Original scientific paper
Journal of Central European Agriculture, 2021, 22(1), p.19-38
DOI: /10.5513/JCEA01/22.1.3051

The effect of ripening medium (goat skin bag or plastic barrel) on the volatile profile, color parameter and sensory characteristics of Tulum cheese

Ali TEKIN[1] (✉), Zehra GULER[2]

[1] Firat University, Keban Vocational School, Department of Food Technology, 23700 Elazıg, Turkey

[2] Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Food Engineering, 31034 Antakya- Hatay, Turkey

✉ Corresponding author: a.tekin@firat.edu.tr

Received: September 29, 2020; accepted: November 13, 2020

ABSTRACT

The objective of this study was to determine volatile compounds, color parameters and sensory characteristics in raw sheep milk cheeses ripened up to 270 days using different packaging materials such as goat's skin bag or plastic barrel. Volatile compounds extracted by solid-phase microextraction were separated, identified and quantified using a gas chromatography (GC)-mass spectrometry detector (MS). Butanoic acid, hexanoic acid, octanoic acid, ethanol, ethyl acetate, ethyl butanoate, ethyl hexanoate, 2-butanol, 2-butanone and 2-heptanone were the predominant compounds in the volatile fraction of the cheeses. Among these, ethanol was the most abundant at the first 180 days of cheese ripening but there were 2-butanol and 2-butanone for cheese in plastic barrel (PB) and 2-heptanone for cheese in goat's skin bag (GS) at the 270 days. To best acknowledge, *m*-cimene, *alpha*-cubebene, *trans*-caryophylene, *delta*-cadinene and 16-oxasalutaridine were identified for the first time in sheep milk and its cheese. Discriminant analysis based on volatile compounds classified the cheeses according to their ripening times and also packaging materials. At the end of the ripening the differentiation between GS and PB cheeses was more evident. The results showed that GS cheese was more preferred by panelists. Cheeses ripened for more 180 days, in particular PB cheese, were much less acceptable to panelists. Therefore, in terms of optimum overall acceptability, 90 and 180 days ripening periods could be advised for the producers for PB and GS cheeses, respectively.

Keywords: sheep milk, tulum cheese, ripening, volatile, color, sensory

INTRODUCTION

Consumer acceptance of a cheese depends on its flavor, namely volatile and non-volatile compounds, and its appearance, namely texture and color. Volatile compounds (VCs) present in cheeses can come from (i) milk, (ii) various biochemical pathways such as glycolysis, lipolysis, proteolysis and citrate metabolism, (iii) subsequent reactions between themselves and (iv) secondary biochemical events, as metabolism of fatty acids and amino acids (Fox et al., 2000). Thus, volatile compounds are the more informative about biochemical pathways during cheese processing and ripening, and also useful to define cheese flavor or off-flavor and to determine the right ripening time.

Tulum cheese is one of the most commonly consumed traditional Turkish sheep cheeses. It is produced mostly from Akkaraman sheep raw milk without using any starter culture. In Turkish goat's skin bag is known as 'tulum', in which the crumbled and dry-salted curds are filled tightly and ripened for at least 90 days at 4-12 °C and 75-85% relative humidity (Guler and Uraz, 2003). However, the use of goat's skin bag for ripening, storage and transportation of Tulum cheese has recently been reduced due to the decreases in animal number, economic reasons and transportation difficulties. Nowadays, hardened plastic barrels are used as an alternative to goat's skin bag (Hayaloglu et al., 2007a). Therefore, the traditional cheese is named as 'skin tulum' or 'plastic barrel tulum' according to its ripening and preserving container. Compared to plastic barrel, goat's skin bag is permeable to air and water due to its porous structure, which can affect various biochemical events such as glycolysis, proteolysis and lipolysis during cheese ripening (Tekin and Guler, 2019).

Volatile compound profile of 90-day Tulum cheese ripened in goat's skin bag or plastic barrel, and the volatile compounds during cheese ripening for 120 or 180 days have been previously reported by some researchers (Hayaloglu et al., 2007b; Cakir et al., 2016; Ozturkoglu-Budak et al., 2016). According to the Turkish regulation (Food Codex, 2015), raw milk Tulum cheese should be ripened for at least 3 months. However, it is not known whether ripening time extended up to 270 days will affect the volatile profile and overall acceptability of Tulum cheeses and how volatile compound profile would be changed during traditional Tulum cheese production, and also what is optimum ripening time for optimum cheese acceptability.

The specific aims of this study, therefore, were: (1) to evaluate effects of different types of packaging materials (goat skin bag and plastic barrel) on volatile profile, color parameter and sensory characteristics throughout cheese ripening; and (2) to predict optimum ripening time for optimum cheese acceptability depending on packaging material.

MATERIAL AND METHODS

Materials

Akkaraman sheep raw milk (total solids 18.65±0.06%, protein 5.56±0.22%, fat 7.25±0.22%, carbohydrate 5.05±0.20%, ash 0.85±0.07% and pH 6.46±0.10) used in the manufacture of Tulum cheese was obtained from a farmer in Yazılı village, Karaman, Turkey. Commercial animal calf rennet at strength 1/15000 (Mandra, Peyma-Hansen; Oksuz chemistry, Konya, Turkey) as coagulant and the hardened plastic barrels (Pektim Inc. Turkey) and goat's skin bags (Karaman Goatskin, Turkey) with approximately 3-kg capacities as cheese ripening containers were used.

Cheese production

Tulum cheese was traditionally produced according to procedure described in Tekin and Guler (2019). Briefly, commercial calf rennet (20 mL/180 L milk) was added at 31-32 °C into raw sheep's milk. After approximately 180 min, curd was irregularly cut using a knife and harvested in a cloth bag. After pressing for removing whey, curd was crumbled by hand, and dry-salted with rock salt (approximately 4.25 kg/100 kg curd) and kneaded. Finally, the curd was divided into two parts. Each part was filled tightly into the plastic barrel or goat skin bags which were tied up tightly. The cheeses were ripened for 270 days at 4 °C and 85% relative humidity. The cheeses were made in three batches. Analyses were conducted on days 0 (Curd), 90, 180 and 270. Duplicate samples from each type of cheese were analyzed for each batch. Tulum cheese samples ripened in a goat's skin bag or in plastic barrel were coded as 'GS' and 'PB', respectively.

Chemical analysis

Cheeses were analyzed for total solid (IDF, 1982), salt (IDF, 1988), fat (IDF, 1986) and total nitrogen (TN) by the Kjeldahl method (IDF, 1993). For pH measurement, grated cheese (10 g) was macerated with 10 mL of distilled water, and the pH of the resultant slurry was measured using a digital pH meter (Orion, Thermo, Austin, TX, USA).

