



Microbiological, physicochemical, textural and volatile characteristics of traditional kashar cheese produced in Muş

Muş'ta üretilen geleneksel kaşar peynirinin mikrobiyolojik, fizikokimyasal ve uçucu bileşen özellikleri

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ABSTRACT

The aim of this study is to investigate physicochemical properties, microbiological qualities and volatile compound contents of traditional aged kashar cheese produced in Muş, Turkey. Vacuum packaged cheeses were purchased from six different local producers. Total aerobic mesophilic bacteria, total yeast and mold counts, mesophilic and thermophilic lactic acid bacteria populations (log CFU g⁻¹) were in similar ranges in most samples ($P>0.05$). Coliform populations stayed below 1 log CFU g⁻¹. All six samples yielded negative *Salmonella* spp. and positive *Listeria* spp. results. The average compositional properties of cheese samples in percentages (w/w) were 55.73 ± 2.47 for total solids, 27.05 ± 1.73 for protein, 25.92 ± 0.98 for fat, 4.11 ± 0.33 for salt. On average, all color parameters were different for interior and exterior parts of cheese samples. The textural hardness levels of cheese samples were between 1548.73-5727.04 g and significantly different from each other ($P<0.05$). A total of 17 volatile compounds were detected by GC-MS. According to the results of this study, production steps and raw materials should be standardized to minimize the diversity among products.

Key Words: Kashar cheese, Microbiological and physicochemical quality, Pasta-filata, Textural analysis, Volatile compound

ÖZ

Bu çalışmanın amacı Muş ilinde üretilen eski kaşar peynirinin mikrobiyolojik ve fizikokimyasal özellikleri ile uçucu bileşen madde içeriğini belirlemektir. Bu amaçla altı farklı yerel üreticiden vakum paketli peynir örnekleri temin edilmiştir. Mikrobiyolojik analizler sonucunda örneklerdeki toplam aerobik mezofilik bakteri, toplam maya-küf sayısı, mezofilik ve termofilik laktik asit bakteri popülasyonları çoğu örnekte benzer aralıklarda belirlenmiştir ($P>0.05$). Koliform popülasyonu 1 log CFU/g'un altında kalmıştır. Bütün örneklerde *Salmonella* türleri negatif ve *Listeria* türleri ise pozitif olarak tespit edilmiştir. Fizikokimyasal analizler sonucunda yüzde olarak ortalama (w/w) toplam kuru madde 55.73 ± 2.47, protein oranı 27.05 ± 1.73, yağ oranı 25.92 ± 0.98 ve tuz oranı 4.11 ± 0.33 olarak saptanmıştır. Ortalama olarak, peynir örneklerinin dış yüzey renk değerleri iç yüzeylerden farklı bulunmuştur. Peynir örneklerinin tekstürel sertlik seviyesi 1548.73-5727.04 g arası olup birbirinden istatistiksel olarak farklı bulunmuştur ($P<0.05$). Örneklerde toplam 17 uçucu bileşen GC-MS kullanılarak tespit edilmiştir. Bu çalışmanın sonuçlarına göre ürünler arasında belirlenen farklılıkların en aza indirilmesi için üretim basamakları ve hammaddelerin standardize edilmesi gerekmektedir.

Anahtar Kelimeler: Kaşar peyniri, Mikrobiyolojik ve fizikokimyasal kalite, Tekstürel analiz, Pasta-filata, Uçucu bileşen

Introduction

The production process and types of cheese have varied depending on technical knowledge, social conditions and wealth of societies throughout the world for centuries (Dugat-Bony et al., 2016). Over one thousand four hundred types of traditional cheese are known all around the world with the specific characteristic aroma, flavor and texture (Dugat-Bony et al., 2016). Under the lead of white and kashar cheese, forty to fifty famed types of cheese including traditionally produced origin-linked cheeses are produced in Turkey (Hayaloğlu et al., 2002; Sert et al., 2007). Demand for famed local cheese types represents the important part of annual consumption and continues to increase regardless of the urbanization and industrialization.

