

# A SURVEY ON THE MICROBIOLOGICAL AND CHEMICAL CHARACTERISTICS OF AKÇAKATIK CHEESE IN THE WEST MEDITERRANEAN REGION

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## Abstract

*Akçakatik cheese is characterized by high fat, high dry material content, salty, butter like aroma, obvious acidic taste. In this study, a total of 15 samples randomly selected from different producers of Akçakatik cheese in Burdur province of the Mediterranean Region were investigated for some of their chemical, biochemical and microbiological analyses. The average content of titratable acidity was found to be 2.24%, pH 3.96, fat 21.00%, dry matter 79.7%1, fat in solid 32.18%, salt 6.16 %, salt in dry matter 4.27%, and  $a_w$  value 0.75. The avarege values in Akçakatik samples were detected as 5.82 log cfu/g for total aerobic mesophilic bacteria, 4.52 log cfu/g for psicrophilic bacteria, 5.31 log cfu/g for Lactobacillus spp., 5.13 cfu log/g for Lactococcus spp., 2.02 cfu log/g for Staphylococcus-Micrococcus spp., 1.99 log cfu/g for coliforms, 2.02 log cfu/g for yeast and 2.05 log cfu/g for mould. E.coli and coagulase positive Staphylococcus were not detected in any samples.*

**Key Words:** Cheese, Cheese properties, Traditional product, Akçakatik, properties of Akçakatik

## 1. INTRODUCTION

Akçakatık cheese, which is matured in “Karın (goat or lamb skin sack), is a common cheese variety native to the district of Burdur province in Turkey. It is produced mostly from goat’s and cow’s milk or mixture of both. The cheese is generally made at homes by use of traditional methods and therefore may vary according to the individual methods of the producer. The manufacturing practices of Akçakatık cheese, in the absence of any rigorous control of temperature and relative humidity during storage, cause variations in the final quality of the product. The variation in Akçakatık cheese indicates the absence of any standardized method for the production of this cheese varieties. The name of the cheese is “aşçı katığı” as known by local population. Its name was changed in time to Akçakatık. Over time the name of cheese was settled in public language as Akçakatık (Kirdar 2004, Kirdar et al., 2013)

Akçakatık cheese is characterized by high fat, high dry material content, salty, butter like aroma, obvious acidic taste and a homogenous structure that is semihard and not easily broken. It is that resembled to labne with producing method and but it is produced locally from torba or süzme yoghurt (concentrated yoghurt) added clove, salt and black cumin (*Syzygium aromaticum*). The type of milk used, the method of production and the amount of salt in the cheese affect its colour. It ranges from cream to light yellow in colour. This cheese variety is usually produced during spring time and is buried in goat’s stomach between September and November for full maturation (Kirdar , 2004; Gün ve Simsek 2006)

The cow milk are used as raw materials. First the non-skimmed milk is boiled (85°C 30 dk), and then is cooled at 40°C. At this temperature, yoghurt is used to as starter culture. The fermentation process takes approximately 2.5-3 hours. After this period, yoghurt is kept for one night at refrigerator temperature (+10°C). The traditional yoghurt production is followed by the production of concentrated yoghurt. The yoghurt which is transferred into cloth bags (a perforated, woven cloth made from 100 % cotton) is exposed to drain for 2-3 days until all the serum is removed (Sometimes it can take up to 5 days). After the filtering process, the salt is added (3-5%). Then, the solid spice (cloves are pounded in a mortar and black seed) is performed. The mixture is filled into the lamb stomach by hand or “soku” compressed. After the compression of the abdomen, the mouths are sewn. Cheeses are kept outdoors and in the shade for 3-4 months. The awarege weight of the cheese in the sack was about 800 g. During this process, they are often turned inside out. The reason for using lamb’s abdomen is to extend the storage period of cheese and to ensure the formation of unique flavor of crips. The storage period of cheese in the lamb skin sack 3-6 months (Kirdar 2004).