Volatile compounds (VCs)

Volatile compounds were analyzed with slight modifications according to the procedure described by Guler (2014). Briefly, a 7 g of cheese was transferred into a 20-mL headspace vial (Agilent, Palo Alto, CA, USA). In case of milk, a 10 g sample was put into the vial containing 2.5 g NaCl. The vials sealed with a TFE-silicone septum (Agilent Palo Alto, CA, USA) immediately frozen at −20 °C until use. Prior to analysis, the frozen samples were thawed at 4 °C overnight. A 75 µm fibre coated with CAR/PDMS (Supelco, Bellefonte PA, US) was used for the adsorption of VCs from head space of milk or cheese samples. For this purpose, the sample vials were put in a water bath at 60 °C with continuous stirring. Vials containing milk and cheese samples were held at 60 °C for 20 min and 30 min without fiber, respectively, and for 40 min with fiber.

The separation of VCs was carried out with an Agilent model 6890 gas chromatography and 5973 N mass spectrometry (MS) (Agilent, Palo Alto, CA, USA) equipped with a HP-INNOWAX capillary column (60 m×0,25 mm id×0.25 µm film thickness). The oven temperature program was initially held at 50 °C for 5 min and then programmed from 50 °C by a ramp of 5 °C/min up to 100 °C and held for 10 min after which increased at a rate of 10 °C/min up to 240 °C and held for 8 min. Total run time was 47 min. The identification of volatile compounds was based on the comparison of their mass spectra with NIST 0.2 (National Institute of Standard and Technology, New York, USA) and WILEY 7n.1 library (Wiley & Sons Inc., New York, USA) Mass Spectral Database. The identity of individual compounds was confirmed by the retention index calculated using the retention times of homologous series of n-alkanes C5–C25, and also by retention time (RT) and MS ion spectra of the available authentic standards (Sigma-Aldrich, Milwaukee, WI, USA) injected under the same operating conditions. Results from the volatile compounds analyses were expressed as the percentage of each compound's integrated area relative to the total integration of the compounds identified. The injection sequence of samples was carried out according to the progressive sampling time for each ripening material. Between both the sampling times and the ripening materials, the SPME fiber was cleaned in the injector at 250 °C for 30 min and blank injections were done until monitoring no signal and impurities.

Color analysis

Cheese color was measured using a Mini Scan portable colorimeter (Hunter Associates Laboratory Inc., Reston, VA). The standardization of instrument was performed using white and black standard plates (Hunter Associates Laboratory Inc.). This color assessment system is based on the Hunter *L**, *a** and *b** values using illuminant *D65*. White index (*WI*), Hue angle (*h°*) and Chroma (*C*) were calculated according to the method of Wrolstad and Smith (2010) as follows:

$$WI = 100 - [(100-L)^2 + a^2 + b^2]^{0,5}$$

$$h° = tan^{-1} [b/a]$$

$$C = (a^2 + b^2)^{0.5}$$

Sensory evaluation

After 90 days of ripening, the cheeses were evaluated using a modified sensory scale described by Bodyfelt et al. (1988). In this sensory system, sensory card comprised color (from white to yellow) as appearance attribute, 'crumbly' and 'firmness' as texture attributes, and 'sharp', 'salty', 'fruity', 'animal-like', 'bitter', 'rancid', 'yeast' and 'mold' as flavor attributes as well as overall acceptability. The sensory panel consisted of twelve academic staffs (six male and six men, aged from 26 to 45) familiar with the Tulum cheese. Each sensory attribute was clearly defined to the panel-members as reported by Bodyfelt et al. (1988). The intensity of sensory characteristics was measured on a 4-point scale where '3' corresponds to 'too much strong' and '0' corresponds to 'none'. By using a 9-point hedonic sale ('1' corresponds to 'dislike extremely', '5' corresponds to neither like nor dislike, '9' corresponds to 'like extremely'), panelists rated overall acceptability. Cheeses removed from refrigerator (4 °C) were kept at room temperature (22±2 °C) for 1 h prior to sensory evaluation. Panelists used water to rinse their mouth.

Statistical analysis

A randomized complete block design was conducted in a 2×3×4 factorial arrangement for 2 treatments (cheese in goat skin and cheese in plastic barrel), 4 ripening periods (0, 90, 180 and 270 days) and 3 blocks (batches). The General Linear Model (GLM) was applied to all data obtained from volatile compounds, color parameter and sensory analysis using SPSS statistical program (Version 17.00, SPSS, IBM, NY, USA). The paired comparisons of means were made using the t-student test. A stepwise discriminant analysis was performed on all the data on volatile compounds obtained from the experimental cheeses (Pallant, 2010).

RESULTS AND DISCUSSION

Basic chemical composition for the cheeses

As shown in Table 1, ripening in goat's skin bag in comparison plastic barrels caused a significant (P < 0.05) increase in dry-matter content. Except for the curd (0 day), the mean value of dry matter (67.68±2.82%) in Tulum cheese ripened in goat's skin bag (GS) was significantly higher than that (60.32±1.37%) in Tulum cheese ripened in plastic barrel container (PB). This finding is in agreement with the results reported by Hayaloglu et al. (2007b). The permeability of goat skin due to its porous structure could have been resulted in losses in moisture of GS cheeses. There was no significant difference in fat-in-dry matter between Tulum cheeses, but there was in ash-in-dry matter, and in protein-in-dry matter at the end of ripening. Protein-in-dry matter of PB cheese was observed a tendency decreasing during ripening, as reported by Hayaloglu et al. (2007b).

All the stages of ripening, ash-in-dry matter was significantly higher in GS cheese than that in PB one. No significant changes were observed in pH values during the ripening. This may have been related to many factors such as the fermentation of residual lactose, the catabolism of lactic acid, the levels of moisture, NaCl and

Table 1. Basic chemical composition of Tulum cheeses ripened in goats' skin bag (GS) and plastic barrel (PB) during ripening

