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Impact of different starter cultures and *Lactobacillus helveticus* on volatile components, chemical and sensory properties of pasta filata cheese

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ABSTRACT

L.helveticus is known to follow mainly similar metabolic pathways to contribute to cheese flavor with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. In this study, the flavor contributions of commercial *S. thermophilus* starter cultures of different brands and *L. helveticus* adjunct culture to pasta filata type fresh Kashar cheese were investigated. *L. helveticus* affected the buttery aroma components of fresh Kashar cheese and resulted in low diacetyl content. In addition, acetaldehyde and other aroma components of *L. helveticus* added cheese was found to be higher than control and modified control cheeses. On the other hand, the modified control sample containing *S. thermophilus* from Danisco instead of Chr-Hansen in the control was closer to the control sample in terms of volatile profile. As the shelf-life progressed, the contribution of alcohols and hydrocarbons to volatile components decreased, while the contribution of ketones, which was the dominant group, increased in all products. When the proteolysis and lipolysis levels were examined, the control sample differed from the other two cheese samples with its high proteolysis and lipolysis rate. In summary, the rates of increase in water-soluble nitrogen and free fatty acid contents in 8 weeks of storage (from 18 to 72 days) were determined as 61% and 47%, respectively, in the control Kashar cheese, while it was 39% and 27% in the *L. helveticus* added sample, and 37% and 28% in the modified control sample. Finally, the sensory scores revealed that cheese flavor and texture preferences could be increased with the addition of *L. helveticus*.

1. Introduction

Lactobacillus helveticus (*L. helveticus*) is a thermophilic lactic acid culture primarily applied in the production of Italian and Swiss cheese varieties. It has a cell wall-bound proteinase released from whole cells without any leakage of intracellular enzymes (Griffiths and Tellez, 2013). Besides the extracellular proteinase, *L. helveticus* strains have peptidases and lipases generally located intracellularly. Some strains of *L. helveticus* liberate the intracellular enzymes through autolysis, which plays a major role in flavor development as a result of the catabolic conversion of amino acids into aroma compounds. The effects of *L. helveticus* on aroma improvement, reduced bitterness, and accelerating proteolysis, especially in cheese, is still of interest to many researchers (Baptista et al., 2018; Cuffia et al., 2018; Sekban and Tarakci, 2020; Skrzypczak et al., 2020).

Kashar, is a type of pasta filata cheese that can be consumed fresh or matured. Starter and non-starter lactic acid bacteria (LAB) are frequently used to increase flavor and yield (Zisu and Shah, 2005; Atasoy et al., 2008). The traditional process of Kashar includes pasteurization or termization of milk, inoculating with lactic cultures up to pH 6.40, coagulation of milk using rennet, cutting and pressing the curd, scalding and stretching at 75–85 °C (Licitra et al., 2018).

In practice, combinations of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*; *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and *L. casei*; *Lactococcus lactis* subsp. *lactis* and *L. casei* are commonly used cultures of Kashar cheese (Licitra et al., 2017). The use of *L. helveticus* is not a common practice. Recent studies on Kashar cheese have mostly focused on cost-reducing with some modifications to the production process, including fat substitutes and enzyme applications (Akın et al., 2012; Çelik et al., 2018; Topcu et al., 2020). However, these applications are not allowed in the Food Regulations of Kashar cheese (Anonymous, 2015); only processed cheeses can be produced with these methods. On the other hand, there is limited information about the volatile components of Kashar cheese. Hayaloglu (2009) analyzed 4 different brands of mature Kashar cheese from the market and determined the volatile component profile range for 6–10 months old Kashar cheeses. Eroglu

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et al. (2016) matured unpacked fresh Kashar cheeses supplied from 4 different local producers and revealed the change in volatiles after maturing unpacked for 90 days. Temizkan et al. (2014) studied similar maturing, but also compared the volatiles of cheeses produced from different milk sources in the study. Çetinkaya (2020) investigated the effects of brining and vacuum packaging on only a few volatile components of fresh and matured Kashar cheeses. In the study of Guidone et al. (2015), *L. helveticus* have been determined to accelerate the ripening of pasta filata type Scamorza cheeses and differentiate the volatile properties of the product when analyzed with an electronic nose. Although there are studies on some pasta filata cheeses, there is no study on the contribution of *L. helveticus* to volatile components in fresh Kashar cheese. It is also known that the ability of LAB to degrade amino acids to aroma compounds is highly strain-dependent. Based on the last two literature data, (i) the effect of company changes in the main culture - *S. thermophilus* on the volatile compounds of fresh Kashar cheeses and (ii) the effect of *L. helveticus* on the product properties were determined in this study.

2. Material and methods

2.1. Cheesemaking

The cheeses were produced in duplicate from the same batch of milk at AK Gıda, the dairy production plant of Group Lactalis in Turkey. The production flowchart followed was based on the industrial production steps of the plant, given in Fig. 1. Before the process, raw cow's milk was standardized to a fat: protein ratio of 1.05 and pasteurized at 72 °C for 15 s. After cooling to 33 °C, cultures at the dosages given in Table 1 and chymosin (Maxiren®, DSM, Holland) diluted 1:10 (w:w) in water at a dosage of 1% were added to the milk. The coagulum was cut into 0.2 cm^3 particles 30 min after chymosin addition and heated to 40 °C. After 3.5 h, whey was discharged at pH 5.80 and curd was left in carts for further acidification to pH 5.05 and adequate whey drainage (Licitra et al., 2017). The filtered curd was heated to 70 °C in 3 min and stretched under hot condensed steam for 5 min. Then the cheese was put into moulds, cooled to 4 °C in 12 h and then vacuum packed.

2.2. Compositional analysis

Cheeses were analyzed for total dry matter (DM) (ISO, 2004), fat in dry matter (ISO, 2008), and protein (IDF, 2001). Salt was measured according to the potentiometric titration method (ISO 2006).