A few types of cheese ripened in an animal skin sack are produced in certain geographic areas and can have different local and regional names such as “Tulum” (Turkey), “sir iz mis`ine” (Croatia) or “sir iz mjeha” (Bosnia and Herzegovina) (Kirdar and Gün, 2012, Tudor Kalit et al., 2010, Yilmaz et al., 2005, Kivanc, 1989).

The first process used in the Akçakatık cheese production for the preparation of the stomach is that the rough dirt of the lamb stomach is to be thoroughly cleaned. The process goes on until the roughness is removed by hand rubbing. Then the stomach is put in the calcium content. At the end of the process, the stomach which is washed with water and is made completely free from calcium content is washed with detergent and soapy water. After it is rinsed with water, it is kept in salty water for 2-3 days and inflated. It is left to dry for 5-6 days. A week later, the dry stomach is used in packing the cheese. Packaged and unpackaged views of Akçakatık are also given in Figure 1.



**Fig 1 Akçakatık cheese**

According to Kirdar (2004), the gross composition of the Akçakatık cheese was as follows: fat, dry matter salt and titratable acidity (% in lactic acid) were found as 10.3-30.2 %, 24.50-59.63 %, 5.38-9.12 %, 0.9-2.16 %, respectively. Very limited information is available in the literature of the production practices of Akçakatık cheese.

The ash content in Akçakatık samples was in the range 4.89-8.53 %. The mean concentration of 49.62, 417.74 and 2842.24 mg/kg was measured for Mg, K and Ca, respectively. The concentration of the tested metals was in the range Zn > Fe > Mn > Cu. The mean concentration of 3.36, 6.15, 12.62 and 13.92 mg/kg was measured for Cu, Mn, Fe and Zn, respectively. In our samples, tested levels were higher than reported in previous studies, except the elements Fe and Ca (Kirdar et al., 2013).

The saturated fatty acid, total unsaturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid of samples were found as  $61.87 \pm 4.067$ ,  $33.246 \pm 2.919$ ,  $30.728 \pm 2.648$  and  $2.519 \pm 0.516$  %, respectively. Palmitic acid levels were found to be the highest of the saturated fatty acid in all samples. Oleic acid content in all cheeses was also considerable higher than those of other unsaturated fatty acids (Gün and Şimşek, 2006).

The objective of this research is to determine it's the chemical, biochemical characteristics and microbiological properties of Akçakatık cheese and also to introduce the manufacture of this kind of traditional product.

## **2. MATERIALS AND METHODS**

15 ripened Akçakatık cheese samples (~250g each) were collected from different producers from Burdur province in the West Mediterranean Region. The age of the ripened cheeses was approx. 3 months.

The samples were transported in ice chest in sterile plastic bags to the laboratory under aseptic conditions. The microbiological and chemical analyses were started immediately after the samples were brought to the laboratory under cold conversion condition (4°C). The analyses were carried out in triplicate.

### **2.1. Physico-chemical analysis**

The pH of the samples was determined using a pH meter (Hanna instruments, Padova, Italy) and Titratable acidity was determined according to the AOAC methods (A.O.A.C., 1980). Fat was determined by the Gerber-Van Gulik method (IDF, 1997). Total solids content was determined by gravimetric method using the oven drying in a laboratory oven at 105°C until constant weight (IDF, 1982). NaCl was determined as suggested by the International Dairy Federation (1979). The values of water activity ( $a_w$ ) was determined using a water activity meter (TESTO-650) (Lang and Sternberg 1980). Duplicate tests were performed for each analysis.

## 2.2. Microbiological analysis

Cheese samples (25 g) were homogenized using a Stomacher 400 with 225 ml of sterile buffered peptone water for at least 2 minutes. Decimal dilutions were prepared in using the same diluent. All selective media were prepared according to the the manufacturers' instructions.

The total aerobic mesophilic bacteria, psychrophilic bacteria, *Lactobacillus* spp., *Lactococcus* spp., *Staphylococcus-Micrococcus*, *Enterobacteriaceae*, coliform bacteria, *E.coli*, *Enterococcus* spp., yeast and mould were quantified on Akçakatik cheese.