Parameters	Curd	Cheese	Ripening Time (Day)			Ripening	Pac*Rip
			90	180	270		
Dry Matter (% w/w)	60.88 ± 0.76^{A}	GS	*$66.09\pm2.24^{b.B}$*	*$66.76\pm1.91^{b.B}$*	$70.19\pm2.96^{a.C}$	*	*
		PB	$59.30\pm0.55^{a.}$A	$60.52\pm1.87^{a.A}$	$61.14\pm1.03^{a.A}$	NS	
		^{1}P	**	*	**		
Fat in DM (% w/w)	56.04±0.81	GS	55.18±0.29	55.86±1.70	55.20±0.75	NS	NS
		PB	56.10±0.65	55.69±1.18	55.89±0.55	NS	
		P	NS	NS	NS		
Protein in DM (% w/w)	39.74 ± 2.59^{AB}	GS	$38.90\pm2.12^{a.AB}$	$38.21\pm1.35^{a.AB}$	$38.55\pm2.63^{a.B}$	NS	*
		PB	$40.02\pm3.90^{a.AB}$	$37.56\pm2.43^{b.A}$	$36.87\pm1.33^{b.A}$	*	
		P	NS	N.S.	*		
Ash in DM (% w/w)	4.32 ± 1.08^{AB}	GS	$5.04\pm1.13^{a.B}$	$5.72\pm0.39^{a.AB}$	$4.93\pm0.25^{a.B}$	*	*
		PB	$3.84\pm0.90^{a.A}$	$4.20\pm0.66^{a.AB}$	$3.81\pm0.76^{a.A}$	NS	
		P	*	**	*		
pH	4.53 ± 0.12^{A}	GS	$4.73\pm0.26^{a.A}$	$4.48\pm0.28^{a.A}$	$4.76\pm0.25^{a.A}$	NS	NS
		PB	$4.79\pm0.21^{a.A}$	$4.68\pm0.10^{a.A}$	$4.90\pm0.44^{a.A}$	NS	
		P	NS	NS	NS		

GS:Tulum cheeses ripened in goat skin bag. PB: Tulum cheeses ripened in plastic barrel. Abbreviation is: DM, dry matter. Values are means ± standard deviation.Different lower case superscript letters at the same row indicate the effects of ripening period. RM*RP indicates the significant of interactions between ripening material and ripening period. ^{1}P indicates the differences between ripening materials at the same ripening stage. NS: not significant, $P > 0.05$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Ca, the presence of buffering compounds and degree of casein proteolysis since the pH decreasing with lactose fermentation can also increase with lactate catabolism or buffering compounds as phosphates can counter reductions in pH as a result of acid production. The values found for the basic chemical composition in the present study were within ranges obtained by Guler and Uraz (2003) for Tulum cheese. However, Ozturkoglu-Budak et al. (2016) reported total protein content ranging from 4.17% to 4.87% of Divle cave Tulum cheese, in where a calculation error may have been made because the values reflect total nitrogen.

Volatile compounds (VCs)

We detected a total of 90 volatile compounds derived from Tulum cheese during production and ripening. As shown in Table 2, the volatile profile consisted of 15 alcohols, 10 carboxylic acids, 17 esters, 11 ketones, 9 terpenes, 11 hydrocarbons, 3 aldehyde and 14 miscellaneous compounds. Among them chemical group esters were not identified in the volatile fraction of milk. Cheese maturation caused a greater number (88) of total volatile compounds than those in milk and curd (31 and 71, respectively).

Figure 1 shows the variation of the volatile compound abundances per chemical group isolated from milk and the cheeses. The carboxylic acids were the most abundant of volatiles in GS cheese at the first 180 day of ripening. They significantly ($P<0.001$) increased from curd to 180-day GS cheese but did not a change in PB cheese at this period. However, there was a marked decrease in both Tulum cheeses at the end of ripening. The level of alcohols increased in both Tulum cheeses at the first 90 day, after that tended to decrease toward the end of ripening. An increase in ester chemical group was observed in both cheeses at the first 90 day, process that continued in the last 90 day. Esters appeared as the most abundant group of volatiles in PB cheese at the end of ripening. Ketones decreased at the first 90 day, after this time increased toward the end of ripening. Moreover, at the end of ripening ketones were the first and second most abundant groups of volatiles in PB and GS cheeses, respectively. Aldehydes, terpenes and hydrocarbons were found in higher levels in raw milk whereas, their levels decreased during cheese making and ripening, and the lowest levels were observed at the end of ripening.

We detected a total of 14 miscellaneous compounds including 4 phenols, 4 sulfurs, 2 dioxalanes, 1 indole, 1 alkoloid, 1 lactone and 1 imine in the headspace of milk and the cheeses (Table 2). Miscellaneous compounds encompassing dimethyl sulphone, phenol, *p*-cresol and 16-oxosalutaridine were the most abundant in the volatile fraction of milk, whereas they decreased or disappeared during cheese ripening (Figure 1).

Regardless of the ripening period, the most abundant groups of volatiles identified in headspaces of GS and PB cheeses were the carboxylic acids (approximately 29% and 22% as relative of total abundance, respectively), alcohols (22% and 28%), ketones (20% and 16%), esters (16% and 23%), hydrocarbons (5% and 4%) and terpenes (2% and 1.5%).

Carboxylic acids

In cheeses, short-chain linear fatty acids such as butanoic acid, hexanoic acid, octanoic acid and decanoic acid were dominant acids and showed a regular increase from day 0 (curd) to day180. This could be related to the action of indigenous the lipoprotein lipase coming from raw milk or microbial lipases on triacylglycerols. Short-chain linear fatty acids could be important compounds contributed to the flavor of Tulum cheese due to their much lower perception thresholds as in raw sheep milk cheeses such as Reggianito Argentino (Wolf et al., 2010). Moreover, fatty acids (C_4-C_8) can be regarded as an index for measuring cheese lipolysis (Guler and Uraz, 2003). Among fatty acids, hexanoic acid is the most abundant acid in headspace of the present Tulum cheeses. It is an important flavor compound in sheep cheeses and blue-type cheeses as reported earlier (Ozturkoglu-Budak et al., 2016; Molimard and Spinnler, 1996; Valdivielso et al., 2016). On the other hand, acetic acid that formed from either lactose or citrate metabolism, or from amino acids and responsible for 'pungent' flavor note (Curioni

Table 2. Carboxylic acid, alcohol, ester, ketone, terpene, hydrocarbone, aldehyde and miscellenous compounds identified in milk, curd and ripened Tulum cheeses (Areas %)