2.3. pH, proteolysis and lipolysis measurements

The pH values were monitored during storage with a pH meter of Mettler Toledo Seven Compact S220 connected to an InLab Solid pro electrode. The water-soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble (TCA-SN) nitrogen fractions were determined according to Kuchroo and Fox (1982) by a modification on the ratio of cheese in deionized water as applied by Topçu and Saldamli (2006). The 5% phosphotungstic acid-soluble nitrogen (PTA-SN) of cheeses was analyzed according to the method of Sousa and Malcata (1997). Results of proteolysis degrees were quantified as percent of total nitrogen (TN). Free fatty acids (FFAs) were measured by titrating the acidity of the

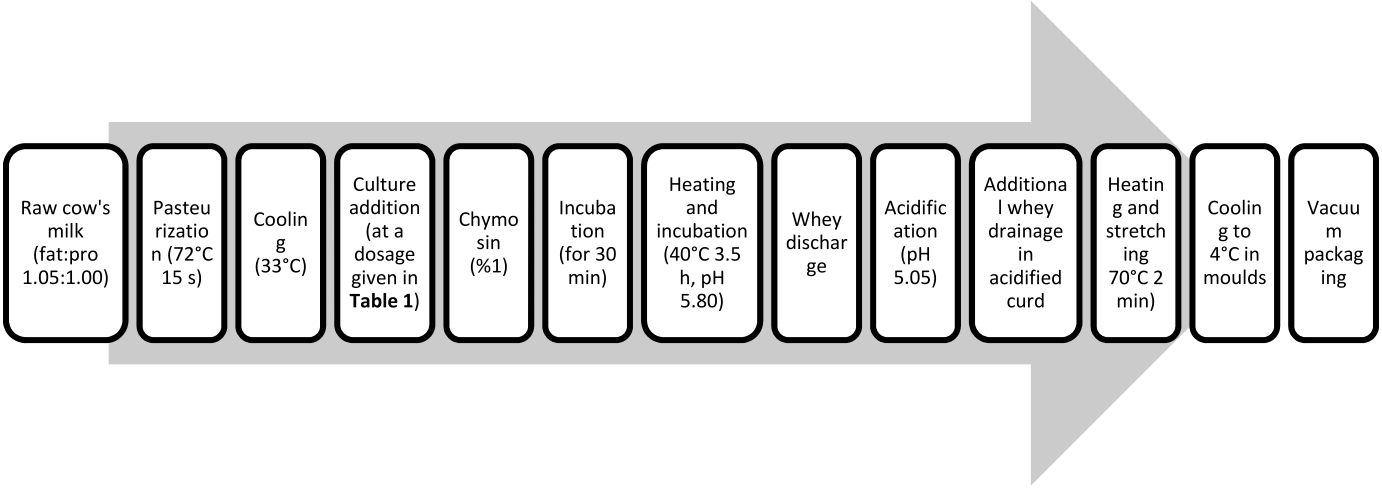

Fig. 1. Flow diagram for cheese manufacture.

Table 1
Cultures used in Kashar cheeses.

Group name	Description	Culture composition	Company and code of culture	Inoculation dosage for a 7-ton batch
C	Control was produced by widely used starter cultures	*L. delbrueckii* subsp. *bulgaricus*	Chr-Hansen LB-12	500 U
		S. thermophilus	Chr-Hansen F-DVS STI-06	500 U
H	*L. helveticus* was added to the control sample (C)	*L. helveticus*	Chr-Hansen LH-B02	50 U
		L. delbrueckii subsp. *bulgaricus*	Chr-Hansen LB-12	500 U
		S. thermophilus	Chr-Hansen F-DVS STI-06	500 U
CM	The starter culture of *S. thermophilus* in the control (C) was replaced with a culture of a different brand.	*L. delbrueckii* subsp. *bulgaricus*	Chr-Hansen LB-12	500 U
		S. thermophilus	Danisco Choozit Swift 18 Fro	500 DCU

cheese fat against potassium hydroxide (KOH) and results were given in terms of oleic acid % in total cheese fat (Nuñez et al., 1986).

2.4. Microbiological analysis

S. themophilus and lactobacilli were analyzed according to the methods in the literature (El-Sayed et al., 2020; Şimşek and Sağdıç, 2009). Ten gram of each batch of cheeses was dissolved in 90 ml of sterile Ringer's solution and a serial 1:9 (v:v) dilution of that stock was prepared to track the microbial counts. *S. thermophilus* was determined using M17 agar (Merck, Germany) and the plates were incubated at 35 °C for 48 h. Lactobacilli were determined by MRS agar (Merck, Germany). The plates were incubated at 37 °C for 48 h under anaerobic condition. The microbiological analysis was enumerated as log cfu/g values.

2.5. Determinations of volatile components by GC-MS

The volatile components of Kashar were extracted by solid-phase microextraction (SPME) technique and analyzed by gas chromatography (GC) coupled with a triple quad mass analyser (Agilent 7000, Agilent Technologies, USA). For analysis, 3 g of grated cheese sample was transferred into a 20 mL vial and sealed with a polytetrafluoroethylene (PTFE)-faced silicone septum (Supelco). Then the vial was kept at 60 °C for 30 min. Then the headspace components were extracted on an 85 μm SPME fiber assembled by carboxen/polydimethylsiloxane (CAR/PDMS) for 30 min at 60 °C.

The extracted volatiles was desorbed by inserting the fiber into the GC injection port, running in splitless mode. Fiber remained for 2 min at a temperature of 250 °C, under helium gas at a flow rate of 1 mL min^{-1}. The components were separated on an Agilent capillary column covered with 5% phenyl/95% dimethylpolysiloxane, 30 m length * 0.25 mm internal diameter * 0.25 μm film thickness. The temperature program was carried out according to the method of Hayaloglu (2009); the oven was maintained at 40 °C for 2 min of desorption, then raised to 70 °C in an increase of 5 °C per min, held for 1 min. Then in 10 °C/min increments, the oven reached 240 °C in a 30 min run time. Comparative values of the volatile components were obtained by elution of peak areas. The results were expressed as peak area (%).

2.6. Sensorial evaluations

The cheese samples were quantified by 20 trained panellists in AK Gıda production plant staff. The panellists scored the flavor and texture of cheeses according to a 9-point hedonic scale. They were also asked to describe the dominant sensation assessments.

2.7. Statistical analysis

Cheese analyzes were performed on days 18, 40 and 72 of shelf-life. The data obtained from two cheesemaking trials were submitted to SPSS (ver. 20.0, SPSS Inc., Chicago, IL) for the analysis of variance (ANOVA) and mean values of triplicate analyzes were compared using Tukey's test with a significance level of $P < 0.05$. The volatile content data were also submitted to principal component analysis (PCA) using Minitab (ver. 16.0, Minitab Inc., State College, PA, USA) to provide further information about the effect of the starter culture on flavor compound production.