Plate Count Agar (PCA) was used to determine total aerobic mesophilic bacteria and psychrophilic bacteria that had been incubated for 48 hours at 35°C and 10 days at 7°C, respectively (Peeler and Maturin, 1992). Baird-Parker agar was used for *Staphylococcus- Micrococcus* spp. at 35 °C for 48 hours. Coagulase-positive staphylococci on Baird-parker agar base supplemented with egg yolk tellurite emulsion, incubated 37°C 48 h (BAM, 2001). Violet Red Bile Dextrose Agar (VRBDA) was used for determination of *Enterobacteriaceae* which had been incubated at 37 °C for 48 hours. The count of the total number of coliforms was performed on standard Violet Red Bile agar (VRBA) that had been incubated at 35±1°C for 24–48 hours (APHA, 1976, ICMSF, 1983). Positive cultures were used to create sub-cultures on Eosin Methylene Blue Lactose Sucrose (EMBLs) agar. They were incubated at 35±1°C for 24 hours. *E. coli* isolates were biochemically characterized by IMVIC tests (MacFaddin, 1991). Potato Dextrose Agar (PDA) was used for determination of yeasts and moulds, which were incubated at 25°C for 5 and 7 days, respectively (BAM, 2001). Enterococci was grown on Slanetz-Bartley Agar plates (SBA) and incubated at 37°C for 48 hours (Facklam and Sahm, 1995). De Man, Rogosa, Sharpe agar (MRS) was used for *Lactobacillus* spp (Dupont et al., 2000) and M17 agar for *Lactococcus* spp. that was incubated at 30 °C for 72 hours (Terzaghi and Sandine, 1975). Identification of the isolates was performed using the criteria of the Bacteriological Analytical Manual (BAM, 2001). All of the media were obtained from Oxoid (Unipath Ltd., Basingstoke, England). All of the selective media were prepared according to the manufacturers' instructions. The samples were analyzed in the Laboratory of Veterinary Faculty of Mehmet Akif Ersoy University. Microbiological analyses were carried out in triplicate.

## 2.3. Statistical analysis

All of the statistical analyses were performed using SPSS Statistical Software (SPSS 19 version, Netherlands). The obtained values were presented as the mean ± standard deviation (SD). Evaluation of significance was performed using analysis of variance followed by Duncan's multiple range tests. The level of significance of differences was determined at  $p < 0.05$  and  $p < 0.01$ . Colony counts were converted to log cfu/g (Draper and Smith, 1998).

## 3. RESULTS AND DISCUSSION

### 3.1. Physico-chemical properties

The results of some chemical and biochemical properties of the Akçakatik samples are presented in Table 1. There were wide ranges in the compositional factors. This seems to indicate that there is no standardized production method for Akçakatik cheese. The cheese is generally made at homes by use of traditional methods and therefore may vary according to the individual methods of the producer.

**Table 1:** Some chemical and physical characteristics of Akçakatık cheese (n=15)

Chemical parameter	M±SD	Minimum	Maximum
%L.A.	2.40±0.5	1.36	2.95
pH	3.96±0.26	3.65	4.56
Fat (%)	21.00±3.96	21.00	34.00
Salt(%)	6.16±1.63	3.96	9.28
Dry matter (%)	79.71±4.92	71.38	85.02
Fat in dry matter(%)	32.18±4.42	24.78	40.90
Salt in dry matter(%)	4.27±1.14	2.87	7.07
Water activity(a <sub>w</sub> )	0.7464±0.04	0.6878	0.8334

Acidity of the Akçakatık samples was obtained 2.40% as average, which has effect on the taste and flavor of the product. In this case, it can be said that Akçakatık is an acidic food (the foods having pH values between 3.65 and 4.56) when pH values were considered. These results can be originated from different production techniques and different storage conditions. Dry matter contents of the samples were found in a wide range (between 71.38-85.02%). High content of dry matter and low amount of fat in the samples resulted with a hard texture. These results were in agreement with the finding of Yıldız (2003) Çelik et. al. (2001).