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
Acids									
				GS	4.14±0.44$^{c.D}$	2.37±0.24$^{b.B}$	0.61±0.05$^{a.A}$	***	***
Etanoic acid[1.2]	1494	5.52±0.56	2.27±0.24^{B}	PB	2.43±0.27$^{b.B}$	3.08±0.20$^{c.C}$	0.55±0.03$^{a.A}$	***	
				^{1}P	***	***	**		
				GS	0.18±0.02$^{b.D}$	0.10±0.02$^{a.B}$	0.09±0.01$^{a.B}$	***	
2-Methyl propanoic	1610	-	0.12±0.02^{C}	PB	0.25±0.03$^{c.E}$	0.07±0.02$^{a.A}$	0.14±0.02$^{b.C}$	***	***
				P	***	*	***		
				GS	7.40±0.47$^{b.E}$	8.49±0.29$^{c.F}$	2.76±0.32$^{a.B}$	***	***
Butanoic acid[1.2]	1676	0.56±0.05	2.98±0.10^{A}	PB	3.78±0.26$^{b.C}$	5.10±0.31$^{c.D}$	2.85±0.32$^{a.B}$	***	
				P	***	***	NS		
				GS	0.53±0.07$^{c.D}$	0.36±0.05$^{b.C}$	0.28±0.03$^{a.AB}$	***	
3-Methyl butanoic acid[2]	1716	-	0.30±0.02^{B}	PB	0.53±0.04$^{b.D}$	0.24±0.02$^{a.A}$	0.51±0.04$^{b.D}$	***	***
				P	***	*	***		
				GS	7.51±0.41$^{b.D}$	8.58±0.54c.E	5.77±0.44$^{a.B}$	***	***
Hexanoic acid[1.2]	1898	1.05±0.10	4.17±0.47^{A}	PB	4.02±0.52$^{a.A}$	5.60±0.44b.B	6.80±0.25$^{c.C}$	***	
				P	***	***	***		
				GS	0.13±0.02$^{b.C}$	0.18±0.02$^{c.D}$	0.06±0.01$^{a.A}$	***	
Heptanoic acid[1.2]	2008	-	0.24±0.02^{E}	PB	0.15±0.02$^{c.C}$	0.09±0.02$^{b.B}$	0.04±0.01$^{a.A}$	***	***
				P	NS	***	NS		
				GS	5.03±0.38$^{b.D}$	7.01±0.41$^{c.E}$	2.71±0.30$^{a.A}$	***	***
Octanoic acid[1.2.3]	2080	0.76±0.07	3.72±0.41^{B}	PB	3.86±0.39$^{ab.BC}$	4.18±0.45$^{b.C}$	3.79±0.26$^{a.B}$	**	
				P	***	***	***		
				GS	4.14±0.44$^{b.D}$	6.85±0.48$^{c.E}$	0.62±0.06$^{a.A}$	***	***
Decanoic acid[1.2]	2283	0.56±0.04	2.79±0.32^{C}	PB	2.64±0.34$^{b.C}$	4.07±0.37$^{c.D}$	1.07±0.10$^{a.B}$	***	
				P	***	***	***		
				GS	0.67±0.06$^{a.C}$	0.91±0.06$^{b.D}$	-	***	***
Dodecanoic acid[1.2]	>22833		0.68±0.07^{C}	PB	0.55±0.0 $^{b.B}$	0.58±0.05$^{b.B}$	0.09±0.01$^{a.A}$	***	
		-		*P*	***	***			
			3.31±0.33^{E}	GS	1.60±0.17$^{a.C}$	1.99±0.28$^{b.D}$	-	***	***
Benzoic acid[1.2.3]	>2283	-		PB	0.72±0.06$^{a.A}$	0.91±0.06$^{b.B}$	-	***	
				P	***	***			
Alcohols									
	933	-	12.12±0.48^{D}	GS	17.25±0.37$^{c.E}$	11.52±0.32$^{b.C}$	3.07±0.36$^{a.B}$	***	***
Ethanol[1.2]				PB	26.96±0.37$^{c.G}$	18.29±0.64$^{b.F}$	2.66±0.34$^{a.A}$	***	
				^{1}P	***	***	*		

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
2-Butanol[2]	1043	-		GS	1.62±0.15$^{a.A}$	3.08±0.26$^{b.C}$	4.35±0.39$^{c.E}$	***	***
			-	PB	2.63±0.15$^{a.B}$	3.75±0.29b.D	8.89±0.34c.F	***	
				P	***	***	***		
1-Propanol[2]	1052	-	-	GS	0.31±0.06$^{a.A}$	1.53±0.13$^{b.C}$	1.61±0.18$^{b.C}$	***	***
				PB	0.30±0.04$^{a.A}$	1.58±0.10$^{c.C}$	1.28±0.13$^{b.B}$	***	
				P	NS	NS	***		
2-Methyl-1-propanol[2]	1127	-		GS	0.20±0.02$^{b.B}$	0.13±0.02$^{a.A}$	0.37±0.04$^{c.D}$	***	NS
			0.28±0.02^{C}	PB	0.18±0.02$^{b.B}$	0.12±0.02$^{a.A}$	0.35±0.04$^{c.D}$	***	
				P	NS	NS	NS		
2-Propenol[2]	1138	-		GS	0.24±0.02$^{a.A}$	0.31±0.05$^{b.B}$	-	***	***
			-	PB	0.50±0.04$^{b.D}$	0.39±0.04$^{a.C}$	-	***	
				P	***	**			
2-Pentanol[2]	1143	-		GS	0.28±0.04$^{a.A}$	0.86±0.06$^{b.B}$	2.20±0.23$^{c.D}$	***	***
			-	PB			0.99±0.06^{c}	***	
				P	***	***	***		
Methyl-1-butanol[2]	1231	0.57±0.06		GS	0.79±0.07$^{b.B}$	0.41±0.03$^{a.A}$	3.67±0.36$^{c.E}$	***	***
			1.93±0.34^{C}	PB	0.87±0.07$^{b.B}$	0.65±0.04$^{a.B}$	2.58±0.28$^{c.D}$	***	
				P	*	***	***		
1-Pentanol[2,3]	1275	6.55±0.57		GS	0.25±0.03$^{b.B}$	0.11±0.02$^{a.A}$	0.15±0.10$^{c.E}$	***	***
			0.35±0.04^{D}	PB	0.22±0.02$^{b.B}$	0.08±0.01$^{a.A}$	0.31±0.03$^{c.C}$	***	
				P	*	***	***		
2-Heptanol[2]	1347	0.21±0.02		GS	0.16±0.02$^{a.B}$	0.33±0.05$^{b.C}$	0.94±0.08$^{c.D}$	***	***
			-	PB	0.12±0.02$^{b.B}$	0.05±0.01$^{a.A}$	0.99±0.09$^{c.E}$	***	
				P	***	***	NS		
2-Nonanol[2]	1552	-		GS	0.19±0.03$^{b.C}$	0.12±0.02$^{a.B}$	0.19±0.02$^{b.C}$	***	***
				PB	0.18±0.02$^{b.C}$	0.07±0.02$^{a.A}$	0.40±0.04$^{c.D}$	***	
				P	NS	***	***		
1.3-Butanediol	1581	3.26±0.34		GS	0.99±0.05$^{b.D}$	0.36±0.04$^{a.A}$	0.39±0.04$^{a.A}$	***	***
			0.55±0.07^{B}	PB	1.51±0.10$^{c.F}$	0.62±0.06$^{a.C}$	1.05±0.08$^{b.E}$	***	
				P	***	***	***		
				GS	0.31±0.02$^{b.E}$	0.19±0.02a.BC	0.20±0.02$^{a.CD}$	***	***
1-Nonanol[2]	1699	-	0.34±0.02^{F}	PB	0.22±0.02$^{c.D}$	0.17±0.02$^{b.B}$	0.10±0.02$^{a.A}$	***	
				[1]***P***	***	NS	***		

