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Determining Some Chemical and Microbiological Changes in the Ripening Process of Kashar Cheese

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ABSTRACT

In the present study, some microbial, chemical, and physicochemical characteristics occurring during ripening were observed in unpackaged and vacuum-packed Kashar cheese samples. Some microbial and chemical properties of Kashar cheese samples were studied Also, the free fatty acid ratio was determined with the SDS-Page Electrophoresis to determine proteolysis during the ripening period and with the High-Performance Thin Layer Chromatography (HPTLC) for lipolysis. Physical, chemical, and microbiological analyzes were made at 0, 15, 30, 45, 60, and 75 days of ripening during which the changes in the number of microbiologically total aerobic mesophilic bacteria (TAMB), Lactobacillus spp., Lactococcus spp., Pseudomonas spp., yeast-mold, lipolytic, and proteolytic bacteria were determined. In the present study, % lactic acid, pH, dry matter percentage, color parameters (L, a and b values) and water activity (aw) were analyzed during ripening and the changes during storage were defined. *Lactobacillus* spp., *Lactococcus* spp., TAMB, and proteolytic bacteria counts and % lactic acid ratios were higher in vacuum-packed Kashar cheeses. It was found that lipolysis and proteolysis were higher in cheese samples stored open during ripening.

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Introduction

Kashar cheese is a traditional cheese known as Kashkaval in Bulgaria, Kasseri in Greece, Caciocavallo in Italy, and is kneaded after the curd is boiled and shaped. It is consumed fresh or after ripening (Atasever et al., 2007). More than fifty different types of cheese are produced in Turkey (Hayaloglu et al., 2002), and Kashar cheese is one of the most consumed traditional cheese types. It is classified as fresh Kashar (semi-hard) and old Kashar (hard) according to its ripening period and texture (Cetinkaya and Atasever, 2015; Çetinkaya and Oz, 2018) Free Fatty Acid (FFA), which forms as a result of the lipolysis reaction, is effective on the flavor and aroma of cheese. FFA is an important and volatile precursor of catabolic reactions producing compounds that contribute to flavor (McSweeney and Sousa, 2000). Fats in foods can undergo hydrolytic or oxidative degradation. However, oxidative changes are limited because of the low oxidation reaction potential in cheese. Triglycerides are hydrolyzed by the effect of endogenous lipases and free fatty acids emerge During ripening in all cheese varieties due to the incoming proteolytic and peptidolytic enzymes (McSweeney and Sousa, 2000). The level of proteolysis in cheese varies depending on the pH, salt, and dry matter content of curd, ripening temperature, and time (Tarakci and Kucukoner 2006; Eroglu et al., 2016)

The present study was conducted to determine the changes during the ripening period, some microbial, chemical, and physicochemical characteristics as well as lipolysis and proteolysis levels of vacuum-packed and storing opened Kashar cheese.

Material and Method

Material

Fresh Kashar cheese samples (approximately 2 kg) in the form of loaves purchased from a local producer in Erzurum were storing opened and vacuum-packed at 4°C for 75 days during the ripening period. On the 1st, 15th, 30th, 45th, 60th, and 75th days of ripening, lipolysis and proteolysis were studied in relation to some chemical and microbial properties.

Methods

Determination of The Chemical Properties of the Kashar Cheese Samples

Total solid content (%) in the samples was determined with the gravimetric method, where the dry matter oil content was divided by the total amount of dry matter (TSE, 2002). The acidity was determined in lactic acid % by means of the titration method. The pH value with a pH meter (WTW3510i). A portable hygrometer (Aqua LAB 4TE) was used to determine the aw value of the samples. Color analysis of the cheese was performed using a chroma meter (Konica Minolta Japan). The results are described using the parameters L (lightness to darkness), a (+a to red, -a to green) and b (+b to yellow, -b to blue). Color measurements were performed at 20°C using a chroma meter (Konica Minolta Japonya). The L, a and b color coordinates were determined.