Fat is among one of the important constituents of milk and contributes quality, taste and flavor and nutritional value to the product. Fat content was obtained an average of 21.00%. Akçakatık is a medium fat cheese category. The fat ratio of Akçakatık samples showed a great variation. The difference in the fat content may be due to the fact that this product does not have a standard production technique and also due to different milk used in cheese making. Akçakatık cheese includes lower fat than our traditional cheese such as White cheese, Kashar cheese, Tulum cheese, Mihalic cheese, Van Herby cheese, Civil cheese (Kaptan and Büyükkılıç 1983, Kurt and Akyüz, 1984, Ergüllü et al., 1998). The results of our study are in accord with the study Yıldız (2003).

Salt is one of the important ingredients that contribute to taste and flavor development (Çelik and Turkoglu, 2007). Salt content in Akçakatık samples was high. It was found that the salt ratio of Orgu cheese and Lavas cheese was consonant with the current results, except Abaza cheese (Uysal et al., 1998). These results were in agreement with the findings of Koçak et al., (1997). Considering salt content, great differences, both among the producers and between the production periods, were observed due to the different amounts of salt used by each producer. There is a positive correlation between the dry-matter values of cheese samples and the fat rates on the level of  $p < 0.01$ . The correlation coefficient between fat values and the salt values in dry matter was calculated as  $r = 0.360$  and was found to be significant on the level of  $p < 0.05$ . As the result of the statistical evaluation, a negative correlation was determined between salt and pH on the level of  $r = -0.599$  ( $p < 0.01$ ).

Water activity ( $a_w$ ) is an important physico-chemical parameter which influences the microbiological and the biochemical evolution during the cheese ripening process (Saurel et al., 2004). According to the values of water activity, a negative correlation was determined between  $a_w$  value and titration acidity ( $r = -0.69$ ), and the correlation was found to be significant on the level of  $p < 0.01$ . A positive correlation ( $r = 0.633$ ) was found between  $a_w$  value and dry matter, and the correlation is significant on the level of  $p < 0.01$ . These results were in agreement with the findings of Maraş cheese (Tekinşen and Nizamlioğlu 2003).

### 3.2. Microbiological properties

In this study, fifteen Akçakatık were examined for total aerobic mesophilic bacteria, psychrophilic bacteria, *Lactobacilli* spp., *Lactococci* spp., Staphylococci-Micrococci, coliform bacteria, *E.coli*, yeast and mold. The results of some microbiological quality of the Akçakatık were given in Table 2, the correlation between the various microorganisms, acidity, pH, aw and salt values were given in Table 3 and the correlation between physical and chemical properties were given Table 4.

**Table 2:** Presence of microorganisms in the Akçakatık (log cfu/g) (n=15)

Microorganisms	M±SD	Minimum	Maximum
<b>Total aerobic mesophilic bacteria</b>	5.82±1.31	4.08	7.20
<b>Psychrophilic</b>	4.52±1.33	3.00	6.90
<i>Lactobasillus spp.</i>	5.31±1.01	3.30	6.68
<i>Lactococcus. spp</i>	5.13±1.08	3.30	6.81
<i>Staphylococcus-Micrococcus</i>	2.02±0.19	1.99	3.30
<b>Coliform bacteria</b>	1.99±0.00	1.99	1.99
<b>Yeast-Mold</b>	2.04±0.19	1.99	3.70

**Table 3:** The correlation between the various microorganisms and chemical properties of Akçakatık

	TAMB	Psychrophilic	<i>Lactobacillus spp</i>	<i>Lactococcus spp</i>
<b>Psychrophilic bacteria</b>	0.810**	-	-	-
<i>Lactobasillus spp.</i>	0.916**	0.866**	-	-
<i>Lactococcus spp.</i>	0.877**	0.840**	0.880**	
<b>a<sub>w</sub></b>	0.434**	0.383**	0.399*	0.565**
<b>Titrateable acidity (%LA)</b>	-0.17	-0.13	-0.12	0.296*
<b>pH</b>	0.501*	0.13	0.301*	0.420**
<b>Salt</b>	-0.638**	-0.367**	-0.586**	-0.406**