Kashar cheese, pasta-filata type of cheese, is the second most preferred cheese type after white cheese due to its specific taste and flavor with longer shelf-life in Turkey (Özdemir and Demirci, 2006; Kamber, 2008). Kashar cheese bears similarities to other pasta-filata type of cheeses including Caciocavallo and Mozzarella from Italy, Kashkaval from Bulgaria, Kasseri from Greece, Kachekavallo from Russia and Kachkavalj from Croatia (De Angelis and Gobetti, 2011; Yılmaz and Dağdemir, 2012; Yuvaşen et al., 2018). Unpasteurized sheep milk or its mixture of cow and/or goat milk is commonly used in the production of traditional kashar cheese (Aydemir, 2010; Temizkan et al., 2014). In general, the process of kashar cheese production can be summarized as scalding and kneading of the curd in hot water after certain levels of acidification (Öksüztepe et al., 2009; Aydemir, 2010; Şengül et al., 2010). Similar to the other pasta-filata types of cheese, the flavor of kashar cheese from unpasteurized milk develops during ripening depending on physicochemical properties of milk, spontaneous fermentation and storage conditions.

Pathogens can be introduced into cheese starting from raw milk to any step of cheese making including production, maturation and

storage before consumption. According to the Turkish Food Codex, kashar cheese products made of raw milk can be consumed after fermentation for at least 120 days during ripening (Turkish Food Codex, 2015). The pH of cheese declines into acidic conditions during maturation period due to the activity of lactic acid bacteria. It is believed that the increase of acidity through fermentation of cheese inhibits the growth of pathogenic microorganism in aged cheeses made of unpasteurized milk (Montel et al., 2014; Johnson, 2017). However, cheese types produced from raw milk in North America and Europa countries were reported as positive for food borne pathogens including *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* after sixty days of fermentation (FDA, 2016). Without a killing step, microbiological safety problems increase in traditional kashar cheese production even though fermentation during ripening is achieved in suggested conditions.

Aged kashar cheese produced in Muş province of Turkey is a traditional pasta-filata type of cheese named after the origin of product location. A mixture of unpasteurized cow, sheep, and goat milks, used for this traditional kashar cheese, comes from animals grazed in an area with 66 endemic plants (Karadağ, 2019). Also, dry salting is used after scalding at mild temperature. Eight to twelve kg of cheese wheels are stored at least for six months in cold storage room for maturation before consumption. No research study has been published about aged kashar cheese produced in Muş in the literature. The aim of this study is to investigate the physicochemical properties, microbiological qualities and volatile compound contents of aged kashar cheese produced in Muş.

Materials and Methods

Collection of cheese samples

Vacuum packaged cheese samples ripened at least for six months were obtained from six different local producers in Muş, Turkey. Two

slices from the same wheel of cheese were requested from each producer. All samples were transferred to laboratory on ice and kept at 4 ± 2 °C until use. One slice of sample from each producer was shipped to Yıldız Technical University on ice in a styrofoam box for volatile compound and texture analysis. The cheese samples were coded as M1, M2, M3, M4, M5 and M6.

Physicochemical analysis

The pH of cheese samples was measured with a portable pH meter (HACH, HQ30d Portable Multi Meter, CO, USA) after 10 g cheese sample was homogenized in 10 mL distilled water. Cheese samples were analyzed for dry matter by a gravimetric method (Anon, 2001) for salt content by Mohr method (Anon, 1983), for protein content by Kjeldahl method (IDF, 1993), and for fat content by Van Gulik method (Kurt et al., 2007). Titratable acidity was determined as described by the Association of Official Analytical Chemists (AOAC, 2000).

Microbiological analysis

Cheese samples were analyzed for a total of eight microbiological parameters. Aseptically, 25 g of cheese samples were weighted and blended for two minutes with 225 mL of 1% (w/v) peptone water (Biolife; Milan, Italy) in a sterilized laboratory blender (Waring Blender 7011, New Hartford, CONN., U.S.A). Before inoculation, homogenized samples were serially diluted in 9 mL of peptone. Dilutions of samples were spread plated on plate count agar (Merck KGaA, Darmstadt, Germany) and incubated for 24 to 48 h at 35 ± 2 °C for total aerobic mesophilic bacteria (TAMB). Spread plated potato dextrose agar plates (Biolife; Milan, Italy) were incubated for 3 to 5 days at room temperature for total yeast and mold count (TYMC). Populations of lactic acid bacteria were enumerated after spread plating dilutions on de Man, Rogosa and Sharpe agar (MRS; Difco Laboratories, USA) incubated at 30 ± 2 °C for mesophilic and at 42 ± 2 °C for thermophilic species for 24 to 48 h, respectively. Violet red bile agar and violet red bile dextrose

agar (Merck KGaA, Darmstadt, Germany) were incubated for 24 ± 2 h at 35 ± 2 °C to count populations of coliform and Enterobacteriaceae. All microbiological enumerations were performed in triplicates (n=3).

