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Changes in volatile composition, proteolysis and textural and sensory properties of white-brined cheese: effects of ripening temperature and adjunct culture

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Abstract The effects of ripening temperature and adjunct cultures (*Lactobacillus helveticus* and *Lactobacillus casei*) on the volatile compounds and sensory and textural properties of white-brined cheese were investigated. Three batches of cheese were produced: cheese A was inoculated with only cheese starter culture (*Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*), cheese B was inoculated with cheese starter culture plus *Lactobacillus helveticus* and cheese C was inoculated with cheese starter culture plus *Lactobacillus casei*. Cheeses were ripened at 6 or 12 °C and analyzed at 30-day intervals up to 120 days of ripening. The use of adjunct culture and ripening temperature significantly influenced the pH and proteolysis of cheeses ($P < 0.05$). Acids, ketones and alcohols were found at high levels in all three cheeses. Volatiles were significantly influenced by the use of the adjunct cultures, ageing and to some extent ripening temperature ($P < 0.05$). Textural parameters of the cheeses were significantly affected by the adjunct culture during ripening ($P < 0.05$). The sensory scores of the cheese samples decreased during the ripening period. An age-related bitterness was detected by the panellists in 90 or 120-day-old cheeses with added adjunct cultures. In conclusion, the use of adjunct culture and ripening at 12 °C enhanced the volatile composition and changed the texture profiles of the cheeses.

Keywords White-brined cheese · Beyaz peynir · Adjunct culture · Volatiles · Proteolysis · Sensory · Texture

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1 Introduction

In Turkey, around 50 different cheeses are produced, and the most important ones from the commercial potentials' and production capacities' point of view are the white-brined cheeses, Kasar, Tulum, Otlu, Dil, Mihalic, Cerkez, Cokelek, Civil and Lor (Hayaloglu et al. 2002). Despite the economical potential of these varieties, little is known about the physical, chemical and microbiological properties and the factors affecting the ripening profiles of these varieties. Therefore, in order to adapt the traditional cheese-making practices to modern applications, it is essential to fully understand the mechanisms of biochemical processes, including proteolysis, glycolysis and lipolysis, that take place during ripening in these varieties, as well as the factors affecting these biochemical events. Proteolysis is the most complex biochemical process and is catalyzed by indigenous milk enzymes (i.e. plasmin, cathepsin D and other proteinases); milk coagulating enzymes (i.e. chymosin, pepsin or fungal acid proteinases) and enzymes from starter bacteria, non-starter bacteria and secondary starter bacteria (Fox and McSweeney 1996). During cheese ripening, caseins are hydrolyzed into large- and intermediate-sized peptides which are further transformed into smaller peptides or amino acids by enzymes released from starter and non-starter bacteria (Hayaloglu et al. 2005). Enzymatic degradation of proteins and lipids in cheese leads to the development of cheese flavour. Especially volatile compounds including amines, alcohols and sulphur-containing compounds, which are formed during proteolytic degradation of medium and small molecular weight peptides, and free amino acids play critical roles in flavour perception of cheese (McSweeney and Sousa 2000). Amino acids contribute directly to the sweet (i.e. Gly, Ser, Thr, Ala, Pro), sour (i.e. His, Glu, Asp) or bitter (i.e. Arg, Met, Val, Leu, Phe) flavour in cheese (McSweeney 2004).

As a result of the metabolism of residual lactose, lactic and citric acids and compounds such as acetate, acetaldehyde, ethanol, 2,3-butanediol and diacetyl are produced (McSweeney and Sousa 2000). Fatty acids can be metabolized to methyl ketones, and fat also acts as a solvent for many of the flavour compounds produced in the cheese (Kondyli et al. 2002). The use of adjunct (secondary) cultures in cheese making is also a common way of improving flavour and texture in various types of cheeses (Kondyli et al. 2002; Bintsis and Robinson 2004; Randazzo et al. 2008). *Lactobacillus* species are the most widely employed adjunct cultures in cheese production for this purpose. The enzymatic activities of cheese microflora are stimulated at high ripening temperatures and thus have long been used to accelerate cheese ripening (Hannon et al. 2005). Cheese texture which is affected by a number of factors, including cheese composition, processing conditions and ripening process, is important for cheese quality and consumers' acceptance (Lawrence et al. 1987). Proteolysis, in particular, degradation of α_{s1} -casein plays a pivotal role in the development of cheese texture (Sallami et al. 2004).

The aim of this study was to determine the effects of adjunct culture (*Lactobacillus helveticus* or *Lactobacillus casei*) and ripening temperature (6 or 12 °C) on volatile composition and textural and sensory properties of white-brined cheese during 120 days of ripening. Proteolysis was monitored in cheese by the determination of soluble nitrogen fractions. The changes in volatiles, texture or sensory scores were discussed by using the proteolysis data.

2 Materials and methods

2.1 Materials

Raw bovine milk supplied from Harran University Dairy (Sanliurfa, Turkey) was used in cheese making. Calf rennet, with declared milk clotting activity of 1:16,000 (Chr. Hansen A.S., Istanbul, Turkey), which means that 1 mL rennet can coagulate 16 L of milk (or 175 IMCU), was used to coagulate milk. Cheese starters including the mixture of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (with reference code FRC 60) and adjunct cultures (*Lactobacillus helveticus* and *Lactobacillus casei*) were also supplied from Chr. Hansen A.S. (Istanbul, Turkey).

2.2 Cheese manufacture

Two cheese-making trials were performed for the manufacture of white-brined cheese. A total of 300 L of raw bovine milk was used and divided into three batches (each 100 L), and all batches were converted to cheese simultaneously as described in Hayaloglu et al. (2002). The milk was heat-treated at 68 °C for 10 min and cooled to 39 °C. Then, the first batch (control, cheese A) was inoculated with industrial cheese starter culture. The second and third batches were inoculated with cheese starter culture plus adjunct cultures of *Lactobacillus helveticus* (cheese B) and *Lactobacillus casei* (cheese C), respectively. The level of inoculum was 1% (v/v) [1% cheese culture for cheese A and 0.5% cheese culture plus 0.5% adjunct culture for cheeses B and C]. The inoculated milk was then rested for an hour to allow the acidity to develop and was coagulated by means of calf rennet (Peyma-Hansen A.S., Istanbul, Turkey) within 100 min. Following the removal of whey, fresh cheese was portioned to blocks (7 × 7 × 7 cm) and stored in a pasteurized brine solution (12% NaCl, w/v) at 6 or 12 °C for 120 days. Cheeses were analyzed in duplicate for the effect of two non-starter adjunct cultures on volatile compound production, texture and sensory properties at 1, 30, 60, 90 and 120 days of ripening.

2.3 Gross chemical composition and proteolysis

Cheese samples were analyzed in duplicate for the pH, moisture, fat, total nitrogen, nitrogen fractions [water soluble nitrogen (WSN) and 12% trichloroacetic acid-soluble nitrogen (TCA-SN)] and salt contents by the methods described in Hayaloglu et al. (2005).

2.4 Headspace analysis of volatiles

Analysis of the volatiles was performed by solid-phase microextraction (SPME) method using gas chromatography-mass spectrometry system (Shimadzu Corporation, Kyoto, Japan), essentially as described in Hayaloglu et al. (2013). The identifications were based on comparing mass spectra of unknown compounds with those in Wiley 7 (7th edition; John Wiley & Sons Inc., 2005) and NIST/EPA/NIH 02 (<http://www.nist.gov>) mass spectral library. Identifications were also confirmed by comparing retention times with reference standards when available. A total of 33 authentic standard compounds

(Sigma Chemical Co., St. Louis, MO, USA) were used to confirm the identities of volatile compounds in the cheese samples. The concentrations were calculated by the comparison of the peak areas of the internal standard containing 81 ppm of 2-methyl-3-heptanone in methanol (Sigma-Aldrich Co., St. Louis, MO, USA) and unknown compounds. Each compound was expressed as $\mu\text{g}\cdot 100\text{ g}^{-1}$ of cheese.

2.5 Texture analysis

Texture Profile Analysis (TPA) of the cheese samples was carried out at 1, 30, 60, 90 or 120 days of ripening using a Texture Analyzer Model LF Plus (Lloyd Instruments Ltd., Hampshire, UK). Cheese samples were cut into cylindrical portions with 20 ± 0.5 mm in diameter and 15 ± 0.5 mm height. Then, the cylinder-shaped cheeses were covered with plastic film and rested at 25 ± 2 °C for about 30 min. The conditions of the analysis were as follows: P/2 aluminum cylinder probe (25 mm diameter), test speed $1\text{ mm}\cdot\text{s}^{-1}$, first test speed $5\text{ mm}\cdot\text{s}^{-1}$, last test speed $1\text{ mm}\cdot\text{s}^{-1}$, suppression 65%, and holding time 5 s. The data obtained were calculated using a software from Nexygen™ FM (Lloyd Instruments Ltd., Hampshire, UK).