\*p<0,05, \*\* p<0,01

**Table 4:** The correlation between physical and chemical properties of Akçakatık

	<b>Titrateable acidity</b>	<b>pH</b>	<b>Fat</b>	<b>Salt</b>	<b>Dry matter</b>	<b>Fat in dry matter</b>	<b>Salt in dry matter</b>
<b>pH</b>	0.606**	--	-	-	-	-	-
<b>Fat</b>	0.354*	0.591**	-	-	-	-	-
<b>Salt</b>	0.038	-0.599**	-0.327**	-	-	-	-
<b>Dry matter</b>	0.2	0.28	-0.529**	-0.417**	-	-	-
<b>Fat in dry matter</b>	0.564**	0.676**	0.907**	-0.26	0.02	-	-
<b>Salt in dry matter</b>	-0.18	0.19	0.360*	0.1	0.01	0.321*	-
<b>A<sub>w</sub></b>	-0.69**	0.431**	0	0.04	0.633**	0.363*	-0.01

\*p<0,05; \*\* p<0,01

In the present study, the microbiological qualities of Akçakatık, a popular dairy product in Burdur province and the consumption of which plays an important role in nutrition of people of the West Mediterranean Region, were determined.

The total aerobic mesophilic bacteria (TAMB) counts ranged from 4.08 to 7.20 log cfu/g, with the average 5.82 log cfu/g (Table 2). It was pointed out that the high TAMB content of Akçakatık samples was due to the quality of raw milk used. A large number of TAMB can be explained by the sufficient change in the environmental conditions which happen during cheese storage and which allows the growth and multiplication of microorganisms, or it could also be due to unsanitary conditions during processing and handling of the cheese. Similar results were reported by Hamed et al. (1992). Similar findings were also reported for other types of cheese. TAMB counts of Akçakatık cheese were lower than those in Carra cheese, Herby cheese, Tulum (Şavak) cheese and White cheese, which are ripened Turkish cheese varieties made from unpasteurized milk (Kıvanc 1989, Aygun et al., 2005). The correlation coefficients between TAMB, psychrophilic bacteria, Lactococcus spp., Lactobacillus spp., yeast-mold, titration acidity, pH, water activity (aw) and salt % pertaining to the cheese samples are given in Table 3. In Akçakatık cheese, the numbers of psychrophilic bacteria were found to be well below the limit of the threat. It could be due to unsanitary conditions during processing and handling of the cheese. There is a positive correlation between the number of TAMB and psychrophilic bacteria on the level of  $r = 0.810$  ( $p < 0.01$ ). In other words; the higher the number of TAMB in cheeses, the higher the number of psychrophilic bacteria in parallel to this. Similar results were reported by Uğur (2001).

Lactic acid bacteria (LAB) count plays a fundamental role in ripening of cheese due to the lactic acid fermentation development, which is a required characteristic of dairy products. It was also determined that lactic acid bacteria formed the dominant flora. These results were in accordance with the findings of Coşkun et al. (2002), Anar et al. (2000).

There is a positive correlation between the number of *Lactobacillus spp.* and the number of TAMB on the level of  $r = 0.916$  ( $p < 0.01$ ) and the number of psychrophilic bacteria on the level of  $r = 0.866$  ( $p < 0.01$ ). Separately, a positive correlation between the number of Lactococcus and the number of TAMB on the level of  $r = 0.877$  ( $p < 0.01$ ) and the number of psychrophilic bacteria on the level of  $r = 0.840$  ( $p < 0.01$ ) and the number of lactobacilli on the level of  $r = 0.880$  ( $p < 0.01$ ) was determined.

*Staphylococcus aureus* may cause food-borne poisoning, when it exceeds  $1.0 \cdot 10^6$  cfu/g; therefore, the presence of *Staphylococcus aureus* is undesirable. The *S.aureus* counts in Akçakatık cheese were also higher than Turkish white cheese (1.30-1.70 log cfu/g) and Herby cheese (0.95 log cfu/g) (Aygun et al., 2005, Sengul et al., 2001). Coagulase positive *Staphylococci* was not detected in Akçakatık cheese.

