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Effect of different lysozyme treatments on the properties of Kashar cheese properties

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SUMMARY: In this study, the solid and liquid forms of microbial lysozyme and egg lysozyme were added to kashar cheese for a 90-day period, and the physicochemical and microbiological features of the cheese were examined. The physicochemical (pH,% LA, DM, fat, protein, TN, WSN, OI, salt), textural, and microbiological characteristics of the cheese were compared to those of control samples (TMAB, coliform, yeast-mold, lactobacilli, spore microorganism, E. coli). Information on free fatty acids (FFA) and volatile compounds was also evaluated. The results showed that goods treated with various lysozyme forms had better physicochemical, microbiological, and textural qualities during the ripening period and decreased microbial loads. The study's findings highlight and suggest employing lysozymes, particularly in microbial form, to increase the shelf life of Kashar cheese and to improve the quality and safety of cheese, as well as obtain better quality characteristics during storage.

KEYWORDS: *Aroma; Kashar cheese; Lysozyme; Physicochemical properties; FFA; Storage*

RESUMEN: ***Efecto de diferentes tratamientos con lisozima sobre las propiedades del queso Kashar.*** En este estudio, se agregaron las formas sólidas y líquidas de lisozima microbiana y lisozima de huevo a quesos kashar durante un período de 90 días, y se examinaron las características fisicoquímicas y microbiológicas de los quesos. Se compararon las características fisicoquímicas (pH, % LA, MS, grasa, proteína, TN, WSN, OI, sal), texturales y microbiológicas de los quesos tratados con las muestras control (TMAB, coliformes, levaduras, lactobacilos, esporas, E. coli). También se evaluaron los ácidos grasos libres (AGL) y compuestos volátiles. Los resultados mostraron que los productos tratados con diversas formas de lisozima tenían mejores cualidades fisicoquímicas, microbiológicas y texturales durante los períodos de maduración y menores cargas microbianas. Los hallazgos del estudio destacan y sugieren el empleo de lisozimas, particularmente en forma microbiana, para aumentar la vida útil del queso Kashar y mejorar la calidad y seguridad del queso, y obtener mejores características de calidad durante el almacenamiento.

PALABRAS CLAVE: *Almacenamiento; Aroma; FFA; Lisozima; Propiedades fisicoquímicas; Queso Kashar*

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1. INTRODUCTION

Kashar cheese is rich in biochemicals and is highly appreciated in Turkey. It is also manufactured in some European and Balkan nations under various names. Kashar cheese is classified as a Pasta-Filata (plastic curd) in the classification made by taking into account specific elements of its processing (coagulation with rennet, boiling the curd, hand molding, and not being pressed) and according to the humidity amount, it belongs to the semihard cheese group. A variety of cheeses known as Pasta Filata are made in Italy, Greece, the Balkans, Turkey, and Eastern Europe. (Çetinkaya, 2020; Topcu *et al.,* 2020). The ripening period is crucial for producing the desired aroma of the cheese because aromatic compounds change during ripening (Eroğlu *et al.,* 2016). However, if conditions in the ripening rooms, such as temperature and relative humidity, are not strictly controlled, the prolonged ripening period could lead to increased surface contamination.

Late blowing (LBD), which results from butyric acid fermentation and affects the aroma and structure of cheese, is the main issue with hard and semihard cheeses during storage. Anaerobic spore-forming bacteria like Clostridium produce certain organic acids (butyric and acetic acid) and gases (H_2 and CO_2) during the ripening process of hard and semihard cheeses and are undesired metabolites in cheese. These products lead to cheese that has undesirable quality flaws such as swelling, uneven eye development, cracks, and taste disturbance. This is a significant economic issue for cheese production (Brandle *et al.,* 2016). These bacteria have the ability to generate spores that withstand the thermal processing used to create hard cheeses, which can then germinate and produce the defect-causing gas (D'Incecco *et al.,* 2016).

Many methods, including lysozymes, a naturally occurring antibacterial and antiviral agent, were suggested to prevent LBD in cheese. Along with g-type (bacterial lysozyme) and i-type (chicken or conventional lysozyme), c-type (chicken or conventional) lysozymes are among the principal types found in the animal kingdom (invertebrate lysozyme). (Callawaert and Michiels, 2010). In-depth research has been done on the impact of lysozyme generated from eggs on the physical and chemical characteristics of semihard and hard cheeses (Conte *et al.,* 2011; Urbienė and Sasnauskait, 2010). However, there is currently no information on the impact of bacterial lysozyme on cheese storage and ripening.

In light of these facts, the purpose of this study was to use bacterial lysozyme and egg lysozyme in various forms (liquid and powder). Since Kashar cheese is a high-cooked pasta-filata variation, the manufacturing procedures for other high-cooked pasta-filata cheeses, like Kashkaval, Mozzarella, or Provalone, could be modified to use bacterial lysozyme for Kashar cheese in order to prevent LBD and enhance physical features.

2. MATERIALS AND METHODS

2.1. Cheese making

Kashar cheese production took place at a nearby dairy facility (Mega Süt Company, Aydın, Turkey). Figure 1 shows the processing procedures for producing Kashar cheese. LysochTM G4 (Code: 0402) is a powdered bacterial lysozyme concentration made by Stretomyces sp. (Handary S.A., 2016, Bruxelles, Belgium). Kashar cheese was produced in three replicas, weighing 500 g, and was vacuum packed. It was kept in storage for 90 days. The supplier of the lysozyme was FMI Gıda Kimya İthalat-İhracat Sanayi ve Ticaret Ltd. in İzmir, Turkey. The Megasüt Dairy Company provided the rennet (Maysa, İzmir), calcium chloride (Merck), polyethylene plastic vacuum packing, rock salt, and other ingredients needed for processing.

2.2. Methods

2.2.1. Gross compositional and textural analyses

Using a pH meter (made by Mettler Toledo), the pH of the milk samples was calculated, and the acidity was assessed using the titrimetric method. Dry matter and ash values were evaluated using the graphimetric method, and the fat contents in the milk samples were determined using the Gerber method (ISO/IDF, 2008). The Gerber test involved visually inspecting the fat content within a Gerber milk-tester butyrometer column. This examination occured after the sulfuric acid hydrolysis of milk components and centrifugation.The Micro Kjeldahl technique was used to determine the total nitrogen content. The total nitrogen amount was multiplied by a coefficient of 6.38 to determine the protein content. At 15 °C, specific gravity values were determined using a lactodensimeter. Standard culture enumeration techniques were