3. Results and discussion

3.1. Compositional analysis

The chemical compositions of the Kashar samples are given in Table 2. According to Table 2 and the variance analysis, the DM and fat values of cheese H produced with *L. helveticus* was significantly different from the other cheeses. The faster acidification rate caused by *L. helveticus* can be explained as the reason for the higher moisture and lower fat in cheese H. As calcium becomes more soluble due to rapid acidification, protein-protein interactions are inhibited, resulting in softer curds at higher moisture content while at the same time causing free fat that cannot be collected in the channels between the protein strands and will be lost in the following processing steps. Protein and salt contents were similar in all groups. Chemical composition results were in line with those reported in the literature for fresh Kashar cheeses (Hayaloglu, 2009; Eroglu et al., 2015), except for salt content. The composition results obtained in the literature varied in a wide range. DM of 30 pieces of cheese varied between 51.5 and 61.5%, fat in DM varied between 25 and 50% and protein content between 20.0 and 28.8% (Eroglu et al., 2015). The salt ratio of 12 pieces of cheese obtained from the market varied between 4.6 and 7.6% in DM (Hayaloglu, 2009). These values are higher than the values in our study, which ranged from 3.4 to 4.7% in DM. In the study of Tuncturk and Coskun (2007), the DM, protein, fat and salt contents of Kashar cheeses were higher than in our study.

3.2. pH, proteolysis, lipolysis and microbial results

The results in Table 3 revealed that the pH of the samples differed significantly, CM had the highest and C had the lowest pH value. The tendency was maintained throughout the shelf-life. In addition, it was observed that the pH value decreased significantly in all groups on the 72nd day. According to the results, the acidity of C and H cheeses progressed faster than CM cheese. Accordingly, it could be concluded that the acidification rate of *S. thermophilus* culture obtained from Danisco was slower than the Chr-Hansen culture. However, the *S. thermophilus,* especially added for biological acidification, had similar counts in C and CM cheeses. When comparing pH results of cheeses C and H, it was thought that the addition of *L. helveticus* might cause an inhibitory (or antagonistic) effect on the other cultures and cause slower acidification. The *S. thermophilus* counts of cheese H was lower at day 18 than the control sample (C), supporting the hypothesis.

The metabolism of amino acids and fatty acids are important secondary biochemical events responsible for the development of many volatile flavor compounds during ripening (McSweeney, 2004). The degree of proteolysis was measured based on the amounts of soluble nitrogen fractions. WSN increased significantly during the storage of all cheeses. TCA-SN, PTA-SN and FFA contents also increased during 72 days of storage in most samples, as expected. The higher the pH above the casein's isoelectric point, the more nitrogen is soluble in water. As the CM sample had higher pH, WSN was also the highest on the 18th day. However, at the end of the storage, the WSN ratios of the C and CM

Table 2
Composition of Kashar cheeses on the 18th day of storage.

Cheese	DM (%)	Fat (%)	Protein (%)	Salt (%)	Fat in DM (%)	Salt in DM (%)
C	55.4 ± 0.30 a	27.3 ± 0.29 a	22.5 ± 0.79 a	1.9 ± 0.47 a	49.3	3.4
H	53.1 ± 0.29 b	24.8 ± 0.29 b	23.6 ± 0.27 a	1.8 ± 0.12 a	46.7	3.4
CM	54.8 ± 0.16 a	27.0 ± 0.50 ab	22.0 ± 0.32 a	2.6 ± 0.16 a	49.3	4.7

Different letters "a & b" in the same column represent statistical difference according to Tukey's test ($P < 0.05$).
*DM: Dry matter.

Table 3
Results of proteolysis-related analyses of Kashar cheeses on the 18th, 40th and 72nd days of storage.

Cheese		pH	WSN (% of TN)	TCA-SN (% of TN)	PTA-SN (% of TN)	FFAs (% oleic acid of cheese fat)	*S. thermophilus* counts (log.cfu/g)	Lactobacilli counts (log.cfu/g)
C	day 18	5.52 ± 0.01 C a	3.63 ± 0.12 B c	0.65 ± 0.07 A b	0.48 ± 0.09 A c	1.14 ± 0.01 A c	4.12 ± 0.12 A a	1.30 ± 0.20 A a
	day 40	5.50 ± 0.01 C a	4.79 ± 0.02 A b	0.82 ± 0.04 A b	0.82 ± 0.09 A b	1.43 ± 0.02 A b	3.97 ± 0.12 A a	1.27 ± 0.17 A a
	day 72	5.45 ± 0.01 C b	5.84 ± 0.24 A a	1.19 ± 0.09 A a	1.08 ± 0.03 A a	1.68 ± 0.03 A a	3.51 ± 0.17 A a	1.70 ± 0.22 A a
Rate of increase from day 18 to 72			*61%*	*83%*	*125%*	*47%*		
H	day 18	5.57 ± 0.01 B a	3.38 ± 0.14 B c	0.65 ± 0.11 A b	0.54 ± 0.11 A b	1.05 ± 0.02 B c	3.22 ± 0.16 B a	0.79 ± 0.13 A a
	day 40	5.56 ± 0.01 B a	4.11 ± 0.01 B b	0.92 ± 0.00 A ab	0.84 ± 0.07 A a	1.13 ± 0.01 B b	3.01 ± 0.22 A a	0.82 ± 0.15 A a
	day 72	5.51 ± 0.01 B b	4.70 ± 0.15 B a	0.95 ± 0.05 A a	0.95 ± 0.06 A a	1.33 ± 0.02 B a	2.71 ± 0.16 A a	1.00 ± 0.12 A a
Rate of increase from day 18 to 72			*39%*	*46%*	*76%*	*27%*		
CM	day 18	5.77 ± 0.01 A a	4.44 ± 0.13 A c	0.73 ± 0.16 A b	0.56 ± 0.00 A b	1.07 ± 0.03 B b	3.70 ± 0.17 AB a	1.12 ± 0.12 A a
	day 40	5.75 ± 0.00 A a	5.14 ± 0.09 A b	1.01 ± 0.08 A ab	0.93 ± 0.08 A a	1.11 ± 0.01 B b	3.30 ± 0.15 A a	1.23 ± 0.17 A a
	day 72	5.55 ± 0.01 A b	6.10 ± 0.20 A a	1.07 ± 0.09A a	1.01 ± 0.04 A a	1.37 ± 0.00 B a	3.19 ± 0.14 A a	1.52 ± 0.21 A a
Rate of increase from day 18 to 72			*37%*	*47%*	*80%*	*28%*		

Capital letters indicate differences between samples on the same storage day. Lowercase letters represent the change in the sample during storage. ($P < 0.05$).
* WSN: Water-soluble nitrogen, TCA-SN: Trichloroacetic acid (12%) soluble nitrogen, TN: Total nitrogen, PTA-SN: phosphotungstic acid (5%) soluble nitrogen, FFAs: Free fatty acids.