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
				GS	1.15±0.09^{c,F}	0.66±0.05^{b,B}	0.33±0.04^{a,A}	***	***
Methionol[2]			0.84±0.09^{C}	PB	0.97±0.05^{a,D}	1.31±0.07^{c,G}	1.08±0.07^{b,E}	***	
				P	***	***	***		
				GS	0.75±0.05^{b,E}	0.73±0.07^{b,E}	0.16±0.02^{a,A}	***	***
Phenyl ethyl alcohol[2]			1.54±0.06^{F}	PB	0.54±0.04^{c,D}	0.38±0.04^{b,C}	0.21±0.03^{a,B}	***	
				P	***	***	**		
Esters									
				GS	6.71±0.49^{c,C}	2.98±0.29^{a,A}	6.10±0.31^{b,B}	***	***
Ethyl acetate[1,2]	889	-	3.16±0.18^{A}	PB	9.05±0.33^{b,D}	8.97±0.55^{b,D}	5.76±0.36^{a,B}	***	
				[1]***P***	***	***	*		
				GS	-	-	1.87±0.18^{B}	***	***
1-Methyl propyl acetate[2]	1008	-	1.97±0.27^{B}	PB	-	-	1.05±0.12^{A}	***	
				P			***		
				GS	1.20±0.09^{a,B}	1.16±0.08^{a,B}	3.66±0.18^{b,D}	***	***
Ethyl butyrate[2]	1056	-	0.41±0.03^{A}	PB	1.76±0.25^{a,C}	3.83±0.32^{b,D}	5.05±0.37^{c,E}	***	
				P	***	***	***		
				GS	-	-	0.15±0.02^{A}	***	***
2-Methyl propyl butanoate[2]	1151	-	-	PB	-	-	2.46±0.26^{b}	***	
				P			***		
				GS	1.63±0.08^{a,B}	2.32±0.25^{b,D}	5.15±0.41^{c,E}	***	**
Ethyl hexanoate[2]	1265	-	0.27±0.04^{A}	PB	1.41±0.13^{a,B}	1.87±0.18^{b,C}	5.35±0.37^{c,E}	***	
				P	***	***	NS		
				GS	-	-	0.16±0.02^{A}	***	***
Butyric acid pentanoate	1293	-	-	PB	-	-	0.25±0.03^{B}	***	
				P			***		
				GS	0.07±0.01^{a,A}	-	0.20±0.03^{b,B}	***	***
2-Methyl propyl hexanoate[2]	1352	-	-	PB	0.06±0.02^{a,A}	0.05±0.01^{a,A}	2.68±0.18^{b,C}	***	
				P	NS	***	***		
				GS	0.30±0.02^{b,C}	0.17±0.04^{a,A}	0.28±0.02^{b,C}	***	***
Ethyl lactate[2]	1382	-	0.18±0.03^{AB}	PB	0.18±0.02^{a,AB}	0.20±0.04^{a,B}	0.17±0.02^{a,A}	***	
				[1]***P***	***	*	***		
				GS	-	-	0.14±0.02^{B}	***	***
Heptyl acetate	1409	-	-	PB	-	-	0.08±0.01^{A}	***	
				P			***		

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
				GS	1.12±0.04^{a,C}	1.44±0.07^{b,E}	1.10±0.09^{a,C}	***	***
Ethyl octanoate[2]	1473	-	0.24±0.03^{A}	PB	0.87±0.02^{a,B}	1.26±0.09^{b,D}	3.22±0.17^{c,F}	***	
				P	***	***	***		
				GS	0.15±0.02^{b,B}	0.13±0.02^{a,B}	-	***	***
Isobutyl octanoate[2]	1560	-	0.14±0.02^{B}	PB	0.09±0.02^{a,A}	0.09±0.02^{a,A}	1.07±0.10^{b,C}	***	
				P	***	***			
				GS	1.88±0.24^{b,E}	2.61±0.21^{c,G}	0.87±0.06^{a,B}	***	***
Ethyl decanoate[2]	1683	-	0.28±0.04^{A}	PB	1.57±0.28^{b,D}	2.25±0.09^{c,F}	1.04±0.16^{a,C}	***	
				P	*	***	**		
				GS	0.28±0.02^{b,CD}	0.30±0.03^{b,D}	0.11±0.02^{a,A}	***	NS
Ethyl benzoate[2]	1736	-	0.44±0.05^{E}	PB	0.22±0.03^{b,B}	0.26±0.03^{a,C}	0.10±0.02^{c,A}	***	
				P	**	*	NS		
				GS	1.68±0.08^{c,E}	1.22±0.03^{b,D}	0.16±0.02^{a,A}	***	***
Phenyl ethyl acetate[2]	1892	-	0.95±0.10^{C}	PB	2.65±0.31^{c,G}	1.90±0.15^{b,F}	0.45±0.03^{a,B}	***	
				P	***	***	***		
				GS	0.09±0.02^{a,A}	0.12±0.02^{b,B}	0.07±0.01^{a,A}	***	***
Butyl butyrate[2]	1950	-	-	PB	0.07±0.02^{a,A}	0.08±0.02^{a,A}	0.18±0.02^{b,C}	***	
				[1]*P*	NS	***	***		
				GS	0.28±0.03^{a,A}	0.55±0.04^{b,D}	-	***	***
Ethyl tetradecanoate[2]	2071	-	0.36±0.04^{C}	PB	0.33±0.02^{a,B}	0.35±0.05^{a,BC}	-	***	
				P	**	***			
				GS	-	-	-		
Ammonium benzoate		-	2.21±0.28	PB	-	-	-		
Ketones									
				GS	-	1.99±0.22^{a,B}	5.89±0.39^{b,E}	***	***
2-Praponone[1,2]	850	31.47±0.63	3.22±0.27^{C}	PB	-	0.39±0.04^{a,A}	3.81±0.29^{b,D}	***	
				P		***	***		
				GS	5.75±0.41^{c,C}	2.68±0.31^{a,A}	4.90±0.37^{b,B}	***	***
2-Butanone[1,2,3]	913	-	7.79±0.24^{D}	PB	8.02±0.37^{a,DE}	10.79±0.59^{b,F}	8.21±0.49^{a,E}	***	
				P	***	***	***		
				GS	0.78±0.04^{a,A}	3.51±0.25^{b,D}	5.65±0.63^{c,E}	***	***
2-Pentanone[2,3]	1000	6.18±0.57	1.29±0.19^{B}	PB	0.69±0.03^{b,A}	0.55±0.05^{a,A}	2.47±0.21^{c,C}	***	
				P	***	***	***		
				GS	0.39±0.04^{a,B}	2.23±0.25^{b,C}	12.89±0.64^{c,E}	***	***
2-Heptanone[2,3]	1215	-	-	PB	0.38±0.04^{b,B}	0.08±0.01^{a,A}	5.57±0.29^{c,D}	***	
				P	NS	***	***		