Remaining cheese and peptone blend were added on 250 mL pre-universal enrichment broth (Biolife; Milan, Italy). The presence of *Salmonella* spp. was tested with a modified FDA Bacteriological Analytical Manual (BAM) (Andrews et al., 2011). After incubation at 35 ± 2 °C for 24 to 48 h, 100 µL of pre-enrichment was transferred into 10 mL Rappaport-Vassilidas (Biolife; Milan, Italy) medium and incubated at 42 ± 1 °C for 48 h. A loopful of enrichment was streaked onto xylose-lysine-desoxycholate agar and incubated at 35 ± 2 °C for 24 h. Following incubation, typical colonies were inoculated in triple sugar iron agar (Merck KGaA, Darmstadt, Germany) and lysine iron agar (Merck KGaA, Darmstadt, Germany) slants. For *Listeria* spp., a loopful of pre-enrichment were streaked into *Listeria* oxford agar (LOX; Liofilchem, Abruzzo, Italy). Contam swabs were used to observe the biochemical changes resulting positive or negative after incubation at 35 ± 2 °C for 24 h.

Texture profile and color analysis

TA.XTplus Texture analyzer (Stable Micro System, London, England) was used to determine the texture profile analysis (TPA). Before analysis, samples were cut roughly in the size of 2.5 cm cubes and cooled to 4 ± 2 °C in the refrigerator. A 100 kg force load cell with a double bite compression was applied to samples. Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience of kashar cheese produced in Muş were determined by the software instrument called Texture Exponent (Stable Micro System, London, England). The color profile of cheese samples was measured with a spectro-Densitometer meter (Techkon GmbH, Germany). Measurements were taken from interior and exterior part measured up to 2 cm from rind (n=3). Color characteristics of six cheese samples were reported as values of L*black (0), white (100); a*: green (-), red (+); b*: blue (-), and yellow (+).

Analysis of volatile compound

Three grams of ground samples were placed into a 20 mL of head space vial. Samples in vials were agitated for 15 min at 70 °C using a CTC-Combi-PAL-Autosampler system (Bender and Hobein, Zurich, Switzerland). Headspace autosampler parameters were adjusted to an incubation condition at 50 °C for 30 min with a syringe at a temperature of 70 °C. Agitation speed and injection volume were set as 500 rpm and 500 µL with a filling speed of 200 µL s⁻¹. Pull-up and pre-injection delays were adjusted to 500 ms. An injection speed of 350 µL s⁻¹ and was used with a post injection delay of 1500 ms. Headspace sample (0.5 mL) was introduced into GC-MS system. HS/GC-MS analyses were performed by using GC/MS-QP2010 (Shimadzu, Milan, Italy) combined with a CTC-Combi-PAL-Autosampler (Bender and Hobein, Zurich, Switzerland). In the GC-MS analyses, a Restec (Bellefonte, USA) Rtx-5MS fused silica capillary column (30 m×0.25 mm ID, 0.25 µm) was used for chromatographic separation in the presence of Helium as carrier gas.

Volatile compound analyses of cheese samples were performed with a modified methodology described by Rzepa et al. (2009). Gradient analysis was performed by using the following temperatures: 35 °C (1 min); 35-80 °C (5 °C min⁻¹); and 80-200 °C (8 °C min⁻¹). The injector temperature was kept at 200 °C, constantly. Pressure value and linear velocity were 14 kPa and 21.6 cm sec⁻¹, respectively. The flow rate of carrier gas was 0.6 mL min⁻¹. The GC-MS interface and ion source temperature were adjusted to 250 °C and 200 °C, respectively. The mass spectrometer was operated with an electron impact ionization voltage of 70 eV. The data was collected in a range of m z⁻¹ 40–300. Analyzes were performed in duplicates for each cheese sample. Major volatile compounds of samples were identified using GC/MS library. Identification of compounds was obtained by the comparison of the mass spectra of detected volatile compounds with the commercial mass spectra libraries (NIST27 and WILEY7).