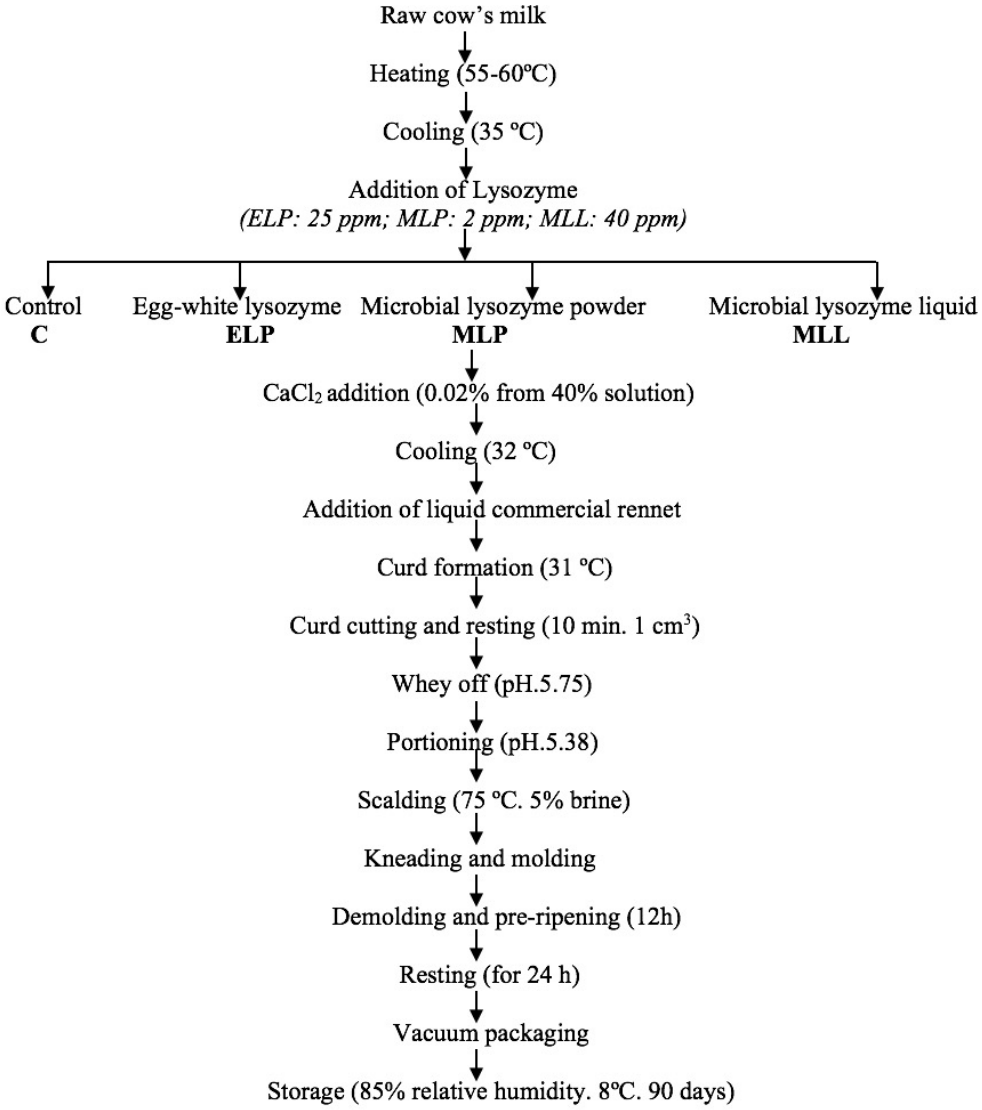

Figure 1: Flow diagram of Kashar cheese production

**C: Control, ELP: cheese treated with eggwhite lysozyme, MLP: cheese treated with microbial lysozyme powder, MLL: cheese treated with microbial lysozyme liquid.*

used to determine the microbial counts in raw milk samples (Halkman and Ayhan, 2000). Sporebacteria were also enumerated on Tryptose Sulfite Cyclocerine Agar (Merck, Germany), incubating at 37 °C for 24-48 h under anaerobic conditions. After 48 hours of incubation at 28–30 °C, the total number of aerobic mesophilic bacteria was counted on Plate Count Agar (PCA) (Merck, Germany). E. coli and total coliform bacteria were counted on Violet Red Bile Agar (VRB) (Merck, Germany) after being incubated for 24 and 48 hours at 35 to 37 °C in an aerobic environment. After 3-5 days of incubation at 25 °C, yeast and mold were counted on Dichloran Rose Bengal Chloramphenicol Agar (DRCB) (Merck, Germany) (VRB). Lactic acid bacteria were counted on MRS Agar and M17 Agar (Merck, Germany) for 48 hours under aerobic conditions at 35–37 °C. Spore bacteria were counted on Tryptose Sulfite Cyclocerine Agar (Merck, Germany), which was incubated at 37 °C for 24–48 hours under anaerobic conditions.

The dry matter and fat contents in the cheese samples were measured using the Gerber (ISO 3433, 2008) and conventional gravimetric (ISO 5534) techniques. Lactic acidity (LA, %) was analyzed according to AOAC (1995). A pH meter was used to measure the pH levels of the cheese samples (Mettler Toledo).

The micro-Kjeldahl method was used to measure the nitrogen content in cheese in terms of total nitrogen (TN), water-soluble nitrogen (WSN), and non-protein nitrogen (NPN). (WSN/TN) x 100 was used to generate ripening index (RI) values. For calculating salt (%), the Mohr method was applied. Six different metrics were used to examine the microbial composition of the cheese samples under the same circumstances as those used for raw milk samples (Halkman and Ayhan, 2000).

Texture profile analyses (TPA) were carried out using a Texture Analyzer (TA-XT plus, Stable Micro Systems, Godalming, UK), (Bourne, 1978). Samples were roughly cut into 2.5 cm cubes before analysis, wrapped in airtight plastic wrap to prevent moisture loss, and brought to assay temperature (25 ºC). The test speed was set at 1.5 mm/sec, and each cut sample was compressed and set to 60%. 2.00 mm/sec and 10.0 g were chosen as the pre-test speed and trigger force, respectively. Values from the texture analysis are the average of three replicates.

2.2.2. Free fatty acid composition and volatiles

Using an Agilent GC (model GC 6890N) equipped with a capillary column (300X250mX0.25m, Agilent 19091F-433 HP-FFAP,CA, USA), the free fatty acid composition was evaluated as ppm in GC in accordance with the capillary gas chromatography method recommended by Deeth *et al.,* 1983. There are two steps to extraction. First, free fatty acids were extracted (using 0.3-0.5 g sample) along with all other lipids in an acidic hexane/diethyl ether solution. Next, free fatty acids were separated from their triglycerides using neutral alumina chromatography on neutral aluminum oxide. Finally, formic acid in ether was used to remove aluminum oxide from the process. The kind and quantity of free fatty acids in cheese samples were lastly identified by injecting them into the gas chromatography apparatus.

Stashenko and Martinez's (2007) description of the static headspace solid phase micro-extraction (SPME) method was used to analyze the volatiles with Agilent GC 7820A gas chromatography-mass spectrometry equipment. For this purpose, 10 ml of sample were kept at 40 °C for 30 minutes in the SPME extraction system. Then the sample was extracted using 50/30 µm Divinylbenzene/ Carboxen/ Polydimethylsiloxane (DVB/CAR/PDMS, Agilent, USA) fiber for another 30 minutes. Desorption was carried out at 250 °C for 10 minutes. The separation was carried out using a DB WAX column (122-7032, Agilent Technologies, USA; 30 m×0.25 mm i.d; 0.25 µm film thickness). Column temperature programme was as folllows: 40 °C (for 5 min) → to 100 °C at 10 °C/min → to 100 °C at 20 °C/min (10 min) NIST/Flavournet library was used to identify the volatile compounds.

Each of the analytical determinations was made in three replicates.

2.2.3. Statistical analyses

One-way analysis of variance (ANOVA) was used to analyze variance and find significant differences. Biochemical and textural data were subjected to an Anova analysis of variance with the following factors: (1) control or lysozyme addition; and (2) storage term. Duncan's test was used to compare significant means (significance $P < 0.05$). The number of replicates was three. The Tukey test was used to compare significant means primarily for textural characteristics (significance $P < 0.05$). Data were examined using SPSS 15.0. (SPSS Inc., Chicago, USA). The 95% confidence interval ($p < 0.05$) was used to signify statistical significance, which was also expressed in terms of P values.