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
2-Octanone[2]	1322	-	-	GS	-	0.09±0.01^{a,B}	0.18±0.01^{b,C}	***	***
				PB	-	0.04±0.01^{a,A}	0.18±0.01^{b,C}	***	
				P		***	NS		
Acetoine[1,2]	1328	-	6.57±0.68^{C}	GS	0.59±0.06^{c,B}	0.18±0.02^{a,A}	0.54±0.05^{b,B}	***	***
				PB	0.61±0.04^{c,B}	0.22±0.02^{b,A}	0.16±0.02^{a,A}	***	
				P	NS	***	***		
2-Nonanone[2,3]	1431	-	0.65±0.08^{C}	GS	0.41±0.03^{a,BC}	2.31±0.36^{b,D}	5.39±0.51^{c,F}	***	***
				PB	0.34±0.04^{b,AB}	0.11±0.02^{a,A}	3.58±0.30^{c,E}	***	
				P	***	***	***		
8-Nonene-2-one	1488	1.75±0.09	0.29±0.04^{E}	GS	0.17±0.02^{a,C}	0.24±0.03^{b,D}	0.46±0.06^{c,F}	***	***
				PB	0.13±0.02^{b,B}	0.09±0.01^{a,A}	0.23±0.03^{c,D}	***	
				[1]*P*	**	***	***		
p-Mentha-1-ene-3-one, semicarbozone	1526	0.59±0.07	0.77±0.04^{D}	GS	0.26±0.02^{c,C}	0.19±0.02^{b,B}	0.16±0.02^{a,A}	***	***
				PB	0.18±0.02b.^{AB}	0.18±0.02^{b,AB}	0.15±0.02^{a,A}	***	
				P	***	NS	NS		
3.5-Octadiene-2-one[2]	1574	-	0.09±0.01^{B}	GS	0.16±0.02^{b,D}	0.05±0.01^{a,A}	-	***	*
				PB	0.14±0.02^{b,C}	0.04±0.01^{a,A}	-	***	
				P	*	NS			
2-Undecanone[1,2]	1647	0.93±0.10	0.41±0.06^{C}	GS	0.48±0.05^{b,D}	0.40±0.15^{b,BC}	0.20±0.03^{a,A}	***	***
				PB	0.24±0.03^{b,A}	0.34±0.04^{c,B}	0.17±0.03^{a,A}	***	
					***	NS	*		
Terpenes									
alpha-Pinene[1,2,3]	1065	3.03±0.48	-	GS	-	-	-		
				PB	-	-	-		
				[1]*P*					
delta-Carene[2]	1184	5.87±0.73	-	GS	-	-	0.08±0.01^{B}	***	***
				PB	-	-	0.06±0.01^{A}	***	
				[1]*P*			***		
m-Cimene	1309	0.78±0.08	1.08±0.08^{E}	GS	0.21±0.03^{b,B}	0.10±0.03^{a,A}	0.55±0.05^{c,D}	***	***
				PB	0.11±0.02^{a,A}	0.12±0.02^{a,A}	0.35±0.02^{b,C}	***	
				P	***	NS	***		
alpha-Cubebene	1507	-	0.60±0.08^{E}	GS	0.36±0.02^{c,D}	0.33±0.04^{b,D}	0.21±0.02^{a,BC}	***	NS
				PB	0.23±0.02^{c,C}	0.19±0.02^{b,B}	0.09±0.01^{a,A}	***	
				P	***	***	***		
Pristane	1540	-	0.12±0.02^{C}	GS	0.10±0.02^{b,B}	0.08±0.02^{a,A}	0.26±0.02^{c,E}	***	NS
				PB	0.08±0.02^{a,A}	0.07±0.02^{a,A}	0.23±0.02^{b,D}	***	

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
				P	*	NS	*		
alpha-Copaene[2]	1548	2.55±0.29	4.19±0.47^{D}	GS	0.83±0.07^{c,C}	0.52±0.04^{a,AB}	0.69±0.07^{b,BC}	***	**
				PB	0.62±0.03^{b,B}	0.41±0.04^{a,A}	0.58±0.06^{b,AB}	***	
				P	***	***	**		
Germakrene-D	1593	-	0.61±0.06^{C}	GS	0.26±0.03^{b,B}	0.19±0.02^{a,A}	0.21±0.02^{a,A}	***	*
				PB	0.20±0.04^{b,A}	0.18±0.02^{a,A}	0.18±0.02^{ab,A}	**	
				P	**	NS	*		
trans-Caryophylene	1668	0.73±0.07	1.04±0.07^{D}	GS	0.17±0.02^{a,A}	0.16±0.02^{a,A}	0.26±0.03^{b,C}	***	*
				PB	0.14±0.02^{a,A}	0.15±0.03^{a,A}	0.21±0.01^{b,B}	***	
				P	*	NS	***		
delta-Cadinene	1832	-	0.50±0.05^{E}	GS	0.25±0.04^{c,D}	0.21±0.02^{b,C}	0.04±0.01^{a,A}	***	*
				PB	0.20±0.02^{b,BC}	0.18±0.02^{b,B}	0.04±0.01^{a,A}	***	
				P	**	**	NS		
Hydrocarbons									
Toluene[2,3]	1071	6.02±0.61	2.36±0.27^{C}	GS	1.81±0.22^{a,B}	2.24±0.30^{b,C}	2.67±0.15^{c,D}	***	***
				PB	1.59±0.14^{a,A}	2.79±0.24^{b,D}	1.53±0.16^{a,A}	***	
				[1]*P*	*	***	***		
Undecane[2]	1133	1.52±0.09	-		-	-	-		
				GS	0.12±0.02^{a,A}	0.18±0.02^{b,B}	0.36±0.04^{c,C}	***	**
6-Aza-5,7,12,14-tetratyapentacene	1165	-	0.56±0.05^{D}	PB	0.13±0.02^{a,A}	0.12±0.02^{a,A}	0.36±0.03^{b,C}	***	
				P	NS	***	NS		
				GS			0.28±0.02^{B}	***	***
Dodecane[2]	1206	-	-	PB			0.07±0.01^{A}	***	
				P			***		
				GS	0.08±0.02^{a,D}	0.07±0.03^{b,CD}	0.02±0.01^{b,A}	***	***
Hexadecene	1469	-	0.30±0.04^{E}	PB	0.05±0.01^{a,BC}	0.06±0.01^{b,CD}	0.04±0.01^{a,B}	***	
				P	**	NS	***		
				GS	0.37±0.03^{b,C}	0.29±0.03^{a,B}	0.43±0.04^{c,D}	***	**
Pentadecene	1533	0.60±0.08	0.56±0.07^{E}	PB	0.28±0.03^{b,B}	0.24±0.03^{a,A}	0.32±0.03^{c,B}	***	
				P					
				GS	0.29±0.03^{c,D}	0.22±0.02^{b,C}	0.02±0.00^{a,A}	***	***
8-Heptadecene	1763	-	0.41±0.05^{E}	PB	0.22±0.03^{b,C}	0.21±0.03^{b,C}	0.09±0.01^{a,B}	***	
				P	***	NS	***		