Statistical analysis

Microbiological populations, physicochemical properties and, textural characteristics of kashar cheese samples were statistically compared with JMP Pro 14.0 (SAS, Cary, NC, USA) using one-way analysis of variance (ANOVA) and Tukey's HSD test. The critical *P*-value was set at 0.05.

Results

Physicochemical analysis

Average values of compositional and chemical properties of all aged kashar cheese samples produced in Muş are depicted with minimum and maximum values in Table 1. The average compositional properties (% (w/w)) of samples were 55.73 ± 2.47 for total solids content, 27.05 ± 1.73 for protein, 25.92 ± 0.98 for fat, 4.11 ± 0.33 for salt and 4.86 ± 0.32 for ash with a limited difference among samples. Saltiness is one of the important sensory attributes of cheese in general. Samples M1 and M4 showed the lowest and highest salt content with a value of 2.93 and 4.74, respectively. All cheese samples had an average of 1.09 ± 0.16 for titratable acidity (%) and 5.35 ± 0.17 for pH.

Table 1. Compositional and chemical properties of six cheese samples (n=3 for each sample)

Property	*Mean± SD	Minimum	Maximum
Total solids (%)	55.73 ± 2.47	52.33	59.71
Fat (%)	25.92 ± 0.98	24.50	27.50
Salt (%)	4.11 ± 0.33	2.93	4.74
Titratable acidity (%)	1.09 ± 0.16	0.86	1.30
Protein (%)	27.05 ± 1.73	24.60	29.79
pH	5.35 ± 0.17	5.13	5.59
Ash	4.86 ± 0.32	4.40	5.47

*Average of all cheese samples

Microbiological analysis

Population of all tested microorganisms in aged kashar cheese samples are shown in Table 2. Total aerobic mesophilic bacteria (TAMB) population ranged from 8.08 ± 0.03 to 6.90 ± 0.88 log CFU g⁻¹ in all six samples. Except for sample M4, population of total yeast and mold counts (TYMC) were between 6.14 ± 1.14 and 7.62 ± 0.13 log CFU g⁻¹. Sample M4 had 4.01 ± 0.41 log CFU g⁻¹

of TYMC population. All cheese samples had up to 1.6 log CFU g⁻¹ higher mesophilic lactic acid bacteria (LAB) population than thermophilic LAB species. The population of mesophilic and thermophilic LAB ranged from 6.28 ± 0.14 to 8.08 ± 0.11 log CFU g⁻¹. Coliform population in all samples and Enterobacteriaceae population in

sample M5 and M6 stayed below detection limits (<1 log CFU g⁻¹). Sample M1, M2, M3 and M4 had the population of Enterobacteriaceae on average of 3.80 ± 0.51 log CFU g⁻¹. No *Salmonella* presence was detected in any samples. All six samples yielded positive results for *Listeria* spp.

Table 2. Microbiological quality of cheese samples (n=3)

	Population (Log CFU/g) ± SD					
	M1	M2	M3	M4	M5	M6
TAMB	7.59 ± 0.33ab	6.90 ± 0.88b	7.91 ± 0.09ab	8.08 ± 0.03a	7.41 ± 0.13ab	7.81 ± 0.19ab
TYMC	6.14 ± 1.14ab	6.78 ± 0.92a	4.01 ± 0.41b	6.71 ± 0.94a	7.62 ± 0.13a	6.89 ± 0.88a
Mesophilic LAB	7.88 ± 0.16abA	6.95 ± 0.93bA	7.84 ± 0.12abA	8.08 ± 0.11aA	7.65 ± 0.08abA	7.81 ± 0.23abA
Thermophilic LAB	6.28 ± 0.14bB	6.46 ± 0.61bA	6.91 ± 0.57abB	7.85 ± 0.19aA	7.14 ± 0.11abB	7.60 ± 0.19aA
Coliforms	<1	<1	<1	<1	<1	<1
Enterobacteriaceae	3.39 ± 0.14b	3.41 ± 0.62b	3.80 ± 0.20ab	4.20 ± 0.22a	<1c	<1c
# <i>Salmonella</i> spp.	-	-	-	-	-	-
# <i>Listeria</i> spp.	+	+	+	+	+	+

Means with different letter within each row are significantly different ($P < 0.05$).