2.6 Sensory analysis

Sensory evaluation of the cheeses was made at 20, 30, 60, 90 and 120 days of ripening. Cheese samples were removed from the refrigerator, cut into pieces and placed on white plates coded with a random three-digit numbers. A total of six different types of cheese were presented to the panellists at one session, and two separate sessions were made for each cheese during ripening. Approximately 200 g of cheese was presented to each panellist. Before sensory evaluation at each session, cheeses were rested for about 60 min at room temperature. The samples were evaluated using the following criteria: colour and appearance (scale 0–5), body and texture (scale 0–5), taste and flavour (scale 0–10) and overall quality (scale 0–5). Water and bread were also provided to the panellists to rinse their mouths between samples. Sensory characteristics of white-brined cheese were evaluated by seven trained panels (from the permanent staff at the Department of Food Engineering, Inonu University, Turkey) who were familiar with the taste and texture of white-brined cheese.

2.7 Statistical analysis

Data obtained from chemical composition, proteolysis, texture and volatiles were subjected to analysis of variance using the GLM procedure of SAS (version 8.0). A split-plot design was used to determine the effects of adjunct culture, ripening temperature and ripening time on the response variables. Duncan's multiple-comparison test was used as a guide for paired comparisons of treatment means. The level of significance of differences between treatments was determined at $P<0.05$. Data from volatile components were also analyzed using multivariate statistical techniques to simplify interpretation of the data from gas chromatography-mass spectrometry (GC-MS). Principal component analysis (PCA) was performed using a covariance matrix and varimax rotation between the cheeses. The concentrations of volatile components (by GC-MS) were used as variables in PCA, which was

performed using SPSS package program version 9.0 for Windows (SPSS Inc., Chicago, IL).

3 Results and discussions

3.1 Gross chemical composition and proteolysis

The mean values of the pH and total nitrogen, fat in dry matter (FDM), WSN, 12% TCA-SN and salt contents of the experimental cheeses are given in Table 1. The pH values of the samples decreased significantly in the samples supplemented with adjunct cultures ($P<0.05$). pH decrease was independent from the ripening temperature, and the fastest and the slowest decreases in pH were noted in the cheese C ripened at 12 °C and the cheese A ripened at 6 °C, respectively. The dry matter contents of the cheeses increased slightly possibly due to the penetration of salt from the brine to the cheese and serum passage from the cheese to the brine (Dagdemiir et al. 2003; Kayagil and Gurakan 2009). The FDM values changed widely in the cheeses (from 46.43% to 67.09%), which were highly dependent to the ripening time and addition of adjunct culture ($P<0.05$). Total protein contents of the cheeses increased during ripening ($P<0.001$), and the addition of adjunct culture had no significant effect on the total protein contents of the cheeses except for cheese B at 90 days and at 12 °C and cheeses B and C at 120 days ($P>0.05$). The FDM and total protein contents of the experimental cheeses were higher than those reported for white-brined (Hayaloglu et al. 2004) and feta (Sarantinopoulos et al. 2002) cheeses due to high level of total solids in these cheese varieties. Salt contents of the cheeses were between ca. 2% and 5% and salt-in-moisture (S/M) contents were between ca. 3.5% and 8% in the cheeses. The concentration of S/M was higher than 7% after 90 days of ripening, and these cheeses were criticized as “quite salty” by the panel group. This salt level (after 90 days) was also a limiting factor for the growth of bacteria; however, the S/M contents were lower than 7% during the first 60 days of ripening. Salt and S/M contents of the cheeses were significantly influenced by adjunct culture, ripening temperature and time ($P<0.001$). These differences may be due to the differences in the pH and total solid contents of the cheeses, which affect salt diffusion into cheese mass from brine (Hayaloglu et al. 2005).

Development of proteolysis in the cheeses during ripening was monitored by measuring the variations in the concentrations of nitrogen fractions (WSN and TCA-SN). It is a well-known fact that the WSN fraction of cheese extract contains high, medium and small molecular weight peptides; free amino acids and nitrogen compounds which are produced mainly by the action of rennet and plasmin (Hayaloglu et al. 2005). The WSN and TCA-SN values of the samples increased at least twofold throughout the ripening period (Table 1). Cheeses B and C had a higher level of TCA-SN than cheese A at 120 days of ripening at both temperatures. Statistical analysis revealed that the levels of WSN and TCA-SN were significantly affected by the ripening time and/or adjunct cultures ($P<0.001$) (Table 1). The increases in both WSN and TCA-SN levels of the cheeses were a result of protein degradation during ripening as previously reported by Folkertsma et al. (1996), Bertola et al. (2000) and Alizadeh et al. (2006).

Table 1 Chemical composition and proteolysis of white-brined cheeses ripened at 6 or 12 °C during ripening

Variables	Days	Cheeses ^a									
		A		B		C					
		6 °C	12 °C	6 °C	12 °C	6 °C	12 °C				
							Adj	Day	P	P	Temp
pH	1	5.10±0.01	5.10±0.01	5.05±0.01	5.05±0.01	5.28±0.01		*	*	*	*
	20	5.04±0.00	5.04±0.00	4.82±0.01	4.72±0.01	5.05±0.01					
	30	5.13±0.01	5.11±0.01	4.54±0.08	4.59±0.01	4.83±0.01					
	60	5.04±0.01	5.12±0.01	4.74±0.01	4.71±0.01	4.95±0.00					
	90	5.07±0.02	5.03±0.00	4.61±0.01	4.51±0.06	4.80±0.01					
	120	5.10±0.01	5.09±0.03	4.81±0.00	4.74±0.01	4.83±0.00					
Dry matter (%)	1	40.25±0.28	40.25±0.28	42.76±0.37	42.76±0.37	42.70±0.56		**	**	**	**
	20	41.46±0.36	43.47±0.20	43.40±0.18	44.05±0.29	41.53±0.45					
	30	43.03±0.02	41.78±4.40	45.07±0.25	43.37±0.83	43.30±0.30					
	60	40.46±0.28	45.06±0.07	42.44±0.02	46.75±0.15	40.73±0.04					
	90	45.18±0.06	45.18±0.06	48.46±0.62	48.46±0.62	39.43±0.80					
	120	40.26±0.09	42.98±0.06	43.16±0.78	45.89±0.00	43.53±0.73					
FDM (%)	1	67.09±0.46	67.09±0.46	59.63±1.14	59.63±1.14	62.06±0.46		*	*	*	*
	20	61.51±1.17	58.66±1.36	65.87±1.91	61.30±0.40	63.22±1.53					
	30	58.11±3.58	62.58±6.59	59.91±2.81	62.24±2.06	64.67±0.17					
	60	63.03±2.19	57.76±6.36	51.84±0.03	51.34±2.86	63.29±6.88					
	90	60.63±1.24	57.55±0.08	50.39±0.74	46.43±0.87	63.44±3.08					
	120	64.03±1.03	59.92±0.73	53.31±0.97	50.12±0.00	58.01±0.04					
Total protein (%)	1	16.06±0.33	16.06±0.33	16.70±1.37	16.70±1.37	15.83±0.47		***	***	***	***
	20	16.11±0.51	18.33±1.91	16.73±0.67	17.90±0.84	17.00±0.13					

Table 1 (continued)

Variables	Days	Cheeses ^a																
		A				B				C								
		6 °C		12 °C		6 °C		12 °C		6 °C		12 °C		Adj	Day	P	Temp	
Salt (%)	30	16.40±1.22	17.72±1.98	16.41±0.58	17.72±0.70	17.96±0.00	18.76±1.73											
	60	16.46±0.55	17.78±0.59	16.89±0.46	18.82±0.45	18.17±0.69	20.43±0.01											
	90	17.27±0.14	18.60±0.06	18.28±1.27	21.80±0.63	18.75±0.61	18.56±0.04											
	120	17.96±0.04	18.90±0.41	18.40±0.08	20.91±1.10	18.37±0.09	19.74±0.37											
	1	5.97±0.00	5.97±0.00	5.32±0.91	5.32±0.91	4.88±0.12	4.88±0.12											
	20	4.27±0.41	3.57±0.08	3.80±0.25	3.74±0.25	3.80±0.08	3.74±0.00											
	30	3.33±0.08	3.10±0.08	2.87±0.08	2.11±0.00	3.39±0.26	2.52±0.08											
	60	4.68±0.00	3.86±0.00	4.56±0.00	3.86±0.00	3.95±0.04	3.69±0.08											
	90	4.39±0.08	4.39±0.08	4.27±0.08	4.62±0.58	3.57±0.08	3.39±0.00											
	120	3.71±0.04	4.15±0.08	4.39±0.08	4.83±0.12	3.45±0.08	3.28±0.00											
	S/M	1	9.08±0.04	9.08±0.04	8.50±1.38	8.50±1.38	7.85±0.11	7.85±0.11										
		20	6.97±0.65	5.94±0.11	6.28±0.37	6.27±0.42	6.11±0.17	6.30±0.06										
30		5.53±0.11	5.07±0.49	4.96±0.16	3.59±0.05	5.65±0.23	4.29±0.10											
60		7.29±0.03	6.57±0.01	7.34±0.00	6.76±0.02	6.25±0.07	6.37±0.28											
90		7.18±0.09	7.41±0.04	7.29±0.21	8.22±0.86	5.56±0.05	5.79±0.02											
120		5.85±0.05	6.79±0.13	7.16±0.21	8.19±0.19	5.76±0.06	5.35±0.05											
WSN (% TN)	1	9.11±0.20	9.11±0.20	6.28±0.96	6.28±0.96	6.32±0.35	6.32±0.35											
	20	16.79±0.44	10.00±1.53	8.55±0.36	10.68±0.36	10.61±1.27	10.92±1.05											
	30	15.80±1.37	14.91±0.09	9.62±0.68	9.21±1.39	13.54±1.22	12.56±0.49											
	60	20.61±0.02	18.30±0.07	11.80±0.80	13.38±0.39	15.65±0.09	15.54±0.24											