Determination of The Microbiological Properties of The Kashar Cheese Samples

For the microbiological analyses, a 25 g cheese sample was weighed in a sterile Stomacher plastic pouch under aseptic conditions, and 225 mL of buffered peptone water was added to the sample before homogenization in a homogenizer. After, appropriate dilutions were prepared from the homogenate to form suitable diluents. Dilutions of the samples were spread on plate counter agar (PCA) and incubated for 48 hours at 35 ± 2°C to grow total aerobic mesophilic bacteria (TAMB). The number of total yeasts and molds was determined on rose bengal-chlorophenicol agar (RBC) and the plates were incubated at 20 ± 1ºC for 5 days. The populations of *Lactobacillus* spp. were counted after spreading dilutions on de Man, Rogosa and Sharpe agar (MRS) incubated at 37 ± 2°C for 72 hours. Purple red bile (VRB) agar plates were incubated at 35 ± 2°C for 24 ± 2 hours to count the populations of coliforms. The number of *Lactococcus* spp. was determined on the M17 agar and on plates incubated for 24 ± 1 hours at 37 ± 1ºC under aerobic conditions. Skim Milk Agar (SMA) medium was used for enumeration of proteolytic bacteria. After 72 hours of incubation at 21±1°C, 1% HCl was added and waited for 1 minute. After removing the remaining acid, the colonies with an open zone were counted. For counting the lipolytic bacteria, tributyrin agar with 10 ml/l glycerol tributyrate was prepared and after 3 days of incubation at 30°C the colonies were counted. The number of *Pseudomonas* spp. was determined in *Pseudomonas* Agar Base (Oxoid, UK) growth medium supplemented with CFC (Cephalothin, Fucidin, Cetrimide). The plates were incubated at 20 ± 1°C for 48±1 hours under aerobic conditions. The bacterial counts determined were expressed as log cfu g^{-1}.

Determination of Lipid Profile of Kashar Cheeses with HPTLC

Each volume of Kashar cheese sample lipids was vigorously shaken with 1 volume of a mixture of n-hexane/2-propanol (3/2) (Merck, Darmstadt/Germany) for extraction of total lipids. After centrifugation of the suspension at +4°C, 2000 x g for 10', the upper phase was aspirated and used for HPTLC analysis (Hara and Radin 1978). High performance thin layer chromatography (HPTLC) plates (20x10 cm) (Merck, Darmstadt/Germany) were used for separation and identification. At the end of the plate, 5 µl of extracted lipids from egg yolk and serum were spotted 2 cm from the bottom of the HPTLC plates using a micropipette. The lipids were spotted 6 cm from the application point with a mobile phase of n-hexane: Diethyl ether: formic acid (80:20:2 (v/v/v) developed. To visualize the lipid classes, the entire plate was sprayed with 10% $CuSO_4$ (w/v) in 8% H_3PO_4 (v/v) (Merck, Darmstadt/Germany) and carbonized at 180°C. After cooling, the HPTLC plates were analyzed using Phoretix 1D (TL120) software (Damyanova, 2002).

Determination of Proteolysis of Kashar Cheeses with SDS-Page of Electrophoresis

The partially purified proteins obtained at the end of the expression of the cheese samples were separated by the SDS-PAGE method using a 12% polyacrylamide gel in a Bio-Rad (Bio-Rad, Hercules, CA, USA). Electrophoresis was performed for approximately 90 minutes in 20 mA/gel constant current mode. The protein gels were stained with Commessie Blue G-250 and then visualized on thegel imaging system (Bio Rad Gel Doc XR, Bio-Rad Laboratories Inc., Hercules, CA, USA) (Laemmli, 1970; He, 2011).

Statistical Analysis

Microbiological populations, physicochemical properties and, textural characteristics of kashar cheese samples were statistically compared with IBM SPSS 20 using one-way analysis of variance (ANOVA).

Results and Discussion

Table 1 shows the changes in the chemical characteristics of vacuum-packed and unpacked Kashar cheeses during storage. When the changes in chemical characteristics of Kashar cheeses during ripening were examined, the changes in pH, % lactic acid content, L-value, and dry matter ratios were statistically significant ($p<0.05$). It was found that the values obtained in the vacuum-packed samples were lower than those that were ripened openly.

When the data obtained in the present study were compared with other studies, it was found that pH was higher than the values obtained by Çetinkaya and Atasever (2015). and be lower than those of Ataveser et al (2007). The pH values recorded in the vacuum-packed cheeses were found to be higher than pH 5.49 by Öksüztepe et al (2009). The 0.50-1.65% lactic acid values obtained in titration acidity were found to be higher than the values obtained in some previous studies.(Atasever, et al., 2007; Öksüztepe et al. 2009; Cetinkaya and Atasever, 2015) About the color values, L, a, and b values were 84.59-75.99, -5.70, -5.07, respectively; and 27.33-31.33 in unpackaged cheeses, and those with vacuum packaging were measured between 79.55-85.03, -5.91, -5.75, and 29.76-28.07, respectively. The measured color values were found to be higher than those reported by Eroğlu et al (2015).