Means with different capitalized letter in lactic acid bacteria within each column are significantly different ($P < 0.05$).

Total Aerobic Mesophilic Bacteria (TAMB).

Total Yeast and Mold Count (TYMC).

#Presumptive results. Positive (+) /Negative (-).

Texture profile and color analysis

Textural profile and color analysis results are shown with the average, minimum and maximum values in Table 3. Hardness and chewiness yielded prominent textural parameter values by all samples as seen Figure 1. Sample M1 had the

highest hardness (5727 g) and chewiness (2634 g cm) values ($P < 0.05$). The lowest hardness (1549 g) was calculated for both sample M2 and M5 ($P < 0.05$). Minimum chewiness was found as 469 for sample M3.

Table 3. Textural and color characteristics of six cheese samples (n=3 for each sample)

	Parameter	*Mean ± SD	Minimum	Maximum
Textural properties	Hardness (g)	2845.28 ± 1481.29	1548.73	5727.04
	Adhesiveness (g.sec)	-0.24 ± -0.11	-0.45	-0.16
	Springiness (cm)	0.74 ± 0.09	0.62	0.88
	Cohesiveness	0.44 ± 0.06	0.36	0.52
	Chewiness (g cm)	1016.91 ± 807.13	469.10	2634.25
	Resilience	0.17 ± 0.05	0.11	0.23
Color	L* (exterior)	56.21 ± 3.21	51.07	61.53
	L* (interior)	65.42 ± 1.87	62.69	68.49
	a* (exterior)	-3.45 ± 0.27	-3.80	-3.08
	a* (interior)	-3.26 ± 0.35	-3.81	-2.69
	b* (exterior)	10.83 ± 1.33	8.42	12.69
	b* (interior)	12.23 ± 1.09	10.34	13.79

Note: Exterior color values of cheese samples were measured up to 2 cm from rind.

L*black (0), white (100); a*: green (-), red (+); b*: blue (-), yellow (+).

On average, all color parameters were higher for interior part of cheese samples than the exterior part as seen on Table 3. The average L* (white) value for interior and exterior part of all cheese samples were 65.42 ± 1.87 and 56.21 ± 3.21, respectively. There was a difference

between interior and exterior part of cheese samples with a* (green/red) value below 1.1. Among all tested cheese samples, average a* (green/red) value was -3.26 ± 0.35 for interior and -3.45 ± 0.27 for exterior. Similarly, differences among b* (blue/yellow) values of interior and

exterior values stayed below 3 and for each sample with an average of 12.23 ± 1.09 and 10.83 ± 1.33 for all samples, respectively.

Analysis of volatile compounds

Table 4 shows the volatile compounds identified in cheese samples. Total of 17 compounds with a 90% and over similarity values were expressed. All samples released cyclohexanol, ethyl alcohol with the exception of sample M5 and 2-pentanone except for sample M3. Trans-β-ionon-5,6-epoxide and 2-butanol were released from three samples including M2, M3, and M4. Among samples, compounds present in only one sample were 1-propanol in Sample M3, n-butane and tetranitromethane in Sample M5, 2-heptanone, acetaldehyde, 4-ethyl-2,2,6,6-tetramethyl-heptane and 2-nonanone in sample M4, octanol and nonanal in Sample M1, and n-nonanal in Sample M2.

Table 4. Volatile compounds identified by GC-MS in cheese samples

Compound	*Sample					
	M1	M2	M3	M4	M5	M6
Acetaldehyde				+		
Ethyl alcohol	+	+	+	+		+
3,3-Dimethyl-2-phenyl-2-azirane	+					+
1-propanol			+			
Cyclohexanol	+	+	+	+	+	+
2-butanol		+	+			+
n-butane					+	
Ethyl acetate	+					
Trans-β-ionon-5,6-epoxide		+	+	+		
2-heptanone				+		
4-ethyl-2,2,6,6-tetramethyl-heptane				+		
Tetranitromethane					+	
Octanal	+					
Nonanal	+					
n-nonanal		+				
2-nonanone				+		
2-pentanone	+	+		+	+	+