Table 1 (continued)

Variables	Days	Cheeses ^a						Temp
		A		B		C		
		6 °C	12 °C	6 °C	12 °C	6 °C	12 °C	
	90	20.77±0.58	18.48±0.68	14.78±1.73	13.44±0.04	16.74±0.48	17.32±0.05	
	120	20.10±1.01	22.33±0.41	14.91±0.14	15.36±0.71	20.58±0.76	17.54±0.41	
% 12 TCA (%TN)	1	2.22±0.09	2.22±0.09	2.18±0.19	2.18±0.19	1.96±0.02	1.96±0.02	***
	20	6.00±0.23	4.92±0.82	5.35±0.22	6.18±0.13	4.47±0.11	4.51±0.08	
	30	6.06±0.16	7.25±0.71	6.00±1.01	6.51±0.63	4.20±0.08	4.79±0.08	
	60	9.37±0.04	9.37±0.84	8.75±0.69	10.26±0.63	7.07±0.27	8.48±0.67	
	90	11.12±0.02	9.33±0.73	10.46±0.80	10.47±0.21	7.98±0.46	9.50±0.30	
	120	11.15±0.06	11.40±0.17	11.48±0.37	11.63±0.38	12.44±0.21	11.47±0.02	

P probability

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^a Cheeses A contains only cheese starter culture (*Lactococcus lactis* ssp. *lactis* plus *Lactococcus lactis* ssp. *cremoris*), B contains A culture plus *Lactobacillus helveticus* and C contains A culture plus *Lactobacillus casei*

3.2 Volatile composition

Twenty-nine compounds were identified in the volatile fractions of the cheeses including three acids, five esters, nine ketones, two aldehydes, eight alcohols and two miscellaneous compounds (Table 2).

3.2.1 Principal component analysis of volatiles

To differentiate the effect of ripening time on the volatile compounds, PCA was performed using volatile compounds as variables. PC1 and PC2 accounted for 28% and 23% of the variance, respectively (Fig. 1). Towards the end of ripening, all samples were separately located on the plot, while young cheeses were grouped together. The cheese samples were located on the negative side of PC1 axis at 1, 30 and 60 days of ripening; however, 120-day-old cheeses were located on the positive side of the PC1. Also, 90 or 120-day-old cheeses were grouped separately on the plot. The cheeses supplemented with adjunct cultures and ripened at 12 °C had higher levels of volatile compounds than the control cheese and those ripened at 6 °C.

3.2.2 Esters

Esters, which are responsible for fruity flavours in cheese, are formed through direct esterification of alcohols and carboxylic acids or via alcoholysis (Hayaloglu et al. 2007). Ethyl esters including ethyl acetate, ethyl butanoate, ethyl octanoate and ethyl hexanoate were detected at the cheeses (Table 2). The ethyl esters were also main esters in Manchego (Fernandez-Garcia et al. 2002), Argentine (Bergamini et al. 2010), Tulum (Hayaloglu et al. 2007) and Kasar (Hayaloglu 2009) cheeses. Similarly, ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl lactate were identified at the highest concentrations in Malatya cheese (a native cheese variety in Turkey) at 90 days of ripening (Hayaloglu and Brechany 2007). Concentrations of ethyl butanoate increased significantly during ripening both in cheeses B and C. Ethyl acetate is responsible for the fruity and pineapple aroma in cheese (Ozer et al. 2011). Ethyl octanoate was found only in 90 or 120-day-old cheeses. Levels of esters were higher in the cheeses with added adjunct than the control cheeses. In general, the use of adjunct culture in white-brined cheeses resulted in an increased level of most of the ester compounds.

3.2.3 Alcohols

Alcohols were the largest group of volatiles identified in the cheeses, and their concentrations increased during ripening. Totally, eight alcohols, dominated by the secondary and branched-chain alcohols, were determined (Table 2). Amongst these alcohols, ethanol, 2-heptanol and 2-pentanol were detected at higher levels in cheeses B or C than cheese A. However, these alcohols except for 2-nonanol and 2-butanol were detected at higher levels in the cheeses ripened at 12 °C than the cheeses ripened at 6 °C. Ethanol was the most abundant alcohol in all cheeses and is mainly produced by the fermentation of lactose and by the catabolism of Ala. Also, it plays an important role in the formation of ethyl esters which may cause a fruity flavour in cheese

Table 2 Volatile compounds in white-brined cheese made using cheese culture and adjunct cultures during ripening ($\mu\text{g}\cdot 100\text{ g}^{-1}$ of cheese)

Volatiles	RI	T	Day 1			Day 30			Day 60			Day 90			Day 120			P Adj	P Day	P Temp
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
Esters																				
Methyl acetate	867	6	0.5±0.1	1.4±0.8	1.6±0.5	0.9±0.8	1.5±0.0	1.1±0.1	0.8±0.2	2.5±1.7	2.4±0.2	2.6±0.0	2.8±0.5	2.6±0.5	0.5±0.0	1.0±1.5	1.1±0.1	**	***	NS
	12	ND	ND	ND	ND	1.3±0.1	2.1±1.2	1.8±0.0	0.5±0.2	1.0±0.0	1.0±0.7	2.5±0.1	3.2±0.5	4.0±0.4	0.9±0.4	1.6±0.8	1.1±0.2	***	***	***
Ethyl acetate	900	6	3.4±0.5	4.7±2.0	3.6±0.0	1.7±0.0	2.2±0.6	1.3±0.5	0.4±0.1	3.7±0.4	2.0±0.1	1.9±2.9	4.6±0.9	1.0±0.6	1.5±0.6	3.6±0.6	2.1±0.2	***	***	***
	12	ND	ND	ND	ND	1.7±0.0	8.3±0.0	3.7±0.0	0.6±0.0	1.5±0.7	0.7±0.1	ND	19.8±2.9	2.9±1.2	0.7±0.1	4.5±0.5	1.7±0.8	***	***	NS
Ethyl butanoate	1,033	6	0.4±0.2	0.8±0.0	0.8±0.4	0.1±0.1	0.3±0.3	0.3±0.1	2.9±0.0	3.1±0.2	4.3±0.1	ND	6.7±1.8	3.7±0.8	ND	1.7±0.2	7.2±1.7	***	***	NS
	12	ND	ND	ND	ND	ND	0.9±0.1	3.1±4.0	ND	0.8±0.7	ND	2.2±1.9	5.2±0.3	3.1±1.6	ND	7.7±0.4	1.3±0.4	***	***	NS
Ethyl hexanoate	1,235	6	ND	0.1±0.0	0.2±0.1	0.1±0.0	ND	ND	0.3±0.0	0.7±0.0	0.4±0.0	***	***	NS						
	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3±0.0	0.6±0.0	0.5±0.0	***	***	NS
Ethyl octanoate	1,438	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.6±1.9	3.0±1.1	2.8±0.2	0.4±0.6	1.7±0.6	0.5±0.0	NS	***	NS
	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.1±0.0	2.6±1.2	2.9±0.2	1.4±0.5	1.5±0.2	1.2±0.3	***	***	NS
Alcohols																				
2-Propanol	933	6	0.5±0.1	0.8±0.8	0.9±0.3	ND	0.1±0.1	0.3±0.1	0.2±0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	12	ND	ND	ND	ND	2.1±2.9	0.2±0.2	0.7±0.2	0.3±0.1	0.5±0.4	ND	ND	0.8±1.1	ND	0.4±0.5	1.3±0.3	0.4±0.5	***	***	***
Ethanol	937	6	2.2±0.9	2.3±1.0	6.5±1.4	1.1±0.0	1.6±0.0	2.2±0.9	13.4±1.7	44.5±0.0	34.3±0.0	12.3±1.1	13.4±2.6	100.7±0.2	15.4±5.4	16.9±2.3	53.4±0.6	***	***	***
	12	ND	ND	ND	ND	15.5±15.1	24.5±0.1	16.4±0.5	35.0±0.2	50.8±0.0	38.7±0.0	13.5±7.2	102.7±3.7	55.9±0.4	18.7±0.8	43.2±2.4	57.3±0.3	***	***	***
2-Butanol	1,026	6	0.7±0.1	ND	ND	0.7±0.5	ND	ND	0.4±0.0	ND	ND	0.1±0.1	ND	ND	2.1±0.1	3.4±0.5	0.9±0.2	**	**	NS
	12	ND	ND	ND	ND	3.9±4.5	ND	ND	2.1±3.0	ND	ND	0.4±0.5	ND	ND	3.1±1.5	1.7±0.1	1.1±0.3	*	***	***
2-Methyl-1-propanol	1,092	6	0.3±0.1	1.0±0.5	0.6±0.1	0.1±0.0	0.3±0.1	0.2±0.3	0.8±0.9	4.1±0.0	2.0±2.0	ND	ND	ND	0.1±0.2	2.3±1.8	1.4±0.9	*	***	***
	12	ND	ND	ND	ND	5.7±0.0	0.8±0.1	1.2±1.0	7.4±0.0	5.3±0.0	3.3±1.1	0.7±0.0	0.8±0.1	ND	5.4±0.0	8.5±1.0	2.3±3.0	***	***	***
2-Pentanol	1,119	6	0.1±0.2	0.3±0.2	0.5±0.1	0.4±0.0	0.9±0.2	1.4±0.1	0.6±0.8	1.0±0.5	2.5±0.0	1.0±0.6	1.7±0.0	1.5±0.0	11.9±2.1	12.1±0.0	12.5±0.3	***	***	***
	12	ND	ND	ND	ND	0.5±0.0	1.3±0.0	2.4±0.3	0.8±2.4	3.7±0.4	4.6±0.2	3.4±0.2	3.8±0.2	6.1±0.0	20.2±3.5	62.5±2.9	24.4±0.4	***	***	*
	1,249	6	0.6±0.4	0.4±0.0	1.4±0.5	0.3±0.4	0.5±0.1	0.6±0.3	0.3±0.1	1.9±0.0	2.1±0.7	ND	ND	0.7±0.0	ND	0.6±0.8	1.2±0.4	***	*	*