3. RESULTS AND DISCUSSION

3.1. Gross composition analysis of cheese

Table 1 lists the gross composition of raw cow's milk. According to the literature (Say and Güzeler, 2008; Göncü *et al.,* 2017), the general characteristics of raw milk were consistent.

Table 1. Parameters of raw milk (n=3).

Parameters	Raw milk
pH	6.56±0.04
LA (%)	0.13±0.00
Dry matter (%)	11.54±0.08
Protein (%)	3.05±0.02
Fat (%)	3.36±0.03
Ash (%)	0.69±0.01
TAMB (log cfu/g)	6.38±0.01
Coliform (log cfu/g)	4.44±0.01
E. coli (log cfu/g)	4.09±0.03
Yeast and mold (log cfu/g)	5.71±0.14
Sporatic bacteria (log cfu/g)	2.13±0.04
Lactobacillus (log cfu/g)	7.21±0.06

Table 2 displays the findings from the examination of the gross compositionand microbiological makeup of the cheese when it was stored. The inclusion of lysozyme had a considerable impact on a number of characteristics. The titratable acidity (measured as g of lactic acid per 100 g of cheese) of 1-day-old Kashar cheese ranged from 1.12 to 1.48%. At the same time, other chemical components were measured, including total solids at 60.30 to 66.43%, protein at 22.18 to 23.6%, fat at28.76 to 34.11%, and salt at 1.51 to 2.86%. The titratable acidity, pH, spore bacteria, coliforms, and E. coli contents in the chese were not significantly impacted by the application of various types and forms of lysozyme ($P > 0.05$). For Kashar cheese, suitable levels of moisture and salt were discovered. The C and MLL in the chese showed the greatest and lowest total solid and protein levels, respectively.

The microbiological quality of the cheese affects how pH and titratable acidity grow. Cheese-treated bacterial lysozyme (MLL, MLP) revealed lower initial microbial counts in TMAB, yeast, mold, and coliform levels. The addition of bacteria which produce lysozyme caused the pH to drop and the acidity to rise, which in turn prevented the growth of coliform bacteria, yeast, and TMAB. The pH value did not change as much since there were fewer living bacteria, which led to less CO_2 being produced (Al Baarri *et al.,* 2018). In essence, the cheese treated with bacterial lysozyme had a lower bacteria count than the control cheese, which was proven to be a significant difference (MLP and MLL). This circumstance demonstrates the antimicrobial effectiveness of lysozyme in Kashar cheese. By dissolving the structural elements on the cell walls of bacteria and fungi, lysozyme is known to exert its antimicrobial effect against these microorganisms as well as viruses and protozoa (Al Baarri *et al.,* 2018). However, the bacterial lysozyme treatment substantially reduced the lactic acid bacteria count in the cheese. The lowest lactic acid bacteria counts were seen in MLP and MLL in the current study at both the beginning and end of ripening. This might be because high salt concentration inhibits LAB (MLP: 2.86-2.40% and MLL: 2.08-2.73%), and lysozyme has a substantial inhibitory effect on all lactic acid bacteria (Silvetti *et al.,* 2017). Badem and Uçar (2016) discovered, in contrast to our findings, that LAB activity was higher at the start of ripening, and the LAB number dropped on the 90th day of Kashar ripening since lysozyme was not used in the study.

Very comparable trends were seen in the fat content of the cheese, which is consistent with the structural impact of egg white lysozyme. When ELP was ripening, the maximum fat percentage was detected (34.11% and 37.77%, respectively). Our results were better than those of previous researchers who studied Kashar cheese (Eroğlu *et al.,* 2016; Ozturkoglu-Budak *et al.,* 2021; Yalman *et al.,* 2017). The composition of the cheese milk and the cheese-making process may be the cause of the variations in the dry matter and fat contents of the Kashar cheese samples.

The cheese (MLP and MLL) treated with bacterial lysozyme showed the highest salt level at 1 and 90 days, while the control had the lowest salt content. The measured values are in line with previous studies, which reported that Kashar cheese has a salt level of 1.16% (Yalman *et al.,* 2017) and 1.22-1.37% (Badem and Uçar, 2016).

The highest protein contents were found in the control cheese (23.66% on day 1, 27.69% on day 60), which showed a drop in protein contents with lysozyme treatments and an increase in protein contents throughout ripening ($p < 0.05$). The protein contents in the cheese showed slight changes as they ripened. As a result of our investigation, it is believed that lysozyme's inhibitory action on microorganisms such as TMAB, coliforms, yeast, and mold, as well as the inhibitory effect of salt on LAB ,is to blame for this condition. These findings were in line with a previous study using four distinct types of Kashar samples that had matured for 90 days (Eroğlu *et al.,* 2015).

In terms of the lysozyme treatment, these values were considerably higher in C and lower in the cheese treated with bacterial lysozyme (MLL and MPP) at the start and end of ripening, respectively ($p < 0.005$). This outcome appears to be connected to lysozyme's inhibitory effect and reduced levels of LAB and salt in MLL and MPP. In comparison to treated cheese, the control cheese may have had a higher proteolysis level and RI value due to a higher microbial count (Ozer and Kesenkas, 2019). All samples underwent the anticipated formation of WSN and TCA-SN,a rise in RI during ripening, and other authors (Sulejmani and Hayaloglu, 2016; Ozturkoglu-Budak *et al.,* 2021) noted the same trend for Kashar cheese.

Table 2. Physicochemical properties of Kashar cheese during ripening (n=3±sd).