The changes in the chemical characteristics of vacuum-packed and unpacked Kashar cheeses during storage are given in Table 2. When the microbiological characteristics of Kashar cheese were examined during ripening, Lactobacillus spp., *Lactococcus spp.*, TAMB, Yeast-Mold, Lipolytic Bacteria, Proteolytic Bacteria, and *Pseudomonas spp.* It was also found that the increases during storage were statistically significant ($p<0.05$). It was observed that the microorganism counts obtained in the vacuum-packed samples were lower than the openly ripened groups.

Table 1. Some chemical analysis results of Kashar cheeses during ripening

Quality		*Days*					
	G	0	15	30	45	60	75
pH	UP	5.69±0.03c	5.81±0.01bc	5.88±0.01bc	6.01±0.02ab	5.87±0.01bc	6.25±0.27a
	VP	5.67±0.07b	5.71±0.09b	5.71±0.03b	5.87±0.04a	5.66±0.03b	5.58±0.04b
Lactic acid %	UP	0.70±0.00b	0.50±0.00c	0.50±0.14c	0.40±0.00c	1.25±0.07a	0.70±0.00b
	VP	0.50±0.00c	0.40±0.00c	0.50±0.00c	0.70±0.14b	1.50±0.00a	1.65±0.07a
L	UP	84.59±2.07a	75.99±3.38c	82.35±0.35ab	81.26±0.09ab	79.89±0.45bc	82.00±0.41ab
	VP	85.03±1.31a	82.13±0.99bc	81.27±1.41bc	79.55±0.59c	82.37±0.45b	81.28±0.74bc
a	UP	-5.58±0.30b	-5.07±0.13a	-5.70±0.09b	-5.57±0.08b	-5.63±0.01b	-5.62±0.16b
	VP	-5.51±0.01	-5.91±0.14	-5.75±0.30	-5.39±0.30	-5.72±0.11	-5.73±0.14
b	UP	28.47±0.54bc	27.33±0.42c	29.27±0.04b	28.41±0.55bc	28.25±1.14bc	31.33±0.25a
	VP	28.07±0.13	29.76±0.42	29.15±1.09	28.43±1.04	29.03±0.44	29.44±0.35
aw	UP	0.979±0.000a	0.971±0.000b	0.971±0.000b	0.967±0.001bc	0.963±0.002c	0.972±0.002b
	VP	0.970±0.000	0.974±0.000	0.969±0.001	0.962±0.003	0.967±0.001	0.984±0.020
Dry Matter (%)	UP	50.82±0.21ab	46.61±0.02c	53.96±2.73a	48.51±1.44bc	49.23±0.37bc	50.91±0.90ab
	VP	49.19±0.84bc	48.78±1.08c	52.08±1.30a	48.93±1.30bc	49.38±0.02bc	51.34±0.25ab

G: Groups; UP:unpackaged ripened cheese; VP: vacuum packed cheese

Table 2. Some microbiological analysis results of Kashar cheeses during ripening (log 10 cfu/g)

	Days						
	G	0	15	30	45	60	75
Lactobacillus spp.	UP	5.25±0.13	5.94±0.01	5.35±0.02	6.14±0.01	6.15±0.01	6.24±0.01
	VP	5.28±0.01	5.13±0.01	6.09±0.01	6.14±0.01	6.16±0.02	6.21±0.30
Lactococcus spp.	UP	4.21±0.01	5.06±0.01	5.39±0.01	5.09±0.02	5.44±0.01	5.34±0.02
	VP	5.15±0.02	5.22±0.02	6.28±0.02	6.46±0.02	6.32±0.02	6.20±0.28
TMAB	UP	4.99±0.01	5.01±0.01	5.68±0.01	5.85±0.01	5.57±0.02	6.72±0.02
	VP	4.53±0.02	5.63±0.01	5.21±0.01	6.44±0.01	6.44±0.01	6.85±0.22
Yeast-Mold Count	UP	2.71±0.02	3.31±0.01	3.77±691	3.78±0.01	3.61±0.01	4.71±0.02
	VP	2.33±0.04	2.49±0.02	3.19±0.02	3.49±0.02	3.71±0.02	4.20±0.28
Lipolytic Bacteria	UP	4.19±0.02	4.43±0.01	4.51±0.01	4.73±0.01	4.73±0.02	5.02±0.04
	VP	4.19±0.02	4.29±0.02	4.31±0.01	4.49±0.02	4.69±0.01	5.00±0.00
Proteolytic Bacteria	UP	4.40±0.01	5.25±0.01	5.71±0.01	5.73±0.02	5.59±0.01	6.15±0.02
	VP	4.81±0.01	5.29±0.01	5.41±0.01	5.87±0.02	6.20±0.01	6.35±0.01
Pseudomonas spp.	UP	1.31±0.01	1.85±0.01	2.31±0.01	2.33±0.01	2.35±0.01	2.54±0.34
	VP	1.31±0.01	2.05±0.07	2.01±0.01	2.05±0.01	2.19±0.02	2.41±0.02