Table 2 (continued)

Volatiles	RI	T	Day 1			Day 30			Day 60			Day 90			Day 120			P Adj	P Day	P Temp
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
3-Methyl-3-buten-1-ol	1,320	6	0.3±0.0	0.8±0.1	0.8±0.4	0.7±0.6	0.8±0.4	3.5±0.0	0.8±0.6	2.6±0.3	2.7±0.3	ND	0.8±0.1	1.3±0.0	ND	0.9±0.1	1.7±0.9			
	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
2-Heptanol	1,320	6	0.3±0.0	0.8±0.1	0.8±0.4	0.1±0.0	0.7±0.1	0.6±0.2	0.7±0.9	2.2±0.0	2.7±0.0	0.1±0.2	2.6±0.6	1.3±2.0	9.9±1.4	13.9±0.0	10.1±4.7	***	***	***
	12	ND	ND	ND	ND	0.2±0.0	0.9±0.1	0.8±0.2	2.9±1.5	3.0±0.3	3.7±0.1	1.2±0.1	2.7±0.0	5.1±5.5	11.7±0.9	81.0±1.4	15.1±4.4			
2-Nonanol	1,518	6	ND	ND	ND	ND	ND	ND	0.1±0.2	ND	0.6±0.0	0.3±0.5	1.5±0.1	2.4±0.0	3.0±1.0	11.6±0.7	0.7±0.1	***	***	NS
	12	ND	ND	ND	ND	ND	ND	ND	0.2±0.3	0.2±0.1	0.2±0.0	5.9±4.1	ND	1.2±1.7	3.8±0.6	9.7±0.2	2.8±0.0			
Aldehydes-ketones																				
Acetaldehyde	6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.5±0.8	2.3±0.5	ND	ND	ND	ND	**	***	***
	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.6±0.8	ND	ND	ND	ND			
2-Pentanone	858	6	6.0±2.1	5.8±3.2	11.6±3.6	3.6±0.0	7.8±0.0	6.3±0.0	5.9±1.3	7.4±0.0	9.5±0.0	17.1±0.0	29.6±0.6	17.9±0.9	5.2±2.8	11.5±1.8	10.9±0.9	***	***	*
	12	ND	ND	ND	4.3±1.4	19.4±0.0	7.1±0.0	8.7±0.5	20.2±0.0	9.8±0.0	17.4±1.8	22.1±0.4	47.8±1.2	32.2±0.7	9.0±0.2	12.3±2.5	11.1±0.1			
2-Butanone	913	6	11.0±3.1	14.0±5.9	17.7±0.0	9.3±0.0	13.5±3.1	14.0±0.0	10.2±0.3	12.9±0.0	29.4±5.4	40.5±2.9	35.3±1.4	44.9±2.5	13.8±5.8	26.1±0.2	24.1±0.3	***	***	NS
	12	ND	ND	ND	4.9±0.0	11.8±0.0	50.2±0.2	6.9±0.3	25.6±0.0	16.7±0.0	ND	39.0±1.6	ND	ND	4.3±1.7	20.8±1.7	34.3±0.4			
3-Methyl-1-butanol	929	6	2.4±3.1	3.2±0.1	2.7±0.1	0.3±0.4	0.4±0.1	0.5±0.0	0.4±0.5	2.3±1.1	1.5±2.1	ND	ND	ND	1.6±0.4	1.6±0.1	0.5±0.0	NS	*	*
	12	ND	ND	ND	0.4±0.3	0.5±0.1	0.7±0.2	0.9±0.1	4.6±0.0	2.2±0.0	1.2±0.0	1.9±0.1	1.6±0.1	1.6±0.1	0.7±0.0	2.0±0.1	0.6±0.0			
Diacetyl	1,054	6	4.9±0.8	3.7±0.9	9.0±0.0	1.9±0.0	2.7±0.0	2.9±0.0	0.3±0.6	5.0±0.0	2.4±0.4	ND	ND	4.2±0.0	0.3±1.7	2.8±0.9	5.8±0.2	***	***	***
	12	ND	ND	ND	2.7±1.0	3.3±3.2	6.0±0.0	0.4±0.5	6.5±0.0	8.3±0.0	2.8±2.1	ND	ND	6.6±0.2	0.6±0.8	3.9±0.1	7.3±0.4			
3-Hexanone	1,100	6	0.6±0.2	0.3±0.0	0.1±0.2	0.1±0.1	0.1±0.1	ND	ND	ND	ND	ND	ND	ND	2.1±0.0	12.9±3.3	1.6±0.2	***	***	***
	12	ND	ND	ND	ND	0.1±0.1	ND	ND	9.4±0.2	18.1±6.6	25.5±0.0									
2-Hexanone	1,542	6	ND	ND	ND	1.8±2.1	ND	ND	ND	2.5±2.8	3.8±1.0	2.3±0.1	3.7±0.1	4.8±1.7	ND	ND	ND	***	***	***
	12	ND	ND	ND	ND	3.1±0.0	ND	8.9±0.0	3.5±1.8	8.1±0.0	11.2±0.0	20.4±0.1	11.5±10.2	ND	ND	ND	ND			
3-Hydroxy-2-butanone	1,291	6	23.7±10.6	21.0±6.4	47.0±0.0	ND	23.7±1.7	25.8±4.5	17.8±4.5	26.3±0.0	73.6±0.0	18.9±2.8	14.4±0.6	24.8±1.0	29.5±27.1	42.1±0.0	21.4±3.3	***	***	NS
	12	ND	ND	ND	24.4±5.0	62.5±0.0	29.7±0.0	13.3±0.0	20.0±0.6	24.7±0.0	1.9±0.2	28.4±0.0	28.9±0.4	22.1±6.5	42.9±3.4	31.0±0.1	18.6±3.3			
2-Nonanone	1,396	6	0.6±0.1	4.2±0.4	4.1±0.9	1.1±0.0	4.8±0.0	2.6±0.0	11.7±0.7	15.3±0.0	18.5±0.0	4.3±0.3	45.8±4.1	13.7±1.1	44.5±7.9	89.9±0.4	18.6±3.3	***	***	***
	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND			

Table 2 (continued)