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day)			R. effect	R*C
					90	180	270		
				GS	0.94±0.06$^{c.D}$	0.77±0.10$^{b.C}$	0.15±0.01$^{a.A}$	***	***
1-Heptadecene	1787	0.53±0.05	1.11±0.09^{E}	PB	0.70±0.04$^{b.B}$	0.71±0.06$^{b.BC}$	0.15±0.02$^{a.A}$	***	
				P	***	NS	NS		
				GS	0.20±0.02^{B}	-	-	***	***
3-Heptadecene-z	1801	-	-	PB	0.12±0.02^{A}	-	-	***	
				P	***				
				GS	0.71±0.04$^{c.F}$	0.34±0.02$^{b.C}$	0.14±0.02$^{a.A}$	***	***
Octadecene	1836	-	0.37±0.04CD	PB	0.40±0.03$^{b.D}$	0.57±0.06$^{c.E}$	0.17±0.01$^{a.B}$	***	
				P	***	***	***		
				GS	0.53±0.02$^{c.D}$	0.42±0.04$^{b.C}$	0.12±0.02$^{a.A}$	***	***
3.7.11.15 tetramethyl 2-hexadecene	1912	-	0.41±0.06^{C}	PB	0.35±0.04$^{b.B}$	0.36±0.05$^{b.B}$	0.13±0.02$^{a.A}$	***	
					***	*	NS		
Aldehydes									
Benzaldehyde2	1591	-	0.29±0.02^{F}	GS	0.38±0.01$^{c.G}$	0.28±0.03$^{a.C}$	0.22±0.04$^{b.D}$	***	***
				PB	0.26±0.02$^{c.E}$	0.15±0.02$^{b.B}$	0.10±0.01$^{a.A}$	***	
				P	***	*	***		
Pentanal[2.3]	1018	5.68±0.40	-		-	-	-		
Heptanal[2.3]	1220	1.31±0.06	-		-	-	-		
Miscellenous									
				GS	0.25±0.01$^{a.B}$	0.29±0.02$^{b.C}$	0.49±0.04$^{c.D}$	***	***
Dimethyl disulfide[2]	1117	-	0.55±0.06^{E}	PB	0.15±0.02$^{a.A}$	0.27±0.02$^{b.BC}$	0.26±0.03$^{b.B}$	***	
					***	*	***		
Dimethyl trisulfide[2]	1435	-	0.38±0.04^{C}	GS	0.16±0.02$^{b.B}$	0.08±0.02$^{a.A}$	0.37±0.03$^{c.C}$	***	***
				PB	0.09±0.01$^{a.A}$	0.10±0.04$^{a.A}$	0.18±0.02$^{b.B}$	***	
				P	***	NS	***		
Allyle methyl sulfide	1691	-	0.13±0.02^{A}	GS	0.38±0.05b.C	0.16±0.02$^{a.B}$		***	***
				PB	0.54±0.05c.E	0.36±0.03$^{a.C}$	0.44±0.03$^{b.D}$	***	
				P	**	***	***		
					-	-	-		
Dimethyl sulphone2.3	2001	0.55±0.07	-		-	-	-		
				GS	2.29±0.24$^{c.D}$	0.74±0.06$^{a.A}$	1.89±0.19$^{b.C}$	***	***
Oxime methoxy phenyl	1774	0.76±0.06	2.19±0.20^{D}	PB	1.52±0.14$^{b.B}$	0.72±0.07$^{a.A}$	1.68±0.26$^{b.B}$	***	
				P	***	NS	NS		
				GS	0.22±0.03$^{b.B}$	0.30±0.04$^{c.C}$	0.04±0.01$^{a.A}$	***	***
Phenol2	2057	1.18±0.07	0.24±0.04^{B}	PB	0.28±0.03$^{c.C}$	0.21±0.03$^{b.B}$	0.05±0.02$^{a.A}$	***	
				P	***	***	NS		

Table 2. Continued

Compounds	RI	Milk	Curd	Cheese	Ripening time (day) 90	180	270	R. effect	R*C
				GS	0.83±0.05^{b,F}	0.97±0.05^{c,G}	0.19±0.02^{a,A}	***	***
p-Cresol	2114	1.01±0.08	0.39±0.06^{C}	PB	0.71±0.07^{c,E}	0.66±0.04^{b,D}	0.31±0.04^{a,B}	***	
				P	***	***	***		
				GS	1.31±0.10^{b,E}	1.75±0.21^{c,F}	0.41±0.05^{a,A}	***	***
m-Cresol[2]	2120	1.64±0.12	0.93±0.10^{C}	PB	1.16±0.07^{b,D}	1.21±0.05^{b,DE}	0.52±0.02^{a,B}	***	
				P	**	***	***		
				GS	0.53±0.05^{c,E}	0.37±0.04^{b,C}	0.06±0.01^{a,A}	***	***
Tymol	2201	-	0.42±0.06^{D}	PB	0.40±0.04^{c,CD}	0.25±0.03^{b,B}	0.05±0.01^{a,A}	***	
				P	***	***	**		
				GS	-	0.06±0.01^{a,A}	0.18±0.03^{b,C}	***	***
Indole	1334	-	0.05±0.01^{a,A}	PB	-	0.04±0.01^{a,A}	0.13±0.01^{b,B}	***	
				P		*	***		
				GS	0.27±0.02^{a,C}	0.34±0.03^{b,D}	-	***	***
16-Oxosalutaridine	1339	6.29±0.64	0.81±0.08^{E}	PB	0.18±0.03^{a,A}	0.23±0.02^{b,B}	-	***	
				P	***	***			
				GS	0.19±0.02^{b,B}	0.27±0.04^{c,C}	0.11±0.03^{a,A}	***	***
1.3-Dioxalane	1932	-	0.29±0.04^{C}	PB	0.19±0.02^{b,B}	0.20±0.03^{b,B}	0.11±0.03^{a,A}	***	
				P	NS	***	NS		
				GS	0.22±0.02^{b,B}	0.24±0.10^{b,B}	0.04±0.01^{a,A}	***	**
cis-5-Hydroxy-2-methyl 1,3 dioxane	2050	-	0.30±0.03^{C}	PB	0.32±0.0^{4c,C}	0.23±0.05^{b,B}	0.04±0.01^{a,A}	***	
				P	***	NS	NS		
				GS	0.56±0.05^{b,D}	0.54±0.05^{b,D}	0.06±0.01^{a,A}	***	***
delta-Decalactone[2]	2255	-	0.43±0.04^{C}	PB	0.52±0.05^{c,D}	0.38±0.04^{b,B}	0.05±0.01^{a,A}	***	
				P	NS	***	NS		