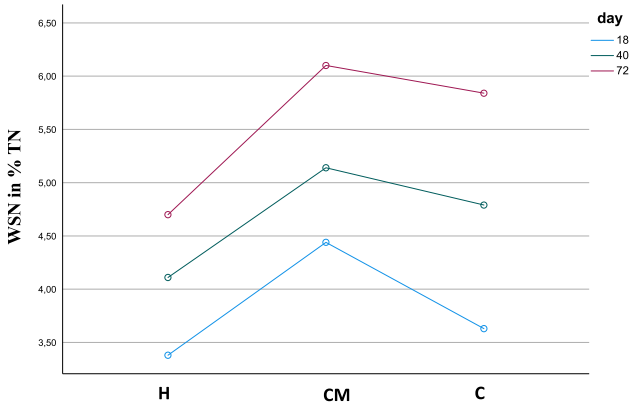

Fig. 2. The graph of changes in WSN rates (% in TN) between samples and storage days.

samples were equalized due to the rapid proteolysis level in the control sample (Fig. 2).

Lactobacilli are mainly responsible for proteolysis in pasta filata cheeses (Kieliszek et al., 2021). In particular, some strains of *L. helveticus* have very high proteolytic activity (Griffiths and Tellez, 2013). In our study, the total counts of lactobacilli were similar between samples and between days, but their ratios to each other and growth curves during incubation were not determined in our study. During storage, the total counts of lactobacilli in the cheeses were lower than the microbiological viability found in the literature, and the proteolytic activity results were lower than the cheeses analyzed by Sert et al. (2007) and Yilmaz and Dagdemir (2012). Temizkan et al. (2014) reported that TCA-SN (% of TN) measurements during the ripening of Kashar cheese were 1.18 and 3.04, respectively, on the 1st and 30th days. These values are also considerably higher than our results. In another study on a pasta filata type cheese, when cheeses were produced by inoculating LAB before the scalding process, as in Kashar cheese manufacturing, the WSN (% of TN) and TCA-SN (% of TN) values were around 10 and the PTA-SN (% of TN) value was around 5 on the 90th days of storage. In the same study, when the LAB was added after scalding, cheeses obtained were not appreciated by panelists, and the ripening values increased considerably to 40 and 15, respectively (Öründü and Tarakçı, 2021).

FFA contents were analyzed to determine the level of lipolysis. According to the pH results and considering the optimum pH required for maximum enzymatic activity, the lowest proteolysis and lipolysis degrees were estimated in group CM, but the level of proteolysis in cheese H with added *L. helveticus* was the lowest throughout the storage, probably due to differences in starter cultures. The observed increase rates in proteolysis and lipolysis from day 18–72 were similar in H cheese with *L. helveticus* and CM cheese with *S. thermophilus* from Danisco (Choozit Swift 18 Fro). However, these values were higher in the control cheese (group C) produced with the *S. thermophilus* strain from Chr-Hansen (F-DVS STI-06).

3.3. The volatile components

Although Kashar is a cooked cheese, it contains thermophilic bacteria and heat-stable enzymes that are active throughout its shelf-life (De Angelis and Gobbetti, 2011). Their activities affect the aroma of Kashar. Total volatile compounds in experimental cheeses consisted of 7 ketones, 3 alcohols, 5 aldehydes, 3 amines, 3 esters, 7 acids, and 3 hydrocarbons. The concentrations of these volatile components are given in Table 4.

Among the ketones (Table 4), the high amount of 2,3-butanendione (diacetyl) and 2-butanone, which is derived from diacetyl, was notable in all cheese samples. According to the 40th day analysis, it was concluded that sample-CM which was produced with *S. thermophilus* from the Danisco company had higher diacetyl content than the samples which were produced with *S. thermophilus* culture of Chr-Hansen. 1-methoxy-2-propanone had also a significant change on day 40, with a higher amount in *L. helveticus* added cheese. It has been reported that 1-methoxy-2-propanone is reduced by heat treatment in pasta filata cheese (Kaminarides et al., 2015). 2-Pentanone was found in all cheese groups at similar concentrations and with minor changes during storage. 2, 3-Pentanedione was found in low concentrations and was not detected on the 72nd-day of analysis. Among all volatile components, the highest concentration belonged to 3-hydroxy-2-butanone (acetoin) and remained in this state during storage in all sample groups. Cuffia et al. (2017) produced a probiotic pasta filata cheese and found the ketones as the largest volatiles group, led of acetoin, as in our study. The flavor contribution of 2-heptanone, known as the blue cheese aroma note, was found to be similarly low in all products.

According to Table 4, 2-Butyl-1-octanol was identified on the 18th day of all samples, and its amount constituted 4–9% of the total volatiles. With the storage, its amount decreased below the detection limits.

LAB can produce acetaldehyde and it can be metabolized to diacetyl and acetoin (Fox et al., 2000). It is known that acetaldehyde obtained by glucose metabolism mainly originates from yogurt bacteria, while *L. helveticus* can also synthesize acetaldehyde, especially through threonine metabolism (Ott et al., 2000; Klein et al., 2001). In our study, the highest acetaldehyde level was determined in *L. helveticus* added cheese H, while the total concentration of diacetyl and acetoin was the lowest in that cheese (Table 4). The heptanal contributing a fatty note to cheese

Table 4
Aroma profile of Kashar cheese samples on the 18th, 40th and 72nd days of storage.[a]