Volatiles	RI	T	Day 1			Day 30			Day 60			Day 90			Day 120			P Adj	P Day	P Temp
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C			
8-Nonen-2-one	1,451	6	2.3±1.6	0.5±0.3	ND	0.4±0.0	0.3±0.4	ND	0.9±1.3	3.3±0.6	4.7±1.6	2.7±0.0	13.9±0.0	2.9±0.5	1.0±0.6	18.1±1.7	3.3±0.6	***	***	***
	12	ND	ND	ND	ND	0.4±0.6	0.5±0.2	0.5±0.0	3.6±1.9	3.7±0.1	5.3±0.0	41.0±0.0	15.8±0.0	9.6±0.3	4.9±1.4	19.8±4.8	22.6±4.5	***	***	***
Acetone	858	6	6.0±2.1	5.8±3.2	11.6±3.6	3.6±0.0	6.3±0.0	5.9±1.3	7.8±0.0	7.4±0.0	9.5±0.0	17.1±0.0	29.6±0.6	17.9±0.9	5.2±2.8	11.5±1.8	10.9±0.9	***	***	*
	12	ND	ND	ND	ND	4.3±1.4	19.4±0.0	8.1±0.0	6.7±0.5	20.2±0.0	10.2±0.0	17.4±1.8	22.1±0.4	47.8±1.2	9.0±0.2	11.3±2.5	12.1±0.1	***	***	***
Miscellaneous compounds																				
Chloroform	1,015	6	1.2±0.1	2.5±1.9	4.5±0.0	0.3±0.1	0.4±0.3	0.2±0.2	ND	0.4±0.6	0.9±0.0	0.6±0.0	2.1±0.6	3.7±0.0	6.1±0.6	11.4±1.0	3.5±0.2	***	***	NS
	12	ND	ND	ND	ND	0.9±0.8	0.8±0.4	ND	2.4±0.7	1.1±0.0	2.0±0.4	4.2±0.5	11.2±0.9	3.4±0.5	ND	ND	ND	ND	***	*
α-Pinene	1,020	6	ND	1.9±1.0	6.2±0.0	ND	2.0±0.9	1.3±0.4	ND	2.3±0.0	0.9±1.3	ND	ND	ND	ND	2.4±0.9	3.3±0.3	***	*	NS
	12	ND	ND	ND	ND	2.7±1.9	2.4±1.2	ND	5.6±0.0	1.3±0.5	ND	1.5±0.2	2.2±0.2	ND	3.1±0.4	3.9±0.5	ND	ND	***	*
Acids																				
Acetic acid	1,478	6	7.3±0.2	13.8±0.0	13.7±0.0	3.4±0.0	10.5±0.8	3.7±0.9	5.7±0.7	25.9±0.0	18.7±0.0	13.3±1.4	22.79±1.3	23.1±1.1	13.8±3.5	16.35±5.7	14.0±8.7	***	***	***
	12	ND	ND	ND	ND	4.2±0.1	25.0±0.0	4.4±0.3	16.7±0.0	12.3±0.0	4.3±0.7	12.5±1.6	25.4±1.2	22.5±0.5	8.6±4.0	15.8±1.7	9.0±3.3	***	***	***
Butanoic acid	1,643	6	7.1±0.8	19.7±0.0	31.0±0.0	8.8±4.7	15.7±0.0	7.1±0.0	37.7±0.0	25.1±1.2	25.4±0.0	41.3±9.4	100.4±3.2	48.4±0.1	84.0±17.4	140.3±0.0	181.8±0.2	***	***	***
	12	ND	ND	ND	ND	31.4±10.3	24.0±0.0	20.4±0.0	30.9±11.1	27.7±2.7	32.4±0.0	137.9±10.7	272.3±12.8	156.4±3.2	62.5±23.8	174.9±3.9	124.4±2.6	***	***	***
Hexanoic acid	1,878	6	1.3±0.1	9.7±0.0	12.5±0.0	7.2±0.7	6.6±0.0	5.3±0.0	16.5±0.0	24.3±0.0	39.1±0.0	10.6±1.7	43.3±0.0	93.9±3.4	29.2±0.0	52.32±2.5	123.7±0.0	***	***	NS
	12	ND	ND	ND	ND	15.5±5.4	26.8±0.0	9.4±0.0	17.9±0.0	11.8±0.0	15.8±0.0	23.0±4.0	109.2±0.0	122.4±0.6	10.1±5.0	66.9±4.4	34.2±7.1	***	***	NS

A, cheese starter culture containing *Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*; B, cheese starter culture + *Lactobacillus helveticus*; C, cheese starter culture + *Lactobacillus casei*

RI retention index, ND not determined, NS not significant, T temperature, P probability

*P<0.05; **P<0.01; ***P<0.001

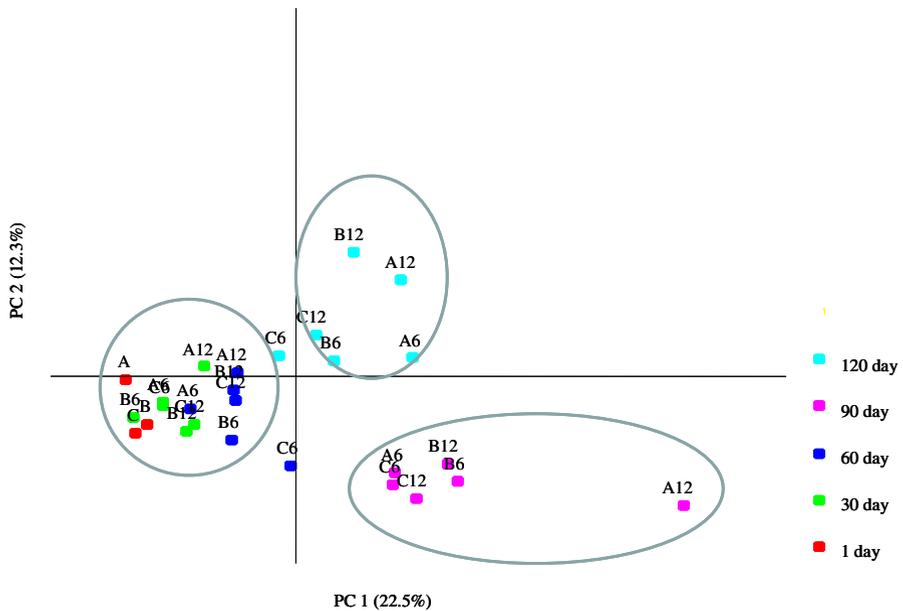


Fig. 1 Score plot obtained by PCA of the volatile composition of white-brined cheeses made using *A* cheese starter culture containing *Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*, *B* cheese starter culture+*Lactobacillus helveticus* and *C* cheese starter culture+*Lactobacillus casei*; 6 or 12 implies ripening temperature of the cheeses

(McSweeney and Sousa 2000). Ethanol is also found as a principal alcohol in other types of cheeses including Pecorino Siciliano (Randazzo et al. 2008) and Malatya (Hayaloglu and Brechany 2007). The use of adjunct culture, ripening temperature and ageing significantly influenced the concentrations of alcohol except for 2-nonanol and 2-butanol ($P < 0.05$). In many cheese varieties, 2-methyl-1-propanol contributes to the development of a slightly sweet and fresh flavour (Kondyli et al. 2002). In cheeses B and C, 2-butanol was identified only at the end of ripening (120 days). Certain primary alcohols, e.g. 2-methyl-1-propanol, can be formed by reduction of the aldehydes produced by Strecker degradation of Val (Hayaloglu et al. 2007).

3.2.4 Aldehydes

Two aldehydes, including 3-methyl-1-butanal and acetaldehyde, were determined in some of the cheese samples (Table 2). Acetaldehyde was identified only in cheeses A and B at 90 days when ripened at 6 °C and only in cheese B at 90 days when ripened at 12 °C. 3-Methyl-1-butanal, which is derived from Leu (García-Cayuela et al. 2012), has been found to be responsible for spicy, cocoa, unripe, apple-like, cheesy, green, malty and harsh flavours in many cheeses such as Proosdji, Parmesan, Camembert, Blue and Cheddar (Sable and Cottencaeu 1999). 3-Methyl-1-butanal was present at high levels in the cheeses inoculated with adjunct cultures (cheeses B and C). This compound is often associated with the formation of off-flavours when the concentration is higher than 18–90 ppm in cheese (McSweeney and Sousa 2000). Similar observation was made by Sable and Cottencaeu (1999) who found a relationship between the levels

of aldehyde and the precursor amino acid concentrations in Cheddar- and Roncal-type cheeses inoculated with lactobacilli as adjunct cultures. The concentration of 3-methyl-1-butanal was higher in the cheeses ripened at 12 °C than the cheeses ripened at 6 °C throughout ripening period ($P < 0.05$). Similarly, Cheddar cheeses ripened at 8 °C were reported to have higher levels of total aldehydes than the cheeses matured at 1 °C (Shakeel-Ur-Rehman Banks et al. 2000).

3.2.5 Ketones

Totally, nine ketones were identified in the cheese samples with high abundance of 2-butanone, diacetyl, 2-pentanone, 3-hydroxy-2-butanone and 2-nonanone (Table 2). Apart from 2-butanone, 3-hexanone and 3-hydroxy-2-butanone, the concentrations of these ketones were significantly higher in the cheeses ripened at 12 °C than in the cheeses ripened at 6 °C. In contrast to the other compounds, the concentrations of 2-butanone and 3-hydroxy-2-butanone decreased when the ripening temperature increased from 6 to 12 °C. Although the concentrations of diacetyl and 3-hydroxy-2-butanone decreased at one stage of ripening ($P < 0.001$), the concentrations of acetone, 2-butanone and 2-nonanone increased continuously during ripening. A similar trend was also noted for 3-hexanone, 8-nonen-2-one. Diacetyl can be reduced to acetoin, which in turn can be reduced to 2,3-butanediol, then to 2-butanone and finally to 2-butanol. According to Urbach (1993), diacetyl and acetoin can be produced by reduction of 2,3-butanediol via the activity of starter or non-starter lactic acid bacteria. Diacetyl gives a creamy flavour and is converted to acetoin and 2-butanone, contributing to cheese flavour. The use of adjunct culture and ripening time caused increased levels of methyl ketones; however, ripening temperature did not significantly affect the concentrations of some ketones.