G: Groups; UP:unpackaged ripened cheese; VP: vacuum packed cheese

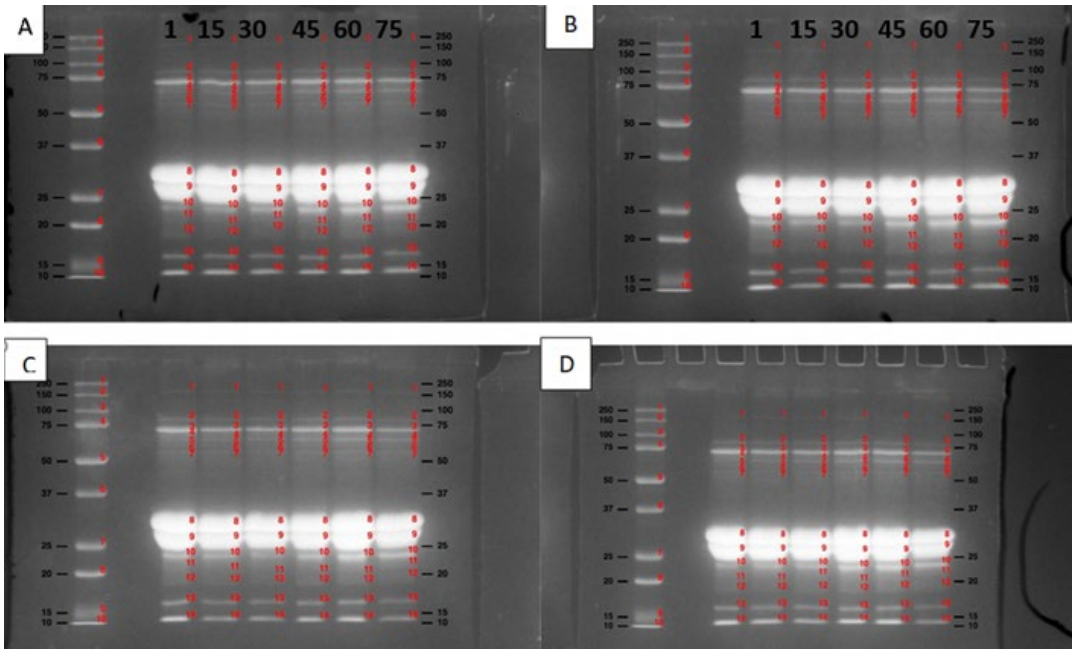

Figure 1. SDS-page electrophoresis images of Kashar cheese samples

A: Samples from the outside of unpackaged ripened cheese; B: Samples from the inside of unpackaged ripened cheese; C: Samples from the outside of vacuum packed cheese; D: Samples from the inside of vacuum packed cheese