	Day 18			Day 40			Day 72		
	C	H	CM	C	H	CM	C	H	CM
KETONES									
2,3-Butanendione	9.88 ± 0.70 A a	8.04 ± 0.61 A a	12.54 ± 1.52 A a	12.09 ± 0.85 AB a	8.09 ± 1.25 B a	19.32 ± 2.10 A a	13.26 ± 0.97 A a	10.45 ± 1.10 A a	15.16 ± 3.32 A a
2-butanone	9.87 ± 0.60 A a	11.86 ± 1.42 A a	8.89 ± 1.10 A a	10.83 ± 0.70 A a	13.97 ± 1.20 A a	12.74 ± 1.24 A a	8.43 ± 0.61 A a	9.25 ± 1.10 A a	8.35 ± 0.91 A a
1-Methoxy-2-propanone	3.16 ± 0.38 A a	4.18 ± 0.90 A a	3.82 ± 0.52 A a	2.04 ± 0.45 B a	7.04 ± 1.10 A a	5.56 ± 0.61 AB a	4.33 ± 0.36 A a	6.85 ± 0.80 A a	4.81 ± 0.48 A a
2-Pentanone	0.31 ± 0.11 A a	0.90 ± 0.30 A a	0.24 ± 0.08 A a	0.50 ± 0.18 A a	0.81 ± 0.24 A a	0.58 ± 0.24 A a	0.54 ± 0.19 A a	0.46 ± 0.14 A a	1.03 ± 0.35 A a
2,3-Pentanedione	0.59 ± 0.15 A a	0.80 ± 0.08 A a	0.23 ± 0.07 A a	0.71 ± 0.31 A a	0.32 ± 0.11 A b	0.38 ± 0.13 A a	ND	ND	ND
3-Hydroxy-2-butanone	46.62 ± 2.37 A a	37.01 ± 2.40 A a	33.73 ± 2.45 A a	51.47 ± 3.11 A a	47.50 ± 3.40 A a	47.05 ± 2.51 A a	48.76 ± 1.45 A a	40.98 ± 2.24 A a	41.15 ± 2.34 A a
2-Heptanone	1.52 ± 0.42 A a	2.63 ± 0.55 A a	1.53 ± 0.45 A a	0.39 ± 0.37 A a	0.95 ± 0.22 A a	0.68 ± 0.65 A a	0.64 ± 0.17 A a	0.65 ± 0.18 A a	1.03 ± 0.33 A a
ALCOHOLS									
2-Butyl-1-octanol	3.41 ± 1.50 a	8.41 ± 2.30 b	5.01 ± 2.80 a	ND	ND	ND	ND	ND	ND
ALDEHYDES									
Acetaldehyde	ND	1.26 ± 0.02 A a	0.63 ± 0.03 B a	ND	0.99 ± 0.01 b	ND	0.19 ± 0.07 A	0.44 ± 0.03 A c	0.20 ± 0.10 A b
Heptanal	0.84 ± 0.09 A a	0.40 ± 0.05 B a	0.76 ± 0.06 AB a	0.42 ± 0.05 AB b	0.27 ± 0.02 B a	0.58 ± 0.06 A ab	0.33 ± 0.04 A b	0.22 ± 0.02 A a	0.37 ± 0.03 A c
3-Methyl-hexanal	ND	ND	ND	ND	0.45 ± 0.01 ab	ND	ND	2.25 ± 0.64 a	ND
3-Methyl-2-butenal	ND	1.21 ± 0.08 a	ND	ND	0.28 ± 0.10 b	ND	ND	1.44 ± 0.09 a	ND
3-Hydroxy-butanal	ND	ND	0.13 ± 0.05 a	ND	ND	0.16 ± 0.04 a	ND	ND	ND
AMINES									
Dimethylamine	0.41 ± 0.23 A a	1.02 ± 0.06 A b	1.02 ± 0.50 A a	0.42 ± 0.24 B a	2.29 ± 0.09 A a	0.67 ± 0.32 B a	0.94 ± 0.37 A a	1.23 ± 0.13 A b	0.97 ± 0.41 A a
4-Methyl-2-hexanamine	0.30 ± 0.03 AB b	0.47 ± 0.06 A c	0.19 ± 0.03 B b	0.72 ± 0.04 B a	1.53 ± 0.09 A a	0.54 ± 0.06 B b	0.71 ± 0.03 B a	0.97 ± 0.09 B b	1.44 ± 0.08 A a
4-Methyl-pentanamine	ND	ND	ND	ND	1.77 ± 0.09 a	ND	0.21 ± 0.10 A	0.37 ± 0.09 A b	ND
ESTERS									
Ethyl acetate	2.40 ± 0.15 A a	ND	2.15 ± 0.20 A ab	2.14 ± 0.05 A a	ND	2.40 ± 0.20 A a	ND	ND	1.16 ± 0.18 b
Ethyl formate	ND	ND	0.45 ± 0.00	ND	ND	ND	0.95 ± 0.04	ND	ND
Docosahexaenoic acid, 1,2,3-propanetriyl ester	0.27 ± 0.02 A a	0.26 ± 0.01 AB a	0.19 ± 0.01 B a	0.17 ± 0.02 A b	0.22 ± 0.01 A a	0.12 ± 0.02 A a	0.19 ± 0.01 A ab	0.25 ± 0.03 A a	0.14 ± 0.01 A a
ACIDS									
Butanoic acid	0.57 ± 0.07 A a	ND	0.57 ± 0.08 A a	0.13 ± 0.06 A b	ND	0.23 ± 0.05 A a	0.22 ± 0.04 A b	ND	0.28 ± 0.04 A a
2-oxopropanoic acid	ND	2.10 ± 1.50	ND	ND	ND	ND	ND	ND	ND
Propylpropanedioic acid	0.75 ± 0.08 A b	0.60 ± 0.10 A	0.49 ± 0.11 A	0.97 ± 0.10 b	ND	ND	2.21 ± 0.08 a	ND	ND
Propanoic acid	ND	ND	ND	ND	1.13 ± 0.10 A b	0.22 ± 0.02 B b	ND	1.80 ± 0.11 B a	5.02 ± 0.03 A a
Octanoic acid	ND	ND	0.90 ± 0.10	0.94 ± 0.01	ND	ND	ND	ND	ND
Nonanoic acid	0.55 ± 0.04 A a	0.51 ± 0.04 A b	0.37 ± 0.04 A a	0.57 ± 0.03 A b	0.48 ± 0.03 A b	0.47 ± 0.03 A b	1.02 ± 0.05 B b	1.39 ± 0.04 A a	0.87 ± 0.05 B b
Decanoic acid	ND	ND	0.30 ± 0.02	ND	ND	ND	ND	ND	ND
HYDROCARBONS									
Tridecane	ND	0.56 ± 0.00 B	0.88 ± 0.06 A a	ND	ND	0.36 ± 0.04 b	ND	ND	0.22 ± 0.04 b
Dodecane	3.57 ± 0.01 C	8.20 ± 0.10 A	5.97 ± 0.00 B	ND	ND	ND	ND	ND	ND
Tetradecane	1.07 ± 0.02 C a	2.04 ± 0.01 A a	1.53 ± 0.02 B a	0.33 ± 0.00 C b	0.41 ± 0.00 A b	0.36 ± 0.00 B b	0.33 ± 0.02 A b	0.24 ± 0.01 B c	0.13 ± 0.01 C c

[a] Different small letters "a, b & c" in the same column represent significant differences in each storage time of the same cheese sample, and the capital letters "A, B & C" in the same column represent significant differences between samples in the same storage time.

was also the lowest in cheese H. Pappa et al. (2019) also found heptanal in Kashkaval cheese on the first day, but not detected on the 90th and 180th storage days. Branched-chain aldehydes were detected only at low concentrations. The branched-chain aldehydes such as 2-methyl--propanal and 3-methyl-butanal are derived from leucine and are potent flavor compounds that are generally perceived as malty, chocolate-like flavors (Smit et al., 2009). 3-Methyl-hexanal and 3-methyl-2-butenal were detected only in cheese H. 3-Hydroxy-butanal was detected only in cheese CM in a small quantity.