3.2.6 Miscellaneous compounds

The concentrations of miscellaneous compounds in the experimental cheeses are shown in Table 2. α -Pinene was the only terpene compound detected at all stages of ripening and it appeared only in the adjunct culture-added cheeses. Terpenes are transferred into the milk through the grazing of the animals in high mountain pastures and are finally detected in the cheese (Fernandez-Garcia et al. 2002). Chloroform, which is of likely external origin volatile was found in the cheese, was detected in the samples at substantial levels, and the effects of ripening time and adjunct culture were significant. The concentration of chloroform was at higher levels at 120 days of ripening (Table 2). Chloroform was also determined in different cheeses including Malatya (Hayaloglu and Brechany 2007), Mihalic (Hayaloglu et al. 2012), Kashar (Hayaloglu 2009) and Feta-type (Bintsis and Robinson 2004).

3.2.7 Carboxylic acids

Carboxylic acids do not only contribute to the volatile fraction of cheese but also serve as precursors of methyl ketones, alcohols, esters and lactones (McSweeney and Sousa 2000). Three carboxylic acids including acetic, butanoic and hexanoic

acids were identified in all cheese samples (Table 2), and their concentrations were significantly influenced by adjunct culture, ripening temperature and ageing ($P < 0.001$). In general, the highest concentrations of these acids were identified at 90 days of ripening (Table 2). Regardless of ripening temperature, levels of acetic, butanoic and hexanoic acids were significantly higher in cheeses B and C than cheese A at 1, 90 and 120 days of ripening (Table 2). The concentrations of carboxylic acids were higher in the cheeses ripened at 12 °C than their counterparts at 6 °C in many stages of ripening. Butanoic acid, which has a rancid taste, was detected at the highest concentration in all samples during ripening. Butanoic acid is mainly produced by lipolysis (McSweeney and Sousa 2000); however, some acids are also produced through other pathways involved in the catabolism of amino acids such as Val, Thr, Glu and Met. Hayaloglu (2009) identified eleven acids in Kasar cheese, being the acetic, butanoic and hexanoic acids most abundant. Acetic acid, which gives a typical flavour of cheeses ripened under brine, is not a product of lipolysis but mainly produced by other biochemical pathways, probably from the fermentation of lactate or the metabolism of amino acids by bacteria (Fox and McSweeney 1996). It contributes to the final flavour of white-brined cheese called “pungent” flavour notes and is occasionally determined in brined cheeses (Hayaloglu et al. 2013). The second most abundant carboxylic acid at 120 days of ripening was butanoic acid, which has rancid, cheesy odours and probably contributes a ripe aroma in cheese (Yvon and Rijnen 2001). Butanoic acid was identified as the most abundant volatile fraction in Feta cheeses (Kondyli et al. 2012), and other carboxylic acids including acetic and hexanoic acids were found to be the major acids in the Turkish white-brined cheeses (Ozer et al. 2011).

3.3 Texture analysis

Texture Profile Analysis (TPA) was performed to evaluate the effects of adjunct starters and ripening temperatures on the textural properties of the samples at 1, 30, 60, 90 and 120 days of ageing (Table 3). The use of adjunct culture significantly influenced the hardness, springiness, gumminess and cohesiveness values of the cheeses ($P < 0.05$) and these parameters decreased significantly throughout ripening ($P < 0.05$). At the end of ripening, a relatively softer texture was observed in all cheeses, and accordingly, the decreases in hardness values were probably due to the hydrolysis of caseins (Sallami et al. 2004) and also the solubility of some of the residual colloidal calcium phosphate in casein matrix of cheese (O'Mahony et al. 2005). The increases in the WSN and TCA-SN values (see Table 1) of the cheeses coincided with the decreases in the hardness values of the samples (see Table 3). These findings are in accordance with those reported by Jooyandeh (2009). The cheese inoculated with adjunct cultures (samples C) had lower hardness values than the control cheese (cheese A) at both ripening temperatures at 120 days of ripening. Softening of the cheese is related to hydrolysis of α_{s1} -casein during ripening, but the role of β -casein hydrolysis is rather limited (Lawrence et al. 1987; Sahan et al. 2008). Several factors affect the cheese texture including pH, calcium level or ionic strength and thermal processing. The texture of the cheese undergoes

Table 3 Texture Profile Analysis parameters in white-brined cheeses made using cheese starter (A) or adjunct cultures (B or C) during ripening

Variables	Days	A ¹		B ¹		C ¹	
		6 °C	12 °C	6 °C	12 °C	6 °C	12 °C
Hardness (N)	1	5.35±0.24		8.54±6.09		6.25±4.41	
	30	4.88±0.53B	6.53±0.58b	12.21±2.62C	12.72±7.62b	6.25±4.09A	8.79±4.98a
	60	3.24±1.70A	5.46±2.09a	12.22±1.23B	11.25±5.74a	3.44±.088A	10.25±3.15a
	90	3.07±0.83A	4.75±0.07a	4.72±0.49A	8.13±0.55a	3.66±0.05A	6.70±2.85a
	120	3.02±0.37B	3.16±0.34b	3.15±0.53B	4.86±4.20b	1.88±0.08A	1.85±0.03a
Cohesiveness	1	0.35±0.04		0.35±0.03		0.37±0.03	
	30	0.40±0.01A	0.36±0.03a	0.35±0.03B	0.34±0.03a	0.41±0.07A	0.35±0.01a
	60	0.30±0.06A	0.34±0.07b	0.32±0.04A	0.32±0.01a	0.34±0.03A	0.31±0.01a
	90	0.32±0.05A	0.26±0.02a	0.30±0.02A	0.25±0.03a	0.31±0.02A	0.21±0.07b
	120	0.30±0.02AB	0.26±0.02b	0.26±0.04B	0.24±0.00a	0.21±0.04B	0.16±0.03c
Springiness (mm)	1	0.70±0.03		0.71±0.03		0.73±0.03	
	30	0.76±0.01A	0.73±0.02a	0.72±0.03A	0.71±0.08a	0.74±0.06A	0.70±0.06a
	60	0.64±0.08A	0.69±0.05a	0.73±0.03A	0.70±0.01a	0.68±0.01A	0.69±0.02a
	90	0.70±0.06A	0.67±0.03a	0.50±0.00B	0.64±0.04a	0.67±0.06A	0.57±0.06a
	120	0.63±0.10B	0.69±0.03b	0.64±0.05B	0.55±0.05a	0.47±0.06A	0.64±0.02ab
Gumminess (N)	1	1.92±0.30		2.85±1.83		2.27±1.48	
	30	1.93±0.14A	2.32±0.03a	4.17±0.51B	3.72±2.50a	2.73±2.14A	3.14±2.85a
	60	1.02±0.70A	1.92±1.11a	3.87±0.02B	3.50±1.70a	1.19±0.40A	3.16±1.07a
	90	0.96±0.27A	1.28±0.24a	1.14±0.13A	3.31±2.01a	1.84±1.09A	1.51±0.99a
	120	0.88±0.01B	1.12±0.04b	1.93±1.14B	1.15±0.95b	0.42±0.21A	1.29±1.09a
Adhesiveness (Nmm)	1	-0.04±0.03		-0.04±0.09		-0.05±0.03	
	30	-0.04±0.01A	-0.04±0.01a	-0.02±0.00A	-0.06±0.01a	-0.04±0.04A	-0.06±0.00a
	60	-0.06±0.06A	-0.03±0.05a	-0.02±0.05A	-0.03±0.00a	-0.06±0.01A	-0.04±0.01a
	90	-0.05±0.04A	-0.03±0.00a	-0.06±0.01A	-0.07±0.03a	-0.05±0.00A	-0.07±0.00a
	120	-0.05±0.02A	-0.05±0.00a	-0.04±0.02A	-0.10±0.02a	-0.09±0.03A	-0.07±0.00a

The capital letters A, B and C indicate means that significantly differ at $P < 0.05$ within cheeses at 6 °C in a row. The lower case letters a, b and c indicate means that significantly differ at $P < 0.05$ within cheeses at 12 °C in a row

¹ A, cheese starter culture containing *Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*; B, cheese starter culture + *Lactobacillus helveticus*; C, cheese starter culture + *Lactobacillus casei*

continuous changes during ripening as a consequence of biochemical reactions, i.e. proteolysis, the decrease in water activity (a_w) due to the release of water-binding ionic groups, redistribution of salt, and, in many cases, evaporation of water as well as changes in pH (Lawrence et al. 1987; McSweeney 2004). Decreases in the pH of the cheese curd are correlated with progressive dissociation of the casein micelles into small aggregates (Jooyandeh 2009). At low pH, casein micelles lose their integrity and cohesion and cheese cohesiveness shows a trend of decreasing as proteolysis increases and pH decreases in cheese

(Dabour et al. 2006). A significant reduction in cohesiveness values of cheeses B and C was observed, being more pronounced with the extended ripening. The decrease in cheese springiness during ripening was reported previously for Cheddar and other cheese types (Dabour et al. 2006). Gumminess is described as the energy required to disintegrate a semi-solid food product to a state ready for swallowing (Gunasekaran and Ak 2003). The use of adjunct culture in cheese production caused an increase in the gumminess values ($P < 0.05$), and the changes were pronounced in cheese B during ripening (Table 3). As expected, gumminess values decreased during ripening regardless of cheese type, and the decrease in this parameter continued with increasing proteolysis which is absolutely a reflection of casein network loosening (Romeih et al. 2002). The adhesiveness values of the samples slightly decreased during the ripening period; however, these changes were not significant ($P > 0.05$) as shown in Table 3. The effects of adjunct culture and ripening temperature were not significant in the cheeses. The adhesiveness is strictly related to the levels of fat and total solid in cheeses (Dimitreli and Thomareis 2007); therefore, the same levels of adhesiveness were recorded due to similar levels of fat in dry matter and dry matter in the cheeses (Table 1).