	Samples	Day 1	Day 30	Day 60	Day 90
pH	C	5.40±0.29Aa	5.63±0.12Aa	5.33±0.12Aa	5.37±0.05Aa
	ELP	5.43±0.05Aa	5.30±0.14Aa	5.37±0.09Aa	5.20±0.16Aa
	MLP	5.50±0.24Aa	5.40±0.14Aa	5.33±0.09Aa	5.17±0.05Aa
	MLL	5.37±0.12Aa	5.30±0.08Aa	5.43±0.12Aa	5.27±0.12Aa
Titratable acidity LA %	C	1.48±0.32Aa	1.48±0.24Aa	1.84±0.08Aa	1.48±0.28Aa
	ELP	1.31±0.13Aa	1.48±0.11Aa	1.49±0.04Aa	1.40±0.45Aa
	MLP	1.12±0.02Aa	1.26±0.08Aa	1.26±0.15Aa	1.76±0.11Aa
	MLL	1.21±0.06Aa	1.41±0.07Aa	1.32±0.03Aa	1.53±0.04Aa
Drymatter %	C	62.19±0.94ABb	63.77±1.39ABb	63.08±0.88ABb	68.52±0.99ABa
	ELP	66.43±3.05Ab	63.82±2.32Ab	65.98±3.04Ab	69.82±0.94Aa
	MLP	60.36±2.06Bb	60.50±1.23Bb	62.02±1.29Bb	66.50±1.13Ba
	MLL	60.30±0.91Bb	62.65±1.65Bb	63.75±2.57Bb	66.54±1.79Ba
Ash %	C	4.22±0.10Ca	3.91±0.05Ca	3.91±0.15Ca	3.66±0.14Ca
	ELP	3.93±0.07Ca	3.99±0.08Ca	4.08±0.16Ca	3.99±0.07Ca
	MLP	5.54±0.18Aa	5.21±0.04Aa	5.19±0.09Aa	5.41±0.26Aa
	MLL	4.36±0.24Bb	4.41±0.07Bb	4.24±0.16Bb	4.69±0.68Bb
Fat %	C	32.80±1.79Bb	32.93±0.32Bb	30.81±0.31Bb	37.23±1.75Ba
	ELP	34.11±3.49Ab	35.45±2.04Ab	36.52±2.58Ab	37.77±1.29Aa
	MLP	28.76±2.42Bb	30.09±1.47Bb	31.31±1.18Bb	35.62±1.30Ba
	MLL	31.68±0.89Bb	32.58±1.76Bb	32.38±2.68Bb	34.11±1.47Ba
Protein %	C	23.66±0.64Ab	25.33±1.29Aab	27.69±1.67Aa	25.01±1.93Aa
	ELP	22.69±1.92Bb	22.56±0.78Bab	23.15±0.52Ba	25.73±0.51Ba
	MLP	23.20±1.56Bb	22.50±0.24Bab	23.07±0.76Ba	23.07±1.28Ba
	MLL	22.18±0.21ABb	23.58±0.27ABab	25.01±0.63ABa	25.01±0.96ABa
TN %	C	3.60±0.22Ab	3.97±0.20Aab	4.34±0.26Aa	3.92±0.30Aa
	ELP	3.56±0.30Bb	3.54±0.12Bab	3.52±0.07Ba	4.03±0.08Ba
	MLP	3.64±0.24Bb	3.53±0.04Bab	3.62±0.12Ba	3.62±0.20Ba
	MLL	3.48±0.03ABb	3.70±0.04ABab	3.92±0.10ABa	3.92±0.15ABa
WSN %	C	0.43±0.02Ac	0.56±0.04Ab	0.69±0.07Aa	0.70±0.08Aa
	ELP	0.39±0.02Bc	0.43±0.04Bb	0.47±0.06Ba	0.53±0.06Ba
	MLP	0.34±0.01Cc	0.36±0.01Cb	0.38±0.03Ca	0.42±0.04Ca
	MLL	0.35±0.01 Bc	0.43±0.03 Bb	0.52±0.05 Ba	0.62±0.01 Ba
Ripening index %	C	12.00±1.10Ac	14.19±1.67Abc	16.06±2.07Aab	17.85±1.24Aa
	ELP	10.97±1.32Bc	12.06±0.91Bbc	13.36±1.92Bab	13.24±1.58Ba
	MLP	9.38±0.46Cc	10.21±0.26Cbc	10.62±0.98Cab	11.59±0.43Ca
	MLL	10.07±0.32Bc	11.73±0.84Bbc	13.39±1.62Bab	15.94±0.93Ba
Salt %	C	1.51±0.44Ca	1.60±0.13Ca	1.66±0.16Ca	1.95±0.29Ca
	ELP	1.69±0.12BCa	1.82±0.24BCa	2.24±0.06BCa	2.32±0.12BCa
	MLP	2.86±0.14Aa	2.70±0.05Aa	2.44±0.11Aa	2.40±0.43Aa
	MLL	2.08±0.14ABa	2.07±0.09ABa	2.13±0.03ABa	2.73±0.40ABa
TMAB log cfu/gr	C	5.01±0.41Aab	5.71±0.32Ab	7.00±0.12Aab	6.52±0.16Aa
	ELP	5.01±0.41Aab	5.28±0.56Ab	5.87±0.11Aab	6.29±0.16Aa
	MLP	4.10±0.15Bab	3.61±0.50Bb	3.99±0.74Bab	4.46±0.24Ba
	MLL	5.58±0.04Bab	3.95±0.46Bb	4.66±0.33Bab	4.89±1.13Ba
Coliform cfu	C	4.20±0.48Ab	1.81±0.58Ac	3.55±0.11Aab	4.23±0.32Aa
	ELP	3.98±0.15Ab	1.59±0.30Ac	3.67±0.11Aab	4.28±0.49Aa
	MLP	3.28±0.20Ab	2.13±0.40Ac	4.11±0.31Aab	4.70±0.07Aa
	MLL	3.58±0.08Ab	2.14±0.20Ac	4.17±0.43Aab	4.04±0.22Aa
Total yeast and mold count (TYMC) cfu	C	2.00±0.00Ab	2.61±0.32Aa	2.87±0.26Aa	2.85±0.21Aa
	ELP	0.87±1.23ABb	2.68±0.08ABa	2.77±0.07ABa	2.62±0.23ABa
	MLP	0.00±0.00Cb	1.63±0.05Ca	2.37±0.30Ca	1.90±0.47Ca
	MLL	0.43±0.61BCb	2.42±0.19BCa	2.69±0.17BCa	2.52±0.20BCa
Lactobacillus cfu	C	6.65±0.12Ac	6.48±0.11Abc	7.11±0.07Ab	7.25±0.12Aa
	ELP	4.83±0.28Ac	6.01±0.09Abc	6.91±0.25Ab	7.27±0.03Aa
	MLP	3.67±0.14Cc	3.83±0.12Cbc	3.77±0.53Cb	6.03±1.07Ca
	MLL	4.33±0.23Bc	4.68±0.21Bbc	5.44±0.39Bb	6.24±0.65Ba
Sporadic microorganism cfu	C	*<1*	*<1*	*<1*	*<1*
	ELP	*<1*	*<1*	*<1*	*<1*
	MLP	*<1*	*<1*	*<1*	*<1*
	MLL	*<1*	*<1*	*<1*	*<1*
E. coli cfu	C	*<1*	*<1*	*<1*	*<1*
	ELP	*<1*	*<1*	*<1*	*<1*
	MLP	*<1*	*<1*	*<1*	*<1*
	MLL	*<1*	*<1*	*<1*	<1

*C: Control, ELP: cheese treated with eggwhite lysozyme, MLP: cheese treated with microbial lysozyme powder, MLL: cheese treated with microbial lysozyme liquid

The Duncan test was used to compare significant means. Different lowercase superscript letters in the same row indicate significant differences during ripening ($P < 0.05$); different uppercase superscript letters in the same column indicate significant differences among sample groups ($P < 0.05$).

In relation to the textural analysis, Table 3 provides changes in the textural characteristics of the cheese as it ripens. Overall, the results show that storage and lysozyme treatment considerably changed the textural characteristics of kashar cheese (P 0.05). The highest hardness and cohesiveness values were found in cheese that had been treated with microbial powder lysozyme (MLP). However, the lowest springiness, gumminess, and resilience values were also found. At the end of storage, a similar tendency was seen in all the cheese.

3.2. Free fatty acids and volatiles in cheese

Table 4 contains the FFA profiles of the cheese after 90 days of ripening. The results show that short-chain fatty acids (C4 to C10, SCFA), medium-chain fatty acids (C12 and C14, MCFA), and long-chain fatty acids (C16, C18, and C18:1, LCFA) were all significantly affected by the lysozym treatment of Kashar cheese, with the exception of butyric acid (C4:0), myristic acid (C14:0), and palmitic acid (C16:0). All samples had significantly greater lev-

Table 3. Texture profile of Kashar cheese during ripening (n=3±sd).