Coliform group bacteria in foods are accepted hygiene index. The presence of coliform bacteria in cheeses is not desired because these cause structural defects in cheeses. The Turkish Food Codex mandates that dairy products must contain no more than 100 cfu/g coliform bacteria, and it must exclude *E. coli* (Anonymous 2010). All of the samples were legal as stated in the Turkish Food Codex. *E.coli* was not detected in 15 samples. Akçakatık can be accepted as a safe food.

The incidence of yeast- mould has been considered a common problem during ripening and refrigerated storage of the cheese. According to the Turkish Food Codex, a maximum 100 cfu/g yeast-mould is allowed. The counts were lower than the limit ( $< 100$  cfu/g) of the Turkish Food Codex. In these samples, yeast-mold counts were lower than those in Herby cheese, Tulum cheese, Carra cheese and White cheese (Kıvanc, 1989, Aygun et al., 2005). Its reason can be accredited to the higher content of salt and dry matter.

Since the inhibition effect of salt on microorganisms is known, the research results support this, as well. The correlation coefficients between salt and the number of TAMBs, psychrophilic bacteria, Lactococci and Lactobacilli are  $r = -0.638$ ,  $r = -0.367$ ,  $r = -0.586$  and  $r = -0.406$ , respectively. The negative correlation is significant on the level of  $p < 0.01$ . In other words, the increase in the amount of salt in cheese reduce the number of TAMBs, psychrophilic bacteria, Lactococci and Lactobacilli bacteria.

The correlation coefficient between the titration acidity and the number of *Lactococcus spp.*, on the other hand, is  $r = 0.296$ . The positive correlation proved to be significant on the level of  $p < 0.05$ . The correlation coefficient between pH and TAMB and *Lactobacillus spp.* is  $r = 0.501$ , and  $r = 0.301$ , respectively. The positive correlation is significant on the level of  $p < 0.01$ . That is to say, as the pH value in cheese increase, so does the number of TAMB and Lactobacilli increase along with it. On the other hand, there is a significant correlation between pH and the number of Lactococci on the level of ( $r = 0.420$ )  $p < 0.01$ .

### 3. CONCLUSIONS

Akçakatık is an important animal protein source in diet of people with low economical income in West Mediterranean Region in Turkey. Traditional Akçakatık cheese implies that the cheese is produced primarily by hand, in small batches, with particular attention paid to the tradition of the cheese maker's art, and thus by use of as little mechanisation as possible in the production of the cheese. For improving the microbiological quality of Akçakatık cheese, pasteurized milk should be used and the processing, ripening, storage and marketing should be carried out under good hygienic conditions. It is necessary to develop a specific starter culture for this cheese type. Salt content was found high in Akçakatık cheese samples; therefore, new studies should be made to reduce the amount. High dry matter and low fat content of the samples were distinct properties, resulting in hard structure in the product. In addition, low  $a_w$ , high salt content, low pH and low count of potential spoilage microorganisms were responsible parameters for long shelf life of the product. No growth of *E.coli* in Akçakatık samples was a good sign of product safety. Akçakatık cheese needs to be organized according to the technological production, that the production in

question must be performed not in family managements but in modern dairy farms and factories, and that the control mechanism, which is one of the quality-affecting factors, should be made functional, as well. The following points must be taken into consideration so as to provide a standard production for Akçakatık cheese:

- Raw milk must be of high quality.
- The milk must be subjected to a heating process of 10 minutes at least at 85<sup>0</sup>C.
- A proper starter bacteria must be used rather than the condensed yoghurt of the previous day.
- In the production of Akçakatık cheese, the filtration process must be performed in a controlled manner instead of keeping the milk waiting for 2-3 days and then filtrating it without determining its acidity.
- After the coagulation of milk, it must be put into standard filter clothes, and the water in it must be removed, and the process must continue until the proper water rate is achieved.
- The rate of salt to be put into cheese must be arranged not by tasting it but by adding a given amount into the cheese.
- The cheese, the filtration process of which has been completed, must be dried not in the open air but under controlled conditions so as to prevent it from being infected by the microorganisms from environmental conditions.

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