3.4 Sensory analysis

Sensory perception is a complex process, which is influenced by factors such as the level of flavour compounds, texture and appearance of cheese (Smit et al. 2005). A comparison of the sensory data for the three cheeses during ripening is given in Table 4. The mean scores for colour/appearance and flavour scores of the experimental cheeses decreased during ripening ($P < 0.05$). Cheese B made by using *Lactobacillus helveticus* received a lower appearance scores than the other cheeses during ripening. The cheeses made by using adjunct cultures and ripened at 12 °C had slightly bitter flavour. This may be probably due to a higher level of proteolysis during ripening, and formation of bitter amino acids may have negatively influenced the flavour scores of the cheeses. The amino acids including Met, Lys, Val, Pro, Ile and Trp were at higher levels in cheese B than other cheeses (Sahingil et al. 2014). These amino acids are mainly produced from the hydrophobic terminus of caseins by chymosin and proteinases originated from the starter bacteria (Cogan and Beresford 2002; Smit et al. 2005). Lowered levels of bitterness have been observed in several studies of added adjunct lactobacilli (Hashemi et al. 2009; Drake et al. 1996; Muir et al. 1996); however, bitterness in cheeses B or C may be due to the higher levels of proteolysis when compared to cheese A. The panellists found that cheeses ripened at 12 °C had higher sour/acid and bitter flavour than the cheeses ripened at 6 °C. The cheeses ripening at 12 °C had a higher concentration of amino acids (Sahingil et al. 2014), so it might give a bitterness note. Moatsou et al. (2004) showed that the development of proteolysis in Graviera Kritis cheeses ripened at higher temperature (i.e. 20 °C) was faster than its counterpart ripened at 16 °C, and the former cheese received lower sensory scores by the panellists. Cheeses B and C ripened at 12 °C had lower flavour scores than cheese A. At 30 and 90 days of ripening, the cheeses made with adjunct cultures had lower overall quality scores than the

Table 4 Sensory properties in white-brined cheeses made using cheese starter (A) or adjunct cultures (B or C) during ripening

Properties	Days	A ¹		B ¹		C ¹	
		6 °C	12 °C	6 °C	12 °C	6 °C	12 °C
Colour and appearance (0–5)	20	4.08±0.35A	4.50±0.24a	3.92±0.35A	3.79±0.18a	4.00±0.35A	4.00±0.35a
	30	4.58±0.12A	4.65±0.21b	4.19±0.15A	3.90±0.14a	4.30±0.42A	4.32±0.02a
	60	4.55±0.07A	4.45±0.21a	3.85±0.64A	4.00±0.42a	4.50±0.42A	4.30±0.71a
	90	3.98±0.26A	3.67±0.24a	3.18±0.02A	2.73±0.09a	3.57±0.38A	3.75±1.06a
	120	3.68±0.02A	3.85±0.49a	3.40±0.57A	3.42±0.38a	3.62±0.40A	3.50±0.71a
Texture (0–5)	20	3.92±0.12A	3.92±0.35a	3.92±0.35A	3.75±0.12a	3.83±0.24A	3.67±0.47a
	30	4.00±0.00A	4.45±0.21b	3.90±0.14A	3.45±0.35a	3.95±0.49A	3.95±0.07a
	60	3.80±0.42A	4.35±0.35a	3.85±0.21A	3.85±0.07a	3.40±0.28A	4.05±1.06a
	90	3.90±0.14A	3.65±0.21a	3.25±0.35A	2.75±0.35a	3.35±0.21A	3.50±0.42a
	120	3.85±0.21A	3.70±0.00a	3.30±0.71B	3.25±0.35b	3.25±0.35B	3.20±0.00b
Flavour (0–10)	20	7.92±0.59A	8.50±0.24a	7.58±0.35A	8.50±0.24a	7.58±0.82A	7.33±0.00a
	30	7.67±0.24A	8.17±0.42b	7.25±0.12A	6.79±0.53b	7.50±0.47A	7.25±0.59a
	60	7.76±0.22A	7.92±0.12a	7.32±0.68A	7.42±0.82a	7.67±0.47A	7.73±0.67a
	90	7.33±0.24A	6.50±0.47a	6.17±0.47A	5.67±0.94b	6.64±0.53A	6.67±0.00a
	120	7.42±0.59A	6.75±1.06a	7.92±0.12A	6.17±1.65a	7.17±0.24A	6.17±1.65a
Overall quality (0–5)	20	3.58±0.12A	4.33±0.47a	3.75±0.12A	3.50±0.24a	3.83±0.59A	3.25±0.71a
	30	4.08±0.00A	4.54±0.18b	4.00±0.35A	3.50±0.35a	3.96±0.29A	3.79±0.18a
	60	4.08±0.25A	4.13±0.18a	3.65±0.21A	3.78±0.16a	4.13±0.18A	3.74±0.93a
	90	3.96±0.41B	3.54±0.06a	3.00±0.00A	3.33±0.24a	3.04±0.29AB	3.54±0.77a
	120	3.54±0.06A	3.50±0.00a	3.46±0.53A	3.25±0.12a	3.88±0.29A	3.08±0.12a

The capital letters A, B and C indicate means that significantly differ at $P < 0.05$ within cheeses at 6 °C in a row. The lower case letters a, b and c indicate means that significantly differ at $P < 0.05$ within cheeses at 12 °C in a row

¹ A, cheese starter culture containing *Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*; B, cheese starter culture + *Lactobacillus helveticus*; C, cheese starter culture + *Lactobacillus casei*

control cheeses ripened at 6 or 12 °C. These may be due to higher levels of some volatiles determined in adjunct culture-added cheeses when compared to control (A) cheese. Methyl ketones contribute to the pungent aroma of Blue cheeses (Hayaloglu et al. 2007), and nine ketones were identified in white-brined cheese. Amongst these, 2-butanone, 3-hydroxy-2-butanone and 8-nonen-2-one were the most abundant ketones which were isolated from adjunct added cheeses (Table 2). Cheeses A and B had better sensory scores with respect to perceived texture than those of cheese C at 120 days of ripening. The lower score in cheese C may be because of high softening of cheese texture. It has been reported that Iranian white-brined cheese made by incorporating *Lactobacillus helveticus* as adjunct culture had higher sensory scores than the control cheese (Hashemi et al. 2009). Similar results were reported for white-brined cheeses made by using *Lactobacillus casei* (Kayagil and Gurakan 2009).

4 Conclusions

This study represented an extensive description and quantification of the volatile and texture profiles of white-brined cheese made by incorporating adjunct cultures. The concentrations of volatile compounds in white-brined cheese significantly increased by raising ripening temperature and extended ripening period ($P < 0.05$). The use of adjunct culture increased the concentrations of some volatiles including alcohols, methyl ketones and acids in the cheeses during ripening. In addition, the concentrations of most of the volatile compounds were the highest in cheeses B and C (adjunct culture-added cheeses) ripened at 12 °C. Significant differences were found amongst the three samples with respect to textural parameters except for the adhesiveness. Hardness was greatly influenced by ripening temperature ($P < 0.05$), and these changes were mainly attributed to the extended proteolysis. The use of adjunct cultures changed the texture and flavour scores of the cheeses; however, a slight bitter flavour was noted by the panellists at 90 days of ripening, and this change in flavour was linked to the formation of some proteolysis products. In conclusion, the use of adjunct cultures changed the texture and sensory profiles of the cheeses as well as enhanced the volatile composition. Keeping period (or shelf life) for white-brined cheese with an adjunct culture should not last longer than 90 days to avoid bitterness.