*Compounds with 90% and over similarity values

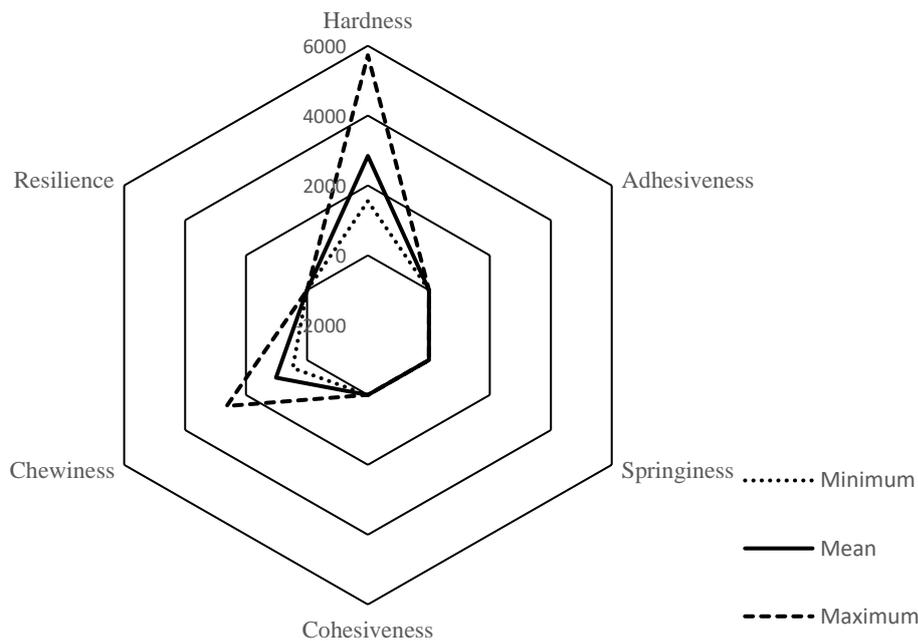


Figure 1. Radarplot showing overall textural properties of cheese samples

Discussion

Cheese types are accepted and preferred by consumers relatively based on taste, flavor, appearance and texture in general. Approximately, five hundred tons of cheese were imported compare to seventeen thousand tons of exported cheese from Turkey in 2016 (FAO stat,

2019). This is an important indicator that traditional cheese varieties are a part of culture meeting consumers' expectations. The yield of final product depends on several factors, including the fat, casein, and moisture contents of milk, the distribution quality of salt in cheese, and the ripening period before consumption (Montel et al., 2014; Johnson, 2017). Kashar cheese needs

to stand for long periods (3-6 months) in order to gain the characteristic aroma, appearance, and texture (Çelik et al., 2018).

All aged kashar cheese samples produced in Muş exposed similar compositional properties with slight differences. Even though no standardized process for aged kashar produced in Muş has been documented, all samples had similar chemical attributes, probably due to the commitment of producers to the traditional ways of cheesemaking. Especially, low standard deviations of mean total solids, fat and protein values can be evaluated as the use of similar compositional mixture of cow, sheep and goat milk. In the Turkish food codex, the fat content of semi-fat cheeses varies between 25 and 45%. In addition, those with a moisture content of 57-64% in the fat-free cheese mass, are defined as semi-hard cheese. When classified according to the milk fat content and firmness, kashar cheese produced in Muş can be classified as semi-fat and semi-hard cheese. Similar to kashar cheese samples in this study, cheese samples produced in the Trakya region of Turkey had contents of 55.2% dry matter, 25% protein, 22.6% fat, and 4.5% ash (Hamzaçebi and Anter, 1978). Kashar cheeses produced in Kars were collected to examine their protein, fat and dry matter contents for chemical quality purposes. The average protein, fat and dry matter contents of the samples were 22.34 ± 0.63 %, 21.59 ± 0.70 %, and 64.40 ± 0.72 %, respectively (Kamber, 2005). The protein and fat percentages of the cheese produced in Muş were higher, but the dry matter content was relatively lower than those produced in Kars.