Three amines were found in low concentrations in the cheese samples (Table 4). Dimethylamine is a common biogenic amine found in cheeses. In some cheese groups, methyl esters of hexanamine and pentanamine were also identified in trace amounts.

In the analyzed samples, esters were found in minor contents (Table 4). Ethyl acetate, which has a fruity note, was detected in C and CM cheeses in similar concentrations ($P > 0.05$) during the storage. In addition, trace amounts of ethyl formate were identified on some days of CM and C cheeses and docosahexaenoic acid, 1,2,3-propanetriyl ester in all cheeses during their 72 days of storage.

There were found some short and medium-chain carboxylic acids in cheese samples (Table 4). Carboxylic acids are known both as aroma components and also are precursors for the formation of methyl ketones, alcohols, lactones, aldehydes, and esters (Collins et al., 2003). Butanoic acid (Butyric acid) has a sweety, cheesy note and is commonly found in cheeses as it can also transmit from milk. In cheese H, it was not detected during the storage, while in the CM and the C samples, its concentration was similar and slightly decreased after day 18. Other acids with pungent and rancid flavors were identified only on some days of storage. 2-oxopropanoic acid was detected only on the 18th day of cheese H. Propylpropanedioic acid was present only on the 18th days of cheese H and CM, while it was found throughout the storage of cheese C. Nonanoic acid was detected in all groups with a significant increase on day 72. Eroglu et al. (2016) analyzed fresh Kashar cheeses and detected a very different volatile aroma profile from our study. According to their results, the Kashar cheeses contained high amounts of butyric acid and acetic acid.

Hydrocarbons do not contribute to the major aroma of cheeses; however, can be precursors for other volatile compounds (Munoz et al., 2003). Tridecane was detected only in cheese CM. Dodecane was detected in high amounts in cheeses only on day 18. (Table 4).

3.4. PCA of the volatile compounds

The PCA was applied to volatile compounds to explicate better the effect of different culture use and storage time on flavor compound production. The two main components explaining 54.7% of the total variance are given as PC1 (30.2%) and PC2 (24.6%), in the x and y axes of Fig. 3, respectively. The addition of *L. helveticus* significantly affected the volatiles of the cheeses; *L. helveticus* added cheese was on the negative side of PC2, while the other samples were on the positive side of PC2. Another distinct group was obtained according to PC1; on the 18th storage day, all samples located in the positive side of PC1, but shifted towards the negative side with progressing shelf life. According to Fig. 3, *L. helveticus* added H sample differed significantly from the control sample (group C), while the modified control sample - CM was closer to the control. The only factor affecting the volatile flavor profile of CM and C was the progressing shelf-life. On the other hand, it was observed that the volatile compounds of cheese H did not change from day 40. The variables that correlate the most with PC2 were acetaldehyde (−0.345), 2-oxopropanoic acid (−0.293), 3-methyl-2-butenal (−0.290), diacetyl (0.278), and 2-heptanone (−0.264). Diacetyl was positively correlated and more prominently found in C and CM samples. Acetaldehyde and the other components mentioned above were more dominant in the H sample.

3.5. The sensory evaluations

The sensory evaluations are given in Table 5. In terms of flavor, on the 18th day of storage, sample H and sample CM differed significantly ($P < 0.05$), while C was similar to both groups. Additionally, Kashar H with *L. helveticus* was found to have good flavor whereas the other cheeses CM and C were found non-aromatic. On the next analysis day, cheese H was still identified as aromatic, but the numerical flavor scores of all samples tended to be closer to each other. On the other hand, storage time was an important factor in the flavor and texture of sample CM, while H and C remained more stable. According to the flavor scores, the flavor composition of CM_18 in Fig. 3 was less favored than the flavor compositions of the other products. However, in general, the flavor compounds given in Fig. 3 were appreciated by the panelists at their current concentrations during storage. Evaluation of the three samples revealed that their textures differed on day 40, but on day 72

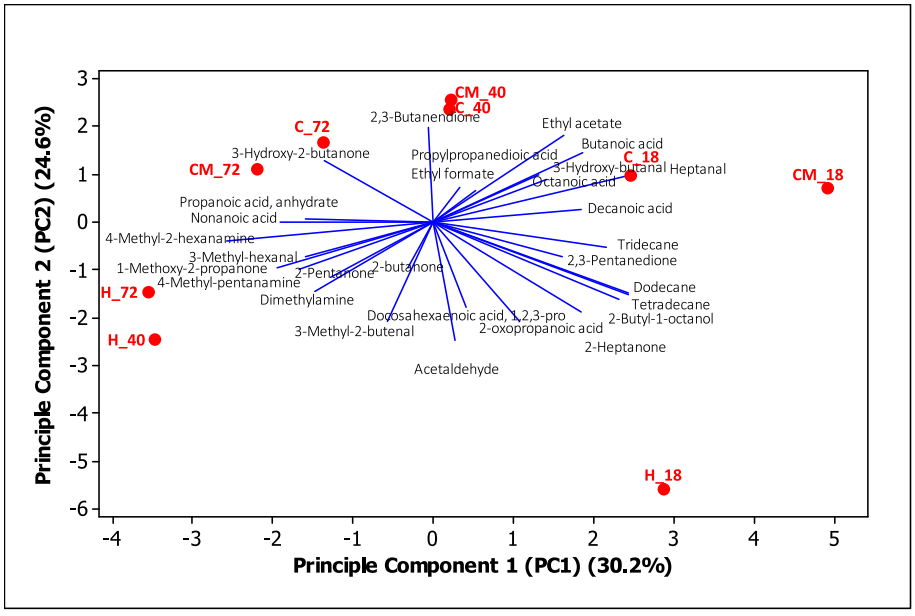

Fig. 3. Biplot of the first two principal components of volatile compounds of Kashar cheeses on the storage days 18, 40 and 72. C, H and CM represent group names, the following numbers represent the storage day.

Table 5
Aroma profile of Kashar cheese samples on the 18th, 40th and 72nd days of storage.

Cheese		Flavor	Texture	Summary of sensory descriptions
C	day 18	6.7 ± 1.2 AB a	6.7 ± 1.0 A a	It was found non-aromatic, plain tasted. Sandy texture was detected from the 40th day of the storage.
	day 40	6.4 ± 1.0 A a	6.4 ± 1.0 B a	
	day 72	6.5 ± 0.8 A a	6.5 ± 1.0 A a	
H	day 18	7.1 ± 1.0 A a	7.0 ± 1.2 A a	Good flavor and good elastic texture.
	day 40	7.1 ± 1.0 A a	7.3 ± 0.9 A a	
	day 72	6.8 ± 1.1 A a	7.0 ± 1.0 A a	
CM	day 18	6.2 ± 1.2 B b	6.2 ± 1.3 A b	It was found non-aromatic, salty, plain tasted. Sandy texture was detected from the beginning of the storage.
	day 40	6.7 ± 1.0 A ab	6.7 ± 1.1 AB ab	
	day 72	7.0 ± 0.9 A a	7.2 ± 0.9 A a	

* Different small letters "a & b" in the same column represent significant differences in each storage time of the same cheese sample, and the capital letters "A & B" in the same column represent significant differences between samples in the same storage time.

the difference was no longer significant. During the storage period, the sandy texture was found in CM and C, while sample H with *L. helveticus* was identified as typical, elastic fresh Kashar cheese.

