJD, Deans SG. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol

88:308-316.

- Durlu-Ozkaya F, Ayhan K, Ozkan G. 2000. Biogenic amine determination in tulum cheese by high performance liquid chromatography (HPLC). *Milchwissenschaft* 55:27-28.
- Duzgunes O, Kesici T, Kavuncu O, Gurbuz F. 1987. Araştırma ve deneme metotları. A.Ü. Ziraat Fakültesi Yayınları: 1021, Ankara, Turkey pp 132 (in Turkish).
- Edwards ST, Sandine WE. 1981. Public health significance of amines in cheese. Symposium; microbial metabolites of importance in dairy products. *J Dairy Sci* 64:2431-2438.
- Erkus O, Okuklu B, Yenidünya AF, Harsa S. 2014. High genetic and phenotypic variability of *Streptococcus thermophilus* strains isolated from artisanal Yuruk yogurts. *LWT-Food Sci Technol* 58:348-354.
- Fox PF. 1989. Proteolysis during cheese manufacture and ripening. *J Dairy Sci* 72:1379-1400.
- Giaschi A, Beretta B, Poieshi C, Conti A, Giuffida MG, Galli LC, Rostani P. 2001. Proteolysis of  $\beta$ -casein as a marker of Grana Padano cheese ripening. *J Dairy Sci* 84:60-65.
- Grippon JC, Desmazeaud MJ, Et Le Beas D, Bergere JH. 1975. Role des micro-organismes et des enzymes du cours de la maturation. *Le Lait* 55:502-516.
- Guler Z, Uraz T. 2003. Proteolytic and lipolytic composition of Tulum cheeses. *Milchwissenschaft* 58:502-505.
- Harrigan FW, McCance EM. 1966. *Laboratory Methods In Microbiology*. Academic Press London, New York, pp 285.
- Hassan HN, El-Deeb SA. 1988. Electrophoretic patterns and thin-layer chromatography of common cheeses in Egypt: comparison and quantification. *Food Chem* 30:245-255.
- Hernandez M, Juarez M, Ramos M. 1988. A ripening and storage study of soft goat cheese with *penicillium candidum* on the surface. *Food Chem* 30(3):191-203.
- Holt GH, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. *Bergey's manual of determinative bacteriology*. Williams and Wilkins Co., Ninth Edition, USA, pp 787.
- Huggins RA. 1984. Progress in dairy starter culture technology. *Food Technology* 38:41-50.
- Ibrahim FS, Dabiza NMA, Abd-El-Lattief OA, Abd-El-Razik ST. 1999. Microstructure, amino acids content and microflora of farm house concentrated fermented milk Laban Zeer. *Egyptian J Dairy Sci* 27:291-300.
- Kırdar SS, Köse Ş, Yurdakul Ö, Ocak E. 2017. A survey on the microbiological and chemical characteristics of Akçakatik cheese in the West Mediterranean Region. *European International Journal of Science and Technology* 6:25-35.
- Kırdar SS. 2004. Akçakatik peyniri üretimi üzerine bir araştırma. *Proceed. Geleneksel Gıdalar Sempozyumu Van, Turkey*, pp 354 (in Turkish).
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685
- Leroy F, De Vuyst L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol* 15:67-78.
- Mayer HK. 1996. Electrophoretic ripening index for the evaluation of proteolysis and the deduction of the age of Parmesan cheese. *Z Lebensm Unters Forsch* 202:465-470.
- Oner Z, Karahan AG, Aloglu H. 2006. Changes in the microbiological and chemical characteristics of an artisanal Turkish white cheese during ripening. *LWT-Food Sci Technol* 39:449-454.
- Oner Z, Simsek B, Sagdic O. 2003. Determination of some properties of Turkish Tulum Cheeses. *Milchwissenschaft* 58: 152-154.
- Onganer AN, Kirbag S. 2009. Microbiological quality of "Cokelek" cheeses as a fresh consuming in Diyarbakır (in Turkish). *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 25:24-33.
- Pawar VC, Thaker VS. 2006. *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses* 49:316-323.
- Ranasinghe L, Jayawardena B, Abeywickrama K. 2002. Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et L.M. Perry against crown rot and anthracnose pathogens isolated from banana. *Lett Appl Microbiol* 35:208-211.
- Renner E. 1983. *Milk and Dairy Products in Human Nutrition*. Volkswirtschaftsverlag (450 pp.) München.

- Roushan ZS, Eskandari MH, Shekarforoush SS, Hosseini A. 2014. Phenotypic and genotypic diversity of dominant lactic acid bacteria isolated from traditional yogurts produced by tribes of Iran. *Iran J Vet Res* 15:347-352.
- Ryshka A, Marcov K, Frece J, Čvek D, Delaš F. 2014. A survey of the microbiological quality of Sharri, a hard mountain cheese from Kosovo. *Int J Dairy Technol* 67:277-282.
- Sagdic O, Simsek B, Kucukoner E. 2003. Microbiological and physicochemical characteristics of Van herby cheese, a traditional Turkish dairy product. *Milchwissenschaft* 58:382-385.
- Salji JP, Sawaya WN, Ayaz M. 1983. The yoghurt industry in the central province of Saudi Arabia. *Cultured Dairy Products Journal* 18:14-18.
- Sener A, Cakici N. 2013. Bacterial contamination in fresh white cheeses sold in bazaars Canakkale, Turkey. *International Food Research Journal* 20:1469-1472.
- Simsek B, Gun I. 2009. Free fatty acid composition of Akcakatik cheese, a traditional Turkish dairy product. *Asian J. Chem* 21:5923-5928.
- Steele J, Broadbent J, Kok J. 2013. Perspectives on the contribution of lactic acid bacteria to cheese flavor development. *Curr Opin Biotechnol.* 24:135-141.
- Tamime AY, Robinson RK. 1985. Yogurt: science and technology. In A. Y. Tamime (Ed.), Introduction (pp. 1-5). Great Britain: Pergamon Press.
- Temiz H, Kılıç S. 2016. A survey of the chemical, biochemical, microbiological and sensorial quality of Aho cheese, a traditional cheese from Eastern Black Sea Region, Turkey. *Int J Dairy Technol* 69:209-216.
- Tunctürk Y, Ozdemir, M. 2005. Production techniques and some chemical, biochemical and microbiological characteristics of Golot cheese (in Turkish). *Gıda* 30:167-172.
- Turkoglu H, Ceylan ZG, Dayisoglu KS. 2003. The microbiological and chemical quality of Orgu cheese produced in Turkey. *Pakistan Journal of Nutrition* 2:92-94.
- Ulberth U. 1991. Headspace gas Chromatographic estimation of some yogurt volatiles. *J Assoc Off Anal Chem* 74: 630-634.
- Uraz T, Simsek B. 1998. Ankara piyasasında satılan beyaz peynirlerin proteoliz düzeylerinin belirlenmesi. *Gıda* 23: 371-375.
- Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA. 2007. Antifungal activities of thyme, clove and oregano essential oils. *J Food Safety* 27:91-101.
- Yetişmeyen A, Yıldız F. 2003. Determination of microbiological, chemical and organoleptic properties of Urfa Cheese (in Turkish). *Gıda* 28:287-294.