Salt content is a very important parameter of cheese, which can directly affect the water activity, moisture, ripening, and fermentation attributes of cheese. Changing salt content of a cheese product can also alter the pH value and protein content (McCarthy et al., 2016). The percentage of salt content and the type of salt are decided by producer for traditional cheese types with a general pH value of 4.5-5.3 (Fox et al., 2017). The pH values of samples, measured

between these limits, are an important indicator of an adequate fermentation. The salt content of samples in this study showed limited differences affecting the development of pH and microbiological quality of final product during maturation. According to the Turkish Food Codex, a maximum of 4% salt in dry matter is allowed in the Kashar cheese. The salt content of kashar cheese produced in Muş is slightly above the limits, specified in the codex. This may be due to the production of kashar cheese is made by small family owned dairies in Muş.

The fermentation of cheese depends on all naturally occurring microorganisms including bacteria, yeast and molds and storage conditions during ripening if any microbial inactivation process is applied (Karagözlü and Karagözlü, 2016). Aged kashar cheese produced in Muş is made from raw milk similar to various traditional types of cheese that directly affect taste and flavor. Population of TAMB around 10^8 CFU g^{-1} and TYMC about 10^6 CFU g^{-1} in kashar cheese produced in Muş, which was an indicator of microbial growth requiring 120 days of maturation period (Cogan, 2011; Turkish Food Codex, 2015). Higher population of mesophilic lactic acid bacteria suggests that all samples were fermented during the ripening at temperature values where mesophilic bacteria grow predominantly. Mesophilic bacteria in cheese samples can include mesophilic lactobacilli, pediococci, and leuconostoc (Mannu et al., 2000; Fitzsimons et al., 2001). Some important characteristics of aged kashar cheese produced in Muş were expected to be developed by mesophilic LAB due to their ability of citrate catabolism and proteolysis (Fox et al., 1998; Crow et al., 2001; Vidojevic et al., 2007). The reduction of pH by LAB during fermentation is believed to eliminate pathogens in cheeses made from raw milk during ripening. In all tested samples, *Salmonella* spp. was not detected similar to fecal indicator of generic *Escherichia coli*. All samples yielded positive *Listeria* spp. results. Similar to the results of this study, *L. monocytogenes* survived during 120 days of kashar cheese ripening despite

of a heat treatment of curd at 75 ± 1 °C for 5 min (Çetinkaya and Soyutemiz, 2004). In another study, *Listeria* spp. were found to survive during the production and ripening of soft and semi-hard cheeses (Ryser and Marth, 1987; Dominguez et al., 1987). However, *Listeria monocytogenes* were not detected in parmesan cheese after 2 to 16 weeks of ripening (Yousef and Marth, 1990). This may be due to the fact that each type of cheese has its own medium that can affect the survival of bacteria. All samples in this study had *Listeria* spp. positive that suggests the need of longer ripening or the use of starter culture after pasteurization of milk or proper packaging.

Milk-derived *Staphylococcus aureus* isolates are reported as more resistant to bacteriocins such as nisin (Can and Hastaoğlu, 2020). However, in a study conducted by Özdemir and Demirci (2006), the number of coliform bacteria and *S. aureus* in kashar cheese decreased while the number of LAB, total yeast and molds, lipolytic bacteria, proteolytic bacteria increased with elongated ripening time. Pasteurization of milk is accepted enough to eliminate foodborne pathogens before cheese making. Also, active packaging technologies including antimicrobial materials can be used to prevent or retard the growth of spoilage microorganisms and enzymatic degradation or chemical deterioration of dairy products related to shelf life (Tacer Caba and Kaya Özkök, 2019). In a study, cheese samples were coated with a lysozyme-containing zein-wax composite film and its effect on survival of *L. monocytogenes* was examined during storage. The substantial decrease (-0.4 decimals) was observed in the initial microbial load of inoculated cheese samples due to sustained release of lysozyme (Ünalın et al., 2013). Development of a standardized aged kashar cheese produced in Muş manufacture is important in terms of public health.