4. Conclusion

One of the difficulties of pasta filata cheeses is its weak flavor due to the inhibition of lactic cultures by hot steam injection and consumption fresh without long ripening. In this study, fresh Kashar cheese was manufactured with common techniques using the starter cultures of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, in addition *L. helveticus* was added as a commercial starter to examine its contribution to flavor. In the cheeses examined, acetoin, diacetyl, 1-methoxy-2-propanone, 2-heptanone, 2-butyl-1-octanol, dodecane, tetradecane predominated. Although acetic acid and ethyl alcohol are commonly found in unripened cheeses, they were not detected in Kashar samples. Ammonia and sulfur compounds were also below the detection limit. According to PCA, the volatile profile of the *L. helveticus* added sample was different from the other groups. In addition, although the aroma profiles were relatively close, the two products manufactured with two different strains of *S. thermophilus* from different companies surprisingly resulted in different degrees of ripening in terms of proteolysis and lipolysis rates.

When all results were taken into account, *L. helveticus* can be recommended as an adjunct culture in pasta filata cheeses. Besides flavor contributions, future studies should examine the effects of different starter cultures on the digestibility of cheese.

CRediT authorship contribution statement

Hatice Sıçramaz: Conceptualization, Writing – original draft, Formal analysis. **Olgu Taylan Güven:** Conceptualization, Writing – original draft, Formal analysis. **Ayşen Can:** Conceptualization, Writing – original draft, Formal analysis. **Ahmet Ayar:** Writing – editing, Supervision. **Yasin Gül:** Writing – editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Akın, M.S., Güler-Akın, M.B., Kırmacı, H.A., Atasoy, A.F., Türkoğlu, H., 2012. The effects of lipase-encapsulating carriers on the accelerated ripening of Kashar cheese. Int. J. Dairy Technol. 65, 243–249.

Anonymous, 2015. Turkish Food codex cheese Regulations. Official newspaper 8, 29261. Feb 2015.

Atasoy, A.F., Yetişmeyen, A., Turkoglu, H., Ozer, B., 2008. Effects of heat treatment and starter culture on the properties of traditional Urfa cheeses (a white-brined Turkish cheese) produced from bovine milk. Food Control 19 (3), 278–285.

Baptista, D.P., Galli, B.D., Cavalheiro, F.G., Negrão, F., Eberlin, M.N., Gigante, M.L., 2018. *Lactobacillus helveticus* LH-B02 favours the release of bioactive peptide during Prato cheese ripening. Int. Dairy J. 87, 75–83.

Celik, O.F., Kurt, S., Tufenk, B., Tarakci, Z., 2018. Efficacy of starter culture application using immersion technique on the characteristics of cooked-curd cheeses: Kashar cheese sample. LWT - Food Sci. Technol. 96, 222–227.

Çetinkaya, A., 2020. Effect of different preservation and salting methods on some volatile compounds and sensory properties of Kashar cheese. Kafkas Univ Vet Fak Derg 26 (3), 435–444. https://doi.org/10.9775/kvfd.2019.23508.

Collins, Y.F., McSweeney, P.L.H., Wilkinson, M.G., 2003. Evidence of a relationship between autolysis of starter bacteria and lipolysis in Cheddar cheese during ripening. J. Dairy Res. 70 (1), 105–113.

Cuffia, F., George, G., Renzulli, P., Reinheimer, J., Meinardi, C., Burns, P., 2017. Technological challenges in the production of a probiotic pasta filata soft cheese. LWT - Food Sci. Technol. 81, 111–117. https://doi.org/10.1016/j.lwt.2017.03.039.

Cuffia, F., Bergamini, C.V., Wolf, I.V., Hynes, E.R., Perott, M.C., 2018. Characterization of volatile compounds produced by *Lactobacillus helveticus* strains in a hard cheese model. Food Sci. Technol. Int. 24 (1), 67–77.

De Angelis, M., Gobbetti, M., 2011. Pasta-filata cheeses: traditional pasta-filata cheese. Encyclopedia Dairy Sci. 1, 745–752. https://doi.org/10.1016/B978-0-12-374407-4.00512-4.

El-Sayed, H.S., Salama, H.H., Edris, A.E., 2020. Journal of the Saudi Society of Agricultural Sciences Survival of Lactobacillus helveticus CNRZ32 in spray dried functional yogurt powder during processing and storage. J. Saudi Soc. Agricult. Sci. 19 (7), 461–467. https://doi.org/10.1016/j.jssas.2020.08.003.

Eroglu, A., Dogan, M., Toker, O.S., Yilmaz, M.T., 2015. Classification of Kashar cheeses based on their chemical, color and instrumental textural characteristics using Principal Component and Hierarchical Cluster Analysis. Int. J. Food Prop. 18 (4), 909–921.

Eroglu, A., Toker, O.S., Dogan, M., 2016. Changes in the texture, physicochemical properties and volatile compound profiles of fresh Kashar cheese (<90 days) during ripening. Int. J. Dairy Technol. 69 (2), 243–253.

Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H., 2000. Fundamentals of Cheese Science. Aspen, Gaithersburg, MD.

Griffiths, M.W., Tellez, A.M., 2013. Lactobacillus helveticus: the proteolytic system. Front. Microbiol. 4 (MAR), 1–9. https://doi.org/10.3389/fmicb.2013.00030.

Guidone, A., Braghieri, A., Cioffi, S., Claps, S., Genovese, F., Morone, G., Napolitano, F., Parente, E., 2015. Effect of adjuncts on microbiological and chemical properties of Scamorza cheese. J. Dairy Sci. 98 (3), 1467–1478.

Hayaloglu, A.A., 2009. Volatile composition and proteolysis in traditionally produced mature Kashar cheese. Int. J. Food Sci. Technol. 44, 1388–1394. https://doi.org/10.1111/j.1365-2621.2009.01968.x.

IDF, 2001. Milk. Determination of Nitrogen Content. Part 5: Determination of Protein Nitrogen Content. International Dairy Federation, Brussels, Belgium. *Standard 020.5*.