	Samples	Day 1	Day 90
Hardness (g)	C	5575.55± 308.99ABa	5647.45± 840.17ABb
	ELP	4232.1± 381.6 Ca	1468.52±199.47Cb
	MLP	6405.08±843.52 Aa	6977.55±404.97Ab
	MLL	6100.56±385.76 Ba	3153.11±509.48Bb
Adhesiveness (g s)	C	—64.11± 18.11Ba	—124.79±15.01Bb
	ELP	—52.3 ± 6.63ABa	—63.28±34.52ABb
	MLP	—23.23 ±17.75Aa	—13.83±9.67Ab
	MLL	—9.97±7.12ABa	—121.57±46.96ABb
Springiness (cm)	C	0.66±0.03Ba	0.55±0.02Bb
	ELP	0.77±0.02Aa	0.62±0.02Ab
	MLP	0.33±0.07Ca	0.34±0.03Ca
	MLL	0.76±0.04ABa	0.55±0.03ABb
Cohesiveness	C	0.49±0.04Ba	0.42±0.01Ba
	ELP	0.53 ± 0.05Aa	0.55 ±0.02Aa
	MLP	0.86±0.01Aa	0.57±0.03Ab
	MLL	0.37 ±0.03Ba	0.46±0.02Ba
Gumminess (g)	C	2744.98±192.18Aa	2338.79 ±394.73Ab
	ELP	2219.61±280.15Ba	817.31±141.13Bb
	MLP	2097.34±400.66Aa	1355.61±93.66Ab
	MLL	2303.34±323.83ABa	1464.54±235.72ABb
Chewiness (g cm)	C	1820.11±106.84Aa	1275.93±214.31Ab
	ELP	1705.92±233.53Ba	504.58±75.07Bb
	MLP	2097.34±400.66Aa	1355.61±93.66Ab
	MLL	1747.57± 321.14ABa	806.68±97.59ABb
Resilience	C	0.19±0.02Ba	0.15±0.01Bb
	ELP	0.26±0.03Aa	0.23±0.03Ab
	MLP	0.15±0.01Ca	0.13±0.02Cb
	MLL	0.22±0.02Ba	0.17±0.01Bb

C: Control. ELP: cheese treated with eggwhite lysozyme, MLP: cheese treated with microbial lysozyme powder, MLL: cheese treated with microbial lysozyme liquid

The Tukey test was used to compare significant means. Different lowercase superscript letters in the same row indicate significant differences during ripening ($P < 0.05$); different uppercase superscript letters in the same column indicate significant differences among sample groups ($P < 0.05$).

Table 4. Free fatty acid profiles of Kashar cheese during ripening (n=3±sd) ppm.

	Samples	Day 1	Day 30	Day 60	Day 90
C4 Butyric acid	C	370.98±66.65Aa	336.27±31.78Aa	296.61±38.62Aa	387.29±24.25Aa
	ELP	182.09±17.60Aa	365.64±82.46Aa	456.12±99.39Aa	465.68±153.61Aa
	MLP	283.22±106.12Aa	347.77±33.34Aa	247.15±48.68Aa	351.63±26.46Aa
	MLL	347.35±68.12Aa	312.99±45.37Aa	410.40±132.89Aa	361.02±50.86Aa
C6 Caproic acid	C	28.28±11.93ABa	33.71±3.42ABa	41.95±1.34ABa	50.88±11.81ABa
	ELP	55.72±17.81Aa	29.85±5.81Aa	43.78±10.59Aa	39.60±2.04Aa
	MLP	14.75±1.99Ca	27.41±9.51Ca	19.32±1.61Ca	24.29±2.94Ca
	MLL	16.01±4.06BCa	27.50±4.06BCa	31.07±1.18BCa	39.27±2.44BCa
C8 Caprylic acid	C	11.64±2.19Ab	13.60±0.77Ab	26.63±0.62Aa	31.60±1.21Aa
	ELP	10.44±1.27ABb	11.34±3.01ABb	23.93±11.62ABa	19.89±0.87ABa
	MLP	10.51±2.26Bb	11.01±0.25Bb	17.22±1.68Ba	20.79±0.82Ba
	MLL	9.05±2.74ABb	11.15±0.32ABb	23.50±0.55ABa	24.83±0.29ABa
C10 Capric acid	C	26.09±7.65Ab	31.80±1.83Ab	48.75±1.39Aa	61.86±1.44Aa
	ELP	13.00±0.80Bb	22.73±3.94Bb	44.78±22.98Ba	33.65±2.18Ba
	MLP	22.79±2.81Bb	22.99±2.10Bb	35.21±4.28Ba	41.32±3.18Ba
	MLL	15.83±9.37ABb	26.74±1.55ABb	44.37±0.51ABa	48.69±0.83ABa
C12 Lauric acid	C	43.44±13.23Ab	47.53±4.26Ab	59.88±1.37Aa	77.76±0.79Aa
	ELP	31.54±6.67Bb	38.91±5.18Bb	59.53±26.15Ba	43.59±2.99Ba
	MLP	30.09±7.14Bb	40.49±0.60Bb	47.56±5.76Ba	56.35±5.38Ba
	MLL	26.77±11.06ABb	44.06±0.23ABb	55.80±1.98ABa	63.66±1.26ABa
C14 Myristic acid	C	143.54±50.54Aa	134.53±19.03Aa	149.41±6.44Aa	198.97±3.41Aa
	ELP	199.28±88.85Aa	124.77±19.41Aa	155.81±59.72Aa	111.11±9.54Aa
	MLP	88.27±11.32Aa	130.89±12.56Aa	119.35±14.36Aa	147.02±15.70Aa
	MLL	85.58±17.84Aa	125.97±6.87Aa	139.96±7.90Aa	165.16±3.24Aa
C16 Palmitic acid	C	609.42±242.27Aa	405.50±52.42Aa	411.54±10.26Aa	525.01±2.99Aa
	ELP	495.66±149.39Aa	481.06±145.21Aa	454.23±140.49Aa	323.45±32.85Aa
	MLP	287.48±17.83Aa	391.92±24.98Aa	373.83±44.37Aa	434.91±45.82Aa
	MLL	304.02±48.61Aa	387.46±10.78Aa	394.80±26.47Aa	460.31±4.37Aa
C18 Stearic acid	C	283.28±103.26Aa	117.23±9.47Aa	132.50±3.17Aa	144.31±8.51Aa
	ELP	108.10±11.35Aa	175.90±64.78Aa	139.80±27.32Aa	106.02±11.98Aa
	MLP	101.45±6.33Aa	112.44±2.03Aa	121.42±12.50Aa	133.81±13.39Aa
	MLL	122.77±19.21Aa	120.97±3.07Aa	120.50±6.10Aa	135.08±3.39Aa
C18:1 Oleic acid	C	861.59±228.62Aa	477.06±76.89Aa	457.11±1.99Aa	549.39±27.60Aa
	ELP	499.64±168.03ABa	587.75±189.12ABa	495.92±114.50ABa	367.88±44.06ABa
	MLP	310.77±9.49Ba	412.03±25.38Ba	406.98±42.07Ba	478.54±49.42Ba
	MLL	376.31±63.09ABa	425.99±13.78ABa	426.20±23.62ABa	501.86±10.50ABa
C18:2 Linoleic acid	C	221.64±72.97Aa	78.37±7.17Aa	80.72±3.62Aa	81.92±3.92Aa
	ELP	74.98±13.85ABa	119.59±48.45ABa	79.19±9.10ABa	59.21±4.56ABa
	MLP	66.77±5.66Ba	69.41±1.39Ba	64.81±5.32Ba	70.85±5.60Ba
	MLL	85.81±15.47ABa	74.35±1.53ABa	68.57±2.52ABa	75.44±1.59ABa

C: Control, ELP: cheese treated with eggwhite lysozyme, MLP: cheese treated with microbial lysozyme powder, MLL: cheese treated with microbial lysozyme liquid