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References

- Alizadeh M, Hamed M, Khosroshahi A (2006) Modeling of proteolysis and lipolysis in Iranian white brine cheese. *Food Chem* 97:294–301
- Bergamini CV, Wolf IV, Perotti MC, Zalazar CA (2010) Characterisation of biochemical changes during ripening in Argentinean sheep cheeses. *Small Rumin Res* 94:79–89
- Bertola NC, Califano AN, Bevilacqua AE, Zaritzky NE (2000) Effects of ripening conditions on the texture of Gouda cheese. *Int J Food Sci Technol* 35:207–214
- Bintsis T, Robinson RK (2004) A study of the adjunct cultures on the aroma compounds of Feta-type cheese. *Food Chem* 88:435–441
- Cogan TM, Beresford TP (2002) Microbiology of hard cheese. In: Robinson RK (ed) *Dairy microbiology handbook*, 3rd edn. Wiley, New York
- Dabour N, Kheadr E, Benhamou N, Lapointe G (2006) Improvement of texture and structure of reduced-fat Cheddar cheese by exopolysaccharide-producing lactococci. *J Dairy Sci* 89:95–110
- Dagdemi E, Çelik S, Özdemir S (2003) The effects of some starter cultures on the properties of Turkish White cheese. *Int J Dairy Technol* 56:215–218
- Dimitreli G, Thomareis AS (2007) Texture evaluation of block-type processed cheese as a function of chemical composition and in relation to its apparent viscosity. *J Food Eng* 79:1364–1373
- Drake MA, Boylston TD, Spence KD, Swanson BG (1996) Chemical and sensory effects of *Lactobacillus* adjunct in Cheddar cheese. *Food Res Int* 29:381–387
- Fernández-García E, Carbonell M, Nunez M (2002) Volatile fraction and sensory characteristics of Manchego cheese. I. Comparison of raw and pasteurized milk cheese. *J Dairy Res* 69:579–593
- Folkertsma B, Fox PF, McSweeney PLH (1996) Accelerated ripening of cheese at elevated temperatures. *Int Dairy J* 6:1114–1117
- Fox PF, McSweeney PLH (1996) Proteolysis in cheese during ripening. *Food Rev Int* 12:457–509
- García-Cayuela T, Gómez de Cadiñanos LP, Peláez C, Requena T (2012) Expression in *Lactococcus lactis* of functional genes related to amino acid catabolism and cheese aroma formation is influenced by branched chain amino acids. *Int J Food Microbiol* 159:207–213

- Gunasekaran S, Ak MM (2003) Cheese rheology and texture. CRC Press, Boca Raton
- Hannon JA, Wilkinson MG, Delahunty CM, Wallace JM, Morrissey PA, Beresford TP (2005) Application of descriptive sensory analysis and key chemical indices to assess the impact of elevated ripening temperatures on the acceleration of Cheddar cheese ripening. *Int Dairy J* 15:263–273
- Hashemi M, Azar M, Mazlumi MT (2009) Effect of commercial adjunct lactobacilli on biochemical and sensory characteristics of Iranian white-brined cheese. *Int J Dairy Technol* 62:48–55
- Hayaloglu AA (2009) Volatile composition and proteolysis in traditionally produced mature Kashar cheese. *Int J Food Sci Technol* 44:1388–1394
- Hayaloglu AA, Brechany EY (2007) Influence of milk pasteurization and scalding temperature on the volatile compounds of Malatya, a farmhouse Halloumi-type cheese. *Lait* 87:39–57
- Hayaloglu AA, Guven M, Fox PF (2002) Microbiological, biochemical and technological properties of Turkish White cheese ‘Beyaz Peynir’. *Int Dairy J* 12:635–648
- Hayaloglu AA, Güven M, Fox PF, Hannon JA, McSweeney PLH (2004) Proteolysis in Turkish White-brined cheese made with defined strains of *Lactococcus*. *Int Dairy J* 14:509–610
- Hayaloglu AA, Guven M, Fox PF, McSweeney PLH (2005) Influence of starters on chemical, biochemical and sensory changes in Turkish White-brined cheese. *J Dairy Sci* 88:3460–3467
- Hayaloglu AA, Cakmakci S, Brechany EY, Deegan KC, McSweeney PLH (2007) Microbiology, biochemistry, and volatile composition of Tulum cheese ripened in goat’s skin or plastic bags. *J Dairy Sci* 90:1102–1121
- Hayaloglu AA, Bansal N, McSweeney PLH (2012) Influence of brine immersion and vacuum packaging on the chemistry, biochemistry and microstructure of Mihalic cheese made using sheep’s milk during ripening. *Dairy Sci Technol* 92:671–689
- Hayaloglu AA, Tolu C, Yasar K, Sahingil D (2013) Volatiles and sensory evaluation of goat milk cheese Gokceada as affected by goat breeds (Gokceada and Turkish Saanen) and starter culture systems during ripening. *J Dairy Sci* 96:2765–2780
- Jooyandeh H (2009) Effect fermented whey protein concentrate on texture of Iranian white cheese. *J Texture Stud* 40:497–510
- Kayagil F, Gurakan C (2009) Effects of starter culture combinations using isolates from traditional cheese on the quality of Turkish white cheese. *Int J Dairy Technol* 62:387–396
- Kondyli E, Katsiari MC, Masouras T, Voutsinas LP (2002) Free fatty acids and volatile compounds of low fat Feta-type cheese made with a commercial adjunct culture. *Food Chem* 79:199–205
- Kondyli E, Pappa EC, Vlachou AM (2012) Effect of package type on the composition and volatile compounds of Feta cheese. *Small Rumin Res* 108:95–101
- Lawrence RC, Creamer LK, Gilles J (1987) Texture development during cheese ripening. *J Dairy Sci* 70:1748–1760
- McSweeney PLH (2004) Biochemistry of cheese ripening. *Int J Dairy Technol* 57:127–144
- McSweeney PLH, Sousa MJ (2000) Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. *Lait* 80:293–324
- Moatsou G, Massouras T, Anifantakis E (2004) Effect of different manufacturing parameters on the characteristics of Graviera Kritis cheese. *Int J Dairy Technol* 57:215–220
- Muir DD, Banks JM, Hunter EA (1996) Sensory properties of cheddar cheese: effect of starter type and adjunct. *Int Dairy J* 6:407–423
- O’Mahony JA, Lucey JA, McSweeney PLH (2005) Chymosin-mediated proteolysis, calcium solubilization and texture development during ripening of Cheddar cheese. *J Dairy Sci* 88:3101–3114
- Ozer B, Kirmaci HA, Hayaloglu AA, Akçelik M, Akkoç N (2011) The effects of incorporating wild-type strains of *Lactococcus lactis* into Turkish white-brined cheese (Beyaz peynir) on the fatty acid and volatile content. *Int J Dairy Technol* 64:494–501
- Randazzo CL, de Luca I, Pitino S, Scifo GO, Caggia C (2008) Effect of wild strains used as starter cultures and adjunct cultures on the volatile compounds of the Pecorino Siciliano cheese. *Int J Food Microbiol* 122:269–278
- Romeih EA, Michaelidou A, Biliaderis CG, Zerfiridis GK (2002) Low-fat white-brined cheese made from bovine milk and two commercial fat mimetics: chemical, physical and sensory attributes. *Int Dairy J* 12:525–540
- Sable S, Cotteceau G (1999) Current knowledge of soft cheeses flavor and related compounds. *J Agric Food Chem* 47:4825–4836
- Sahan N, Yasar K, Hayaloglu AA, Karaca OB, Kaya A (2008) Influence of fat replacers on chemical composition, proteolysis, texture profiles, meltability and sensory properties of low-fat Kashar cheese. *J Dairy Res* 75:1–7

- Sahingil D, Hayaloglu AA, Kirmaci HA, Ozer B, Simsek O (2014) Changes of proteolysis and angiotensin-I converting enzyme-inhibitory activity in white-brined cheese as affected by adjunct culture and ripening temperature. *J Dairy Res*. doi:[10.1017/S0022029914000326](https://doi.org/10.1017/S0022029914000326)
- Sallami L, Kheadr EE, Fliss I, Vuilleumard JC (2004) Impact of autolytic, proteolytic and nisin-producing adjunct cultures on biochemical and textural properties of Cheddar cheese. *J Dairy Sci* 87:1585–1594
- Sarantinopoulos P, Kanaltzopoulos G, Tsakalidou E (2002) Effect of *Enterococcus faecium* on microbiological, physicochemical and sensory characteristics of Greek Feta cheese. *Int J Food Microbiol* 76:93–105
- Shakeel-Ur-Rehman Banks JM, Brechany EY, Muir DD, McSweeney PLH, Fox PF (2000) Influence of ripening temperature on the volatiles profile and flavour of Cheddar cheese made from raw or pasteurised milk. *Int Dairy J* 10:55–65
- Smit G, Smit BA, Engels WJM (2005) Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiol Rev* 29:591–610
- Urbach G (1993) Relations between cheese flavour and chemical composition. *Int Dairy J* 3:389–422
- Yvon M, Rijnen L (2001) Cheese flavour formation by amino acid catabolism. *Int Dairy J* 11:185–201