ISO, 2004. Cheese and Processed Cheese—Determination of the Total Solids Content (Reference Method). International Standardization Organization, Geneva. *ISO 5534: 2004 (IDF 4: 2004)*.

ISO, 2006. Cheese and Processed Cheese—Determination of Chloride Content, Potentiometric Titration Method. International Standardization Organization, Geneva. *ISO 5943:2006 (IDF 88: 2006)*.

ISO, 2008. Cheese, Determination of Fat Content—Van Gulik Method. International Standardization Organization, Geneva. *ISO 3433:2008 (IDF 222: 2008)*.

Kaminarides, S., Litos, I., Massouras, T., Georgala, A., 2015. The effect of cooking time on curd composition and textural properties of sheep Halloumi cheese. Small Rumin. Res. 125, 106–114. https://doi.org/10.1016/j.lwt.2020.109341.

Kieliszek, M., Pobiega, K., Piwowarek, K., Kot, A.M., 2021. Characteristics of the proteolytic enzymes produced by lactic acid bacteria. Molecules (Basel, Switzerland) 26 (7), 1858. https://doi.org/10.3390/molecules26071858.

Klein, N., Maillard, M.-B., Thierry, A., Lortal, S., 2001. Conversion of amino acids into aroma compounds by cell-free extracts of *Lactobacillus helveticus*. J. Appl. Microbiol. 91 (3), 404–411.

Kuchroo, C.N., Fox, P.F., 1982. Soluble nitrogen in cheddar cheese: comparison of extraction procedures. Milchwissenschaft 37, 331–335.

Licitra, G., Radulović, Z., Miočinović, J., Uzunsoy, I., Özer, B., Bintsis, T., Alichanidis, E., Herian, K., Jelen, P., 2017. Pasta-filata cheeses. In: Global Cheesemaking Technology: Cheese Quality and Characteristics. Wiley-IEEE Press, pp. 368–391. https://doi.org/10.1002/9781119046165.ch8.

McSweeney, P.L.H., 2004. Biochemistry of cheese ripening. Int. J. Dairy Technol. 57, 127–144. https://doi.org/10.1111/j.1471-0307.2004.00147.x.

Munoz, N., Ortigosa, M., Torre, P., Izco, J.M., 2003. Free amino acids and volatile compounds in Ewe's milk cheese as affected by seasonal and cheese-making plant variations. Food Chem. 83, 329–338.

Nuñez, M., García-Aser, C., Rodríguez-Martin, M.A., Medina, M., Gaya, P., 1986. The effect of ripening and cooking temperatures on proteolysis and lipolysis in Manchego cheese. Food Chem. 21, 115–123.

Öründü, S., Tarakçı, Z., 2021. Effects of different starter culture applications pre- and post-scalding on the biochemical and sensory properties of pasta filata type cheeses. LWT - Food Sci. Technol. 136 (Part1), 110288.

Ott, A., Germond, J.-E., Chaintreau, A., 2000. Origin of acetaldehyde during milk fermentation using 13C-labeled precursors. J. Agric. Food Chem. 48 (5), 1512–1517. https://doi.org/10.1021/jf9904867.

Pappa, E.C., Kondyli, E., Samelis, J., 2019. Microbiological and biochemical characteristics of Kashkaval cheese produced using pasteurised or raw milk. Int. Dairy J. 89, 60–67. https://doi.org/10.1016/j.idairyj.2018.08.011.

Sekban, H., Tarakci, Z., 2020. Effects of different starter cultures on the ripening characteristics of Golot cheese. Nutr. Food Sci. https://doi.org/10.1108/NFS-06-2020-0230.

Sert, D., Ayar, A., Akın, N., 2007. The effects of starter culture on chemical composition, microbiological and sensory characteristics of Turkish Kaşar Cheese during ripening. Int. J. Dairy Technol. 60 (4), 245–252. https://doi.org/10.1111/j.1471-0307.2007.00339.x.

Şimşek, B., Sağdıç, O., 2009. Keçi Sütünden Üretilen Çökelek Peynirinin Fizikokimyasal, Mikrobiyolojik ve Duyusal Özellikleri ile Yağ Asidi Kompozisyonu Üzerine, Laktik starter Kültür Tipi ve Farklı Sıcaklık Uygulamanın Etkisi. Kafkas Universitesi Veteriner Fakultesi Dergisi 18 (2), 177–183. https://doi.org/10.9775/kvfd.2011.5190.

Skrzypczak, K., Gustaw, W., Wasko, A., Banach, T., 2020. Changes in biochemical properties during ripening process of Swiss-type cheeses produced with different *Lactobacillus helveticus* strains. J. Agric. Sci. Technol. 22 (3), 693–707.

Smit, B.A., Engels, W.J.M., Smit, G., 2009. Branched chain aldehydes: production and breakdown pathways and relevance for flavor in foods. Appl. Microbiol. Biotechnol. 81, 987–999.

Sousa, M.J., Malcata, F.X., 1997. Comparison of plant and animal rennets in terms of microbiological, chemical and proteolysis characteristics of ovine cheese. J. Agric. Food Chem. 45, 74–81.

Temizkan, R., Yasar, K., Hayaloglu, A.A., 2014. Changes during ripening in chemical composition, proteolysis, volatile composition and texture in Kashar cheese made using raw bovine, ovine or caprine milk. Int. J. Food Sci. Technol. 49, 2643–2649. https://doi.org/10.1111/ijfs.12597.

Topçu, A., Saldamli, I., 2006. Proteolytical, chemical, textural and sensorial changes during the ripening of Turkish white cheese made of pasteurized cows' milk. Int. J. Food Prop. 9 (4), 665–678. https://doi.org/10.1080/10942910500542238.

Topcu, A., Bulat, T., Özer, B., 2020. Process design for processed Kashar cheese (a pasta filata cheese) by means of microbial transglutaminase: effect on physical properties, yield and proteolysis. LWT - Food Sci. Technol. 125, 109226.

Tuncturk, Y., Coskun, H., 2007. The effect of homogenized lactic cultures on the development of proteolysis in Kashar cheese. Food Sci. Technol. Res. 13 (4), 356–361.

Yilmaz, F., Dagdemir, E., 2012. The effects of beeswax coating on quality of Kashar cheese during ripening. Int. J. Food Sci. Technol. 47 (12), 2582–2589. https://doi.org/10.1111/j.1365-2621.2012.03137.x.

Zisu, B., Shah, N.P., 2005. Textural and functional changes in low-fat Mozzarella cheeses in relation to proteolysis and microstructure as influenced by the use of fat replacers, pre-acidification and EPS starter. Int. Dairy J. 15, 957–972.