Duncan test was used to compare significant means. Different lowercase superscript letters in the same row indicate significant differences during ripening ($P < 0.05$); different uppercase superscript letters in the same column indicate significant differences among sample groups ($P < 0.05$).

els of LCFA overall than short- and medium-chain free fatty acids (MCFA an SCFA). Linoleic acid (C18:1), palmitic acid (C16), and butyric acid (C4) were the three free fatty acids found in the highest concentrations, respectively. These outcomes might be explained by lysozym's inhibiting impact on the cheese's microbial population. Cheese treated with egg white lysozym (ELP) had much higher levels of the enzyme than cheeses treated with bacterial lysozym (MLP, MLL). This observed value is consistent with the fat % result presented in Table 2, where the highest ELP was discovered (34.11% on day 1 and 37.77% on day 90). This conclusion is consistent with that of Urbienė and Sasnauskait (2010), who studied the effects of 0.01% lysozyme in 18-day-old cottage cheese. As can be seen in Table 4, there were generally no appreciable variations in the FFA profiles of the samples during ripening; the same pattern was noted in all the cheese. Güler (2005) claims that when Kashar cheese's short-chain FFA concentrations were high, flavor intensity increased, but flavor quality was inconsistent. Despite the fact that palmitic, stearic, and oleic acids were the most prevalent FFAs in Kashar cheese, they did not add as much to the cheese's flavor and aroma as short- and medium-chain fatty acids, which both had very high perception threshold values and a less distinctive flavor.

In that order, butyric acid, caproic, capric, and caprylic acids were the most prevalent short-chain fatty acids (FFA). Although the pattern was not continuous in every instance, the levels of each unique SCFA significantly increased during the course of the storage period and decreased with lysozym treatment ($p < 0.05$). At the end of ripening, the control (C) cheese had the greatest FFA concentrations. These ratios were C4 (387.29), C6 (50.88), C8 (31.60), and C10 (61.86), respectively. This outcome can be explained by the increased proliferation of lactobacilli and yeast during storage (as noted in Table 2). The breakdown of amino acids and the lipolysis of milk fat may produce fatty acids with four or more carbons (Urbach, 1993). For Kashar cheese and Muenster-type cheese, De Leon-Gonzalez *et al.* (2000) and Temiz *et al.* (2010) reported that the concentration of FFAs, namely, C4, C8, and C10 acids, considerably rose with the storage period.

Initial lauric acid (C12 ratio) and myristic acid (C14 ratio) levels for medium-chain fatty acids (C12 and C14, MCFA) were 43.44-26.77% and 199.28 -85.58%, respectively. The control cheese finished ripening with higher levels of C12 and C14 than other cheese (MLP, MLL, ELP). According to these data, lauric acid (C12) was strongly affected by the treatments (lysozym and storage duration) in MCFA (p 0.05). According to Temiz (2010) the FFAs in this situation might have hydrolyzed into specific molecules such as methyl ketones, alcohols, lactones, aldehydes, etc..

Long-chain fatty acids (C16, C18, and C18:1) declined significantly during ripening in the current study, and the cheese treated with lysozyme likewise displayed lower values than the control cheese at both the beginning and the end of the ripening. All the cheese included long-chain fatty acids, but oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids predominated. Furthermore, different lysozyme treatments had no appreciable impact on the samples of Kashar cheese's palmitic acid (C16:0) or stearic acid (C18:0) ($p > 0.05$). However, lysozyme caused a decrease in oleic acid (C18:1) and linoleic acid (C18:2) ($p < 0.05$). Because of this, the cheese treated with bacterial lysozym (MLP) had the lowest amount of oleic acid (C18:1) and linoleic acid (C18:2) at the start of storage (861.59 and 221.64), while cheese under the control (C) conditions had the largest amount of these acids (310.77 and 66.77). After 90 days of storage, the amounts of palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids in all Kashar cheese dropped. When compared to Kashar cheese which had been treated with lysozyme at the beginning and final stages of storage, the control sample (C) had the highest LCFA values. During ripening, longer free fatty-acid chains are broken down into shorter ones, and methyl ketones are produced, which may be the cause of the shift in fatty acid composition (Caglar and Cakmakci 1998).Temiz *et al.* (2010) found similar findings for Kashar cheese that had matured for 120 days; while Güler (2005) reported similar findings for Kashar cheese that had been in the market for at least three months.

Tables 5a and 5b display the volatile composition range of the Kashar cheese samples at the start and the completion of the ripening process. 37 distinct volatile chemicals were identified in all, including acids, alcohols, esters, terpenes, and hydrocarbons. Kashar cheese included 10 hydrocarbons, 7 terpe-

Table 5a. Aroma profiles of of Kashar cheese during ripening (n=3±sd) microgram/gram*.