Textural properties of cheese are important for the consumer acceptance and attributes including packaging and handling, shaping, and using as an ingredient (Ercan et al., 2011; Fox et al., 2017). In

this study, samples showed high differences texture attributes of hardness (firmness) and chewiness. The hardness of cheese changes with dry matter, fat content and the length of maturation (Grawtney et al., 2002; Brown et al., 2003; Ercan et al., 2011). The proportion differences in sheep, goat and cow milk mixture, non-standard maturation period and storage conditions are the probable reasons for the high differences particularly in hardness among the samples. The use of a fat replacer increased the cohesiveness and decreased the hardness, springiness, gumminess and chewiness of full fat and low fat kashar cheeses produced by using carbohydrate during the storage period for 90 days (Koca et al., 2004). In addition, as a result of the ripening process, the textural properties of full-fat kashar cheese were very similar to those produced in Muş. In a different study, Aday et al. (2010) examined how the ripening process affects the texture properties of Ezine cheese. The results revealed that firmness, springiness, cohesiveness and chewiness of cheese samples were diminished, but its adhesiveness was considerably improved during ripening. Also, the color values of cheese are affected by the similar factors as textural properties. Cheese has a wide range of color, from white/yellow to even red. Carotenoids, occurring in photosynthesis, are the secondary pigments and major responsible pigments in milk (Fox et al., 2017). Since lactic acid bacteria and several internal enzymes, including lipase, are responsible for the sensory attributes of cheese, color is also highly depended on the milk quality, such as raw or pasteurized, affecting initial microbial load and enzyme activity. Types of cheese made of raw milk, as aged kashar cheese produced in Muş, have stronger taste and aroma than compared to the pasteurized counterparts. Factors, including microbial activities, milk quality and process conditions/steps, directly affect ripeness, aroma and taste development of cheese (Bertuzzi et al., 2018).

Volatile compounds may directly transfer from milk to product, or, as in general, occur during

ripening of cheese. Most of them are produced via lipolysis, proteolysis, and lactose, lactate, and citrate metabolism (Bertuzzi et al., 2018). Cheese is not a common product for production of straight chain aldehydes, such as hexanal, heptanal, octanal, nonanal 2- decanal, and 2-undecanal. These aldehydes are produced by non-enzymatic autooxidation of unsaturated fatty acids. In this study, samples M1 and M2 had most of these mentioned aldehydes. Moreover, the M4 sample possessed acetaldehyde due to the breakdown of threonine or the lactate metabolism or the oxidation of ethanol. The result was similar to findings by Eroğlu et al. (2016). Six different aldehyde compounds were identified in the kashar cheese samples produced in Muş: isovaleraldehyde, benzaldehyde, hexanal, nonanal, pentanal and decanal. However, in a study carried out by Hayaloğlu (2009), six different aldehydes were detected in traditionally produced mature kashar cheese including 2-methyl propanal, 2-methyl butanal, 3-methyl butanal, acetaldehyde, 2-propenal and benzaldehyde. These were slightly different than those found in kashar cheese produced in Muş.

Starter lactic acid bacteria can produce lactate from lactose during cheese ripening. Following that, the same bacteria convert lactate into ethanol and acetic acid (Bertuzzi et al., 2018; van Mastrigt et al., 2018). In aged kashar cheese production in Muş, no starter culture was used, so differences among identified volatiles may be explained with the naturally occurring microbiota. The sample M4 exposed both volatiles whereas M5 did not have any of them. Rest of the samples had only ethanol from a possible lactate metabolism. Only sample M3 had 1-propanol, which might have occurred from triglycerides or synthesized from fatty acids (van Mastrigt et al., 2018). Secondary alcohols, such as 2- butanol and 2-pentanol, might have produced from metylketones via the triglyceride metabolism (McSweeney and Sousa-Gallagher, 2000). Samples M2, M3, and M6 exposed secondary alcohols. In other studies, among the alcohols detected in kashar cheese, ethanol was found to

be the most abundant alcohol (Eroğlu et al., 2016; Hayaloğlu, 2009). Ethyl acetate and ethyl butyrate are esters produced from tryglicerides and synthesized from fatty acids during surface ripening (Bertuzzi et al., 2018). Samples M1, M4, and M6 exposed at least one of these compounds. All samples, except sample M3, include 2-pentanone, a colorless liquid ketone occurring as a metabolic product of mold growth in blue cheese (Walker and Mills 2014).

Conclusion

Various types of cheese with different appearance, odor, taste and texture are recognized and accepted by consumers regardless to the origin of the product. This study introduces kashar cheese to literature with some attributes of commercial traditional aged kashar cheese samples produced in Muş. Differences may occur related to the raw materials, production, ripening, and storage of cheese. The diversity among producers can be minimized and production parameters should be standardized to increase market value.

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