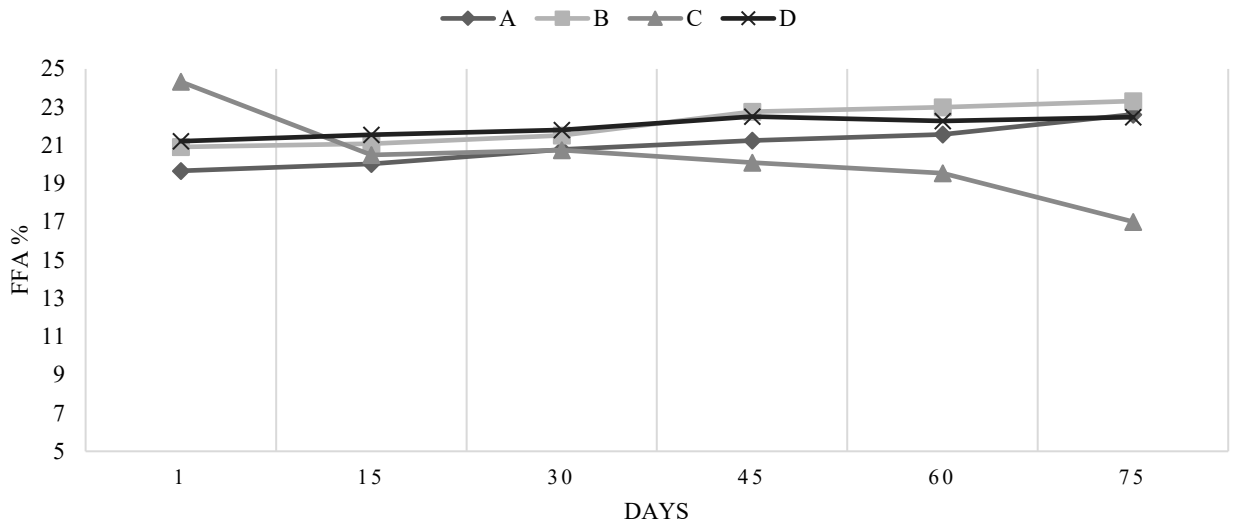

Figure 2. TLC results of Kashar cheese samples
A: Samples from the outside of unpackaged ripened cheese; B: Samples from the inside of unpackaged ripened cheese; C: Samples from the outside of vacuum packed cheese; D: Samples from the inside of vacuum packed cheese

The number of TMAB, *Lactobacillus spp.,* and *Lactococcus spp.* was found lower than the values obtained in other studies (Atasever et al., 2007; Öksüztepe et al., 2009; Cetinkaya and Atasever, 2015). Although the number of yeasts and molds was found to be higher than in some other studies (Atasever et al., 2007; Öksüztepe et al., 2009; Cetinkaya and Atasever, 2015), it was found to be lower than some others (Cetinkaya and Soyutemiz, 2006).

Casein degradation, which starts at the cheese production stage, continued and changes occurred in casein fractions in the case of proteolysis (αs1, αs2, b, and κ casein). As a result of these changes, short-chain peptide groups and free amino acids were exposed, which are involved in the formation of cheese flavor (McSweeney 2004). The proteolysis occurring during the storage and the ripening of Kashar cheese samples was determined with the SDS-Page Method. When the obtained results were evaluated, it was observed that α-CN and β-CN fractions were broken down during ripening and lower molecular weight products occurred. During the ripening, the degradation of casein can be caused by chymosin, plasmin, and enzymes synthesized by microorganisms (Ardö et al., 2017). Also, the degree of degradation of αs-CN is higher than that of β-CN. The β-CN is especially degraded in cheese by milk plasmin and microorganism enzymes instead of chymosin (Mane et al., 2019). There is a positive relationship between the plasmin activity of rennet and the degree of β-CN hydrolysis in cheese (Cortellino et al., 2006) High salt content adversely affects the degradation process of β-CN (Rasouli Pirouzian et al., 2012; Xia et al., 2020).

High-Performance Thin Layer Chromatography (HPTLC) is used commonly for the analysis and quality control of amino acids (protein quality), lipids and fatty acids (quality and contribution of fat), carbohydrates in the structure of foods (Sherma 2000). Cholesterol esters, Triacylglycerol, free fatty acids, free cholesterol, diacylglycerol, and phospholipids are detected with the HPTLC method (Fuchs al., 2011). In the present study, an increased FFA % was observed in both open and vacuum-packed Kashar cheese samples during the ripening period. It was found that free fatty acid formation was high in openly-ripened cheese samples. When the Free Fatty Acid (FFA) ratios obtained in the HPTLC Method that was performed during the ripening process of the Kashar cheese samples were evaluated, it was found that the values obtained were similar.

Conclusions

In conclusion, it was found in the present study that the ripening speed of the Kashar cheese samples that were stored in vacuum packaging was slower. For this reason, it was seen that vacuum packaging application is suitable for extending the shelf life during storage.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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