Compound	Day 1				Day 30			
	C	ELP	MLP	MLL	C	ELP	MLP	MLL
Acids								
Hexanoic acid	1.27±1.53Aa	4.22±4.60Aa	2.24±0.29Aa	2.58±0.64Aa	2.25±1.10Aa	1.20±0.35Aa	0.93±0.26Aa	3.55±0.98Aa
Octanoic acid	2.70±1.77Aa	4.95±6.22Aa	1.09±0.14Aa	1.51±0.17Aa	0.79±0.35Aa	0.66±0.15Aa	0.50±0.15Aa	1.64±0.37Aa
n-Decanoic acid	2.74±1.72Aa	4.55±5.88Aa	0.71±0.08Aa	1.08±0.37Aa	0.43±0.17Ab	0.41±0.09Ab	0.33±0.11Ab	0.77±0.40Ab
Tetradecanoic acid	NA	0.84±7.66Aa	NA	NA	NA	NA	NA	NA
Propanoic acid, 2-methyl-	NA	NA	NA	NA	0.07±0.10	NA	NA	NA
Butanoic acid	NA	0.42±0.40Ab	1.38±0.04Ab	2.44±0.34Ab	2.67±0.75Aab	0.98±0.29Aab	1.19±0.46Aab	3.45±0.56Aab
Butanoic acid, 3-methyl-	NA	NA	NA	NA	1.53±1.23	NA	NA	NA
Alcohols								
1-Hexadecanol	NA	1.90±1.90	NA	NA	NA	NA	NA	NA
1-Butanol, 3-methyl-	NA	NA	NA	NA	3.73±1.38Ab	0.46±0.46Aa	NA	1.80±0.73Aa
Linalool	NA	NA	NA	NA	NA	NA	0.08±0.11Abc	NA
Benzylalcohol	NA	NA	NA	NA	NA	NA	NA	0.16±0.00Aa
PhenylethylAlcohol	NA	NA	NA	0.50±0.10ABb	10.09±2.02Aa	0.66±0.15Ba	2.52±0.74Ba	3.37±0.86ABa
Silanediol, dimethyl-	6.54±4.53Aa	10.65±14.46Aa	1.05±0.05Aa	1.02±0.74Aa	0.27±0.04Aa	0.34±0.05Aa	0.34±0.06Aa	0.30±0.04Aa
Esters								
Arsenousacid. tris (trimethylsilyl) ester	NA	NA	0.43±0.61Aa	0.92±0.00Aa	NA	NA	0.33±0.00Aa	0.53±0.00Aa
Decanoicacid, methyl ester	NA	NA	NA	NA	1.54±0.88A	0.57±0.13A	0.35±0.20A	2.01±1.17A
Decanoicacid, ethyl ester	NA	NA	NA	NA	NA	NA	NA	NA
Hexanoicacid, methyl ester	NA	NA	NA	NA	0.44±0.35a	NA	NA	NA
Hexanoicacid, ethyl ester	NA	NA	NA	NA	0.10±0.14Aa	NA	NA	NA
Octanoicacid, ethyl ester	NA	NA	NA	NA	0.09±0.12Aa	NA	NA	NA
Octanoicacid, methyl ester	NA	0.13±0.19Bb	0.19±0.26Bb	1.09±0.35Ab	3.05±1.29Ba	1.75±0.38Ba	2.09±0.90Ba	5.05±0.57Aa
Methyltetradecanoate	NA	NA	NA	NA	NA	NA	NA	NA
Terpenes								
p-Xylene	13.51±19.10Aa	8.89±8.89Aa	1.56±1.32Aa	NA	2.05±2.23Aa	2.85±2.41Aa	3.81±3.05Aa	NA
o-Xylene	0.57±0.80Aa	NA	NA	NA	0.49±0.42Aa	NA	NA	NA
D-Limonene	12.86±8.19Ac	10.44±14.77Ac	22.60±18.69Ac	28.66±13.19Ac	27.06±7.86Ab	24.47±3.97Ab	56.21±14.86Ab	42.35±17.91Ab
p-Cymene	NA	NA	0.38±0.54Aa	1.22±0.00Aa	NA	0.39±0.55Aa	1.07±0.77Aa	1.23±0.06Aa
β-Pinene	NA	NA	NA	NA	0.51±0.36Ba	0.55±0.39Ba	1.35±0.36Aa	0.74±0.11Aa
γ-Terpinene	NA	0.57±0.40ABd	0.85±0.06Ad	0.75±0.00Bd	1.14±0.01Cb	1.40±0.10Ab	1.69±0.23Ab	1.14±0.09Bb
o-Cymene	NA	0.69±0.49Aa	NA	NA	0.90±0.63Aa	0.65±0.65Aa	NA	NA
Hydrocarbons								
Ethylbenzene	4.60±6.50Aa	1.69±1.69Aa	0.41±0.58Aa	NA	0.69±0.49Aa	NA	0.72±1.01Aa	0.97±0.69Aa
1.3 dimethyl-benzene	5.13±3.67A	7.23±9.36A	4.29±2.85A	3.42±2.43A	2.01±1.94A	3.08±2.52A	5.81±2.19A	2.40±2.22A
Bicyclo[3.1.0]hexane. 4-methylene-1-(1-methylethyl)-	NA	NA	0.16±0.22Ab	NA	NA	NA	0.43±0.43Ab	NA
Oxime-. methoxy-phenyl-_	3.91±5.27Aa	6.64±8.59Aa	1.79±0.52Aa	2.05±0.50Aa	0.53±0.07Aa	1.13±0.89Aa	0.86±0.47Aa	0.59±0.08Aa
Styrene	NA	NA	NA	NA	0.39±0.08Aa	NA	0.24±0.18Aa	0.43±0.00Aa
3.3-Dimethyl-1,2-epoxybutane	NA	NA	NA	3.25±0.00Aa	NA	NA	NA	NA
Cyclododecane	NA	1.37±8.47Aa	NA	NA	NA	NA	NA	NA
Cyclotetradecane	NA	1.37±8.47Aa	NA	NA	NA	NA	NA	NA
Benzene. 1,2-dichloro	NA	0.91±0.67a	NA	NA	0.99±0.70a	2.33±0.08a	NA	NA
Benzene. 1,3-dichloro	NA	NA	NA	NA	NA	NA	1.41±1.07A	2.05±0.51A

*C: Control. ELP: cheese treated with eggwhite lysozyme. MLP: cheese treated with microbial lysozyme powder. MLL: cheese treated with microbial lysozyme liquid. Duncan's test was used to compare significant means. Different lowercase superscript letters in the same row indicate significant differences during ripening ($P < 0.05$); different uppercase superscript letters in the same column indicate significant differences among sample groups ($P < 0.05$).

Table 5b. Aroma profiles of of Kashar cheese during ripening (n=3±sd) microgram/gram*

Compound	Day 60				Day 90			
	C	ELP	MLP	MLL	C	ELP	MLP	MLL
Acids								
Hexanoic acid	2.76±0.46Aa	0.84±0.13Aa	1.18±0.07Aa	1.40±0.07Aa	5.03±2.10Aa	6.76±7.32Aa	2.52±0.87Aa	2.40±0.57Aa
Octanoic acid	1.07±0.66Aa	0.51±0.07Aa	0.38±0.27Aa	0.77±0.01Aa	1.91±0.75Aa	2.41±2.36Aa	1.03±0.29Aa	0.96±0.19Aa
n-Decanoic acid	0.52±0.01Ab	0.17±0.01Ab	0.13±0.10Ab	0.22±0.02Ab	0.62±0.31Ab	1.33±1.33Ab	0.34±0.15Ab	0.37±0.10Ab
Tetradecanoic acid	NA	NA	NA	NA	NA	NA	NA	NA
Propanoic acid. 2-methyl-	NA	NA	NA	NA	NA	NA	NA	NA
Butanoic acid	2.93±0.80Ab	0.45±0.07Ab	0.76±0.13Ab	0.96±0.03Ab	5.07±2.67Aa	4.58±5.08Aa	1.72±0.65Aa	2.42±0.75Aa
Butanoic acid. 3-methyl-	NA	NA	NA	NA	NA	NA	NA	NA
Alcohols								
1-Hexadecanol	NA	NA	NA	NA	NA	NA	NA	NA
1-Butanol. 3-methyl-	NA	NA	0.15±0.21Ab	NA	0.63±0.63Aab	NA	0.55±0.78Aab	0.91±0.00Aab
Linalool	NA	NA	0.36±0.13Aa	0.28±0.05Aa	NA	NA	0.21±0.16Aab	0.29±0.00Aab
Benzylalcohol	NA	0.05±0.04Ba	0.02±0.03Ba	0.12±0.00Aa	NA	NA	NA	0.23±0.00Aa
Phenylethyl Alcohol	0.80±0.13Ab	0.11±0.03Bb	0.24±0.11Bb	1.24±0.15ABb	0.57±0.17Ab	0.82±0.82Bb	0.48±0.20Bb	0.54±0.22ABb
Silanediol. dimethyl-	0.68±0.10Aa	0.35±0.10Aa	0.36±0.07Aa	0.40±0.08Aa	1.09±0.34Aa	3.15±3.20Aa	0.79±0.44Aa	1.22±0.67Aa
Esters								
Arsenousacid. tris (trimethylsilyl) ester	NA	NA	0.17±0.00Aa	0.30±0.00Aa	NA	NA	0.42±0.13Aa	0.25±0.00Aa
Decanoic acid. methyl ester	NA	NA	NA	NA	NA	NA	NA	NA
Decanoic acid. ethyl ester	0.06±0.08	NA	NA	NA	NA	NA	NA	NA
Hexanoic acid. methyl ester	NA	0.16±0.00Aa	NA	NA	NA	NA	NA	NA
Hexanoic acid. ethyl ester	0.48±0.11a	NA	NA	NA	0.96±0.62a	NA	NA	NA
Octanoic acid. ethyl ester	0.29±0.26Aa	NA	NA	NA	0.08±0.08Aa	NA	NA	NA
Octanoicacid. methyl ester	0.49±0.11Bb	NA	0.14±0.19Bb	0.67±0.38Ab	NA	0.10±0.10Bb	0.22±0.19Bb	0.22±0.00Ab
Methyltetradecanoate	NA	NA	NA	NA	NA	NA	NA	NA
Terpenes								
p-Xylene	9.29±3.47Aa	6.51±5.54Aa	9.69±6.05Aa	NA	0.92±0.92Aa	NA	7.11±3.82Aa	NA
o-Xylene	0.25±0.35Aa	1.36±0.07Aa	NA	NA	0.87±0.61Aa	0.43±0.61Aa	NA	NA
D-Limonene	62.34±3.87Aa	65.35±3.01Aa	45.77±32.37Aa	63.01±1.33Aa	39.94±9.31Ab	41.44±9.33Ab	31.32±19.90Ab	34.52±24.01Ab
p-Cymene	NA	1.02±0.74Aa	0.14±0.20Aa	1.47±0.21Aa	NA	0.74±0.00Aa	0.78±0.56Aa	NA
β-Pinene	NA	NA	1.09±0.77Aa	1.60±0.02Aa	0.36±0.51Bab	NA	0.29±0.42Aab	1.06±0.19Aab
γ-Terpinene	1.50±0.14Ca	2.09±0.10ABa	1.93±0.08Aa	1.62±0.20Ba	0.91±0.17Cc	0.99±0.50ABc	1.21±0.10Ac	1.20±0.15Bc
o-Cymene	0.16±0.23Aa	0.86±0.86Aa	NA	NA	0.65±0.47Aa	0.72±0.72Aa	NA	NA
Hydrocarbons								
Ethylbenzene	1.42±1.23Aa	3.20±0.42Aa	NA	3.66±0.00Aa	1.58±1.12Aa	2.40±1.74Aa	2.33±0.03Aa	2.50±0.02Aa
1.3 dimethyl-benzene	1.86±0.86A	10.96±1.33A	8.38±4.00A	16.79±2.58A	7.27±5.15A	1.12±1.58A	4.80±3.96A	3.18±1.51A
Bicyclo[3.1.0]hexane. 4-methylene-1-(1-methylethyl)-	NA	NA	1.73±0.06Aa	NA	NA	NA	0.56±0.48Ab	NA
Oxime-. methoxy-phenyl-	0.78±0.23Aa	0.29±0.02Aa	0.31±0.04Aa	0.53±0.12Aa	1.07±0.43Aa	3.71±4.22Aa	0.66±0.35Aa	0.95±0.47Aa
Styrene	NA	NA	NA	NA	NA	NA	NA	1.20±0.00Aab
3.3-Dimethyl-1.2-epoxybutane	NA	NA	NA	0.51±0.51Aa	NA	NA	0.13±0.18Aa	NA
Cyclododecane	NA	NA	NA	NA	NA	NA	NA	NA
Cyclotetradecane	NA	NA	NA	NA	NA	NA	NA	NA
Benzene. 1.2-dichloro-	NA	0.26±0.22a	NA	NA	0.10±0.13a	2.22±2.22a	NA	NA
Benzene. 1.3-dichloro-	NA	NA	0.20±0.15A	0.46±0.03A	NA	NA	0.07±0.10A	NA

*C: Control. ELP: cheese treated with eggwhite lysozyme. MLP: cheese treated with microbial lysozyme powder. MLL: cheese treated with microbial lysozyme liquid. Duncan test was used to compare significant means. Different lowercase superscript letters in the same row indicate significant differences during ripening ($P < 0.05$); different uppercase superscript letters in the same column indicate significant differences among sample groups ($P < 0.05$).

nes, 6 alcohols, 7 esters, 7 terpenes, and 7 acids, among their volatile constituents. Other researchers have previously examined the volatile compounds in Kashar cheese (Eroğlu *et al.,* 2016; Sulejmani and Hayaloğlu, 2016). However, the lysozyme treatment of Kashar cheese has not been examined. Therefore, the present study's results may be considered valuable information for future studies of this sort of processed dairy product. The type of lysozyme treatment and cheese age impacted some chemicals, but not all of them in a statistically significant way.

Acids. The highest concentration of the seven acids observed in the Kashar cheese was butanoic (butyric), and after 30 days of ripening, there were significant differences between its concentrations and those of the other cheeses ($p < 0.05$). In addition, the most prevalent acids in all samples at day 90 were butyric and hexanoic acids, which contrasts with the findings of Eroğlu *et al.* (2016) but is consistent with research of Suleymani and Hayaloğlu (2016).

Alcohols. The alcohols with the highest concentrations in Kashar cheese after ripening were 3-methyl-1-butanol, linalool, phenylethyl alcohol, and silanediol dimethyl. The kind of lysozyme treatment or ripening period had no effect on the concentrations of these alcohols. In general, the relative abundance of alcohols declined at the end of ripening and was higher in the control cheese than in other types. This might be connected to how lysozyme treatment affects the formation of alcohol in cheese.

Esters. The ester makeup of the cheese samples remained unchanged after the lysozyme and ripening procedures. This could be because there were not any appreciable variations in any of the pH samples throughout ripening (Table 2), which shows a similar pH trend for all samples. Eroğlu *et al.* (2016) hypothesized that the composition of milk and reaction parameters like pH might significantly impact the synthesis of ester compounds.

Terpenes: The volatile percentage of Kashar cheese contained seven terpenes, of which pinene, cymene, terpinene, and xylene derivatives were the main ones (Table 4). These substances were detected in significant amounts in the cheese and the limonene. Terpenes in milk are derived from plants found in the feed combination or pasture (Curioni and Bosset, 2002), and the kind and quantity of cheese are mostly dependent on the quality of the milk used to make it. On the other hand, during the ripening and lysozyme treatments, the concentrations of hydrocarbons fluctuated, but their abundance did not statistically alter.

4. CONCLUSIONS

The findings of this study demonstrate how the use of bacterial and egg lysozymes in Kashar cheese altered the cheese's quality, whether in liquid or in powder form. The application of milk lysozyme did not significantly change the cheese's titratable acidity, pH, spore bacteria, coliforms, or E. coli contents, according to physicochemical data ($P > 0.05$). However, the microbial analysis showed that cheeses treated with bacterial lysozyme (MLL, MLP) were effective against TMAB, yeast and mold, coliform levels, and lactic acid bacteria counts. When compared to control cheese, significant variations in microbe counts during ripening were found. The texture profile showed that lysozyme-treated cheese had improved textural characteristics, with a notable increase in hardness and cohesiveness but a decrease in springiness, gumminess, and resilience during storage, especially in lysozyme-treated cheese made with microbial powder (MLP). It is believed that the decrease in fatty acids observed in the lysozym-treated cheese throughout the ripening phase is connected to the microbial population of the cheese due to the inhibitory impact of lysozym. Of all the samples, the control cheese scored the highest in terms of free fatty acids. With the exception of C4, C14, C16, and C18, the free fatty acid fractions generated in kashar cheese's short chains (SCFA), long chains (LCFA), and fatty acids were all decreased by the application of lysozyme forms. Ripening and lysozme variables did not significantly affect the majority of the volatile profiles.

As a result, we firmly advocate the use of lysozyme, particularly microbial powder lysozyme, as a feasible substitute for enhancing the physical, textural, and compositional aspects of Kashar cheese production as well as other highly-cooked pasta filata type cheese like Mozzarella or Provalone. In addition, the present work provides precise knowledge on the types of lysozyme to be employed while producing cheese that could inhibit microbial proliferation while also improving cheese quality and ensuring consumer safety and health when cheese is ripening. To make Kashar cheese with a longer shelf life, the viability of the solutions suggested in this work could be encouraged at an industrial level.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this work.

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