RI, retention index based on retention time (RT) of each identified compound, is calculated from a linear equation between each pair of straight alkanes (C5-C25). GS and PB indicate Tulum cheese ripened in a goat's skin bag and in a plastic barrel, respectively. R. effect: Effect of ripening period. R*C; interaction between ripening time and packaging material. [1]*P*; Differences between cheeses at each ripening day. *P<0.05; **P< 0.01; ***P<0.001 significant levels. -: not detected. NS: not significant. Different lower case superscript letters at the same row indicate the effects of ripening period. Different upper lower case superscript letters indicate the differences between all cheeses during ripening starting from the curd, regardless of ripening material. [1]Compounds verified with authentic standards.[2, 3]Compounds were previously determined in Tulum cheeses and raw sheep milk, respectively

and Bosset, 2002) showed the irregular changes during manufacturing and ripening. Although acetic acid was the principal acid found in raw milk, it markedly decreased in curd (0 day). This could be attributed to the removal together with whey during the pressing since acetic acid is a water-soluble acid. Acetic acid, which reached the highest levels in PB and GS cheeses on days 90 and 180, respectively, decreased significantly (P<0.001) towards the end of ripening. In this case, the decreases in acetic acid were consistent with the remarkable increases in acetate esters (Table 2).

Branched-chain carboxylic acids (2-methyl propanoic acid and 3-methyl butanoic acid) were routinely detected during ripening whereas found in the less abundance than the other acids. These two carboxylic acids are synthesized from amino acids valine and leucine (Yvon

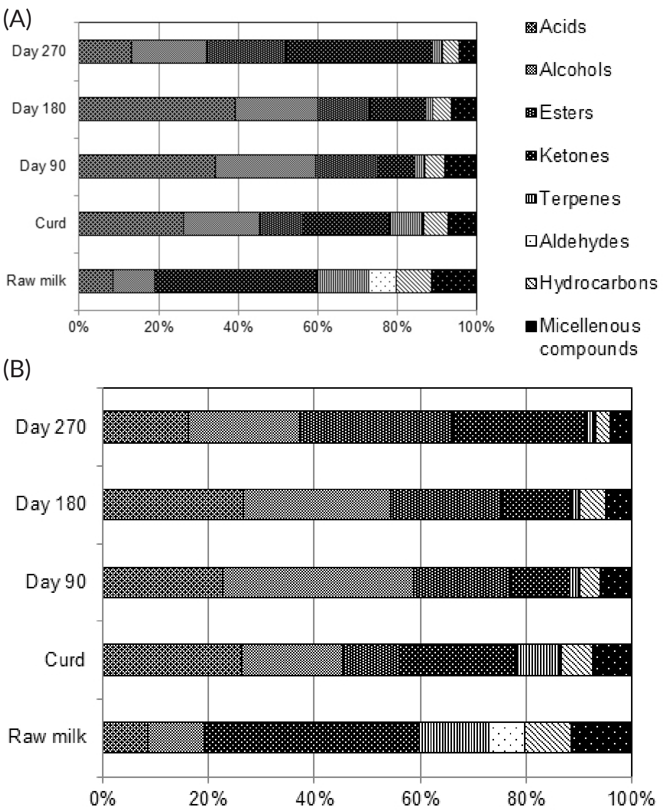

Figure 1. Variation of the main chemical groups of volatile compounds isolated from raw sheep milk, curd, Tulum cheeses ripened in a goats' skin bag (a) and in a plastic barrel (b) for 90, 180 and 270 days

and Rijnen, 2001) and have a characteristic effect on flavor of sheep cheeses (Curioni and Bosset, 2002). The higher level of branched chain acids was found to be in PB cheese compared to GS one. It is probable that precursor amino acids of them are high in PB cheese. This was confirmed by the findings of Tekin and Guler (2019).

Benzoic acid, a simple aromatic carboxylic acid, was identified in the cheeses only, as reported earlier (Cakir et al., 2016). When compared with the ripened cheeses, curd (0 day) had benzoic acid at the highest level. This could be attributed to the oxidation of toluene, a degradation product of β-carotene present in milk fat (Molimard and Spinnler, 1996), or the breakdown of phenylalanine (Urbach, 1997) during cheese making since raw milk had toluene at the high level but not benzoic acid. During ripening period, benzoic acid was the more abundance in GS cheese than PB one. This is probably related with the accelerating conversion of toluene to benzoic acid due to the oxygen permeability of goat's skin bag.

Alcohols

Three alcohols with the domination of 1-pentanol were observed in the volatile fraction of raw sheep's milk (Table 2). O'Callaghan et al. (2016) reported that ewes fed pastures with great botanic diversity were presented higher amount of 1-pentanol in milk. Regardless of ripening period, alcohol chemical group was the first and second most abundant group in the volatile fraction of PB and GS cheeses, respectively. Alcohols are primarily formed by the reduction of the corresponding aldehydes and ketones at the low redox potential of cheese. Both ripening period and ripening container influenced significantly (P<0.001) the levels of most alcohols. An increase in alcohol chemical group was observed until day 90 of ripening, turned into a decrease at day 180. A similar tendency was found for Manchego (Fernandez-Garcia et al., 2002) cheese when manufactured with raw sheep's milk. Alcohols have been found to be the dominant volatile group until day 180 in PB cheese. The high moisture and low salt-in-moisture in PB cheese medium compared to GS could be encouraged alcohol production by nonstarter lactic acid bacteria, enterococci and yeast commonly found in raw milk cheeses, as reported by Avila et al. (2017). Among the 15 species of alcohols identified, ethanol, 2-butanol and 3-methyl-1-butanol, final reduction products of acetaldehyde, 2-butanone and 3-methyl-1-butanal, respectively, were the most abundant alcohols during cheese ripening. Ethanol that formed during lactose fermentation and amino acid catabolism showed an increase at day 90 and turned into a decrease at day 180. However, ethanol was the principal VC at the first 180 days, accounted for approximately 14% and 23% of the total VCs identified in the headspaces of GS and PB cheeses, respectively. The tendency to decrease in ethanol was in line with increases in ethyl esters since ethanol is a precursor of ethyl esters. On the other hand, secondary alcohol 2-butanol, stems from mainly citrate metabolism, and branched-chain alcohol 3-methyl-1-butanol, comes from leucine amino acid catabolism, significantly (P<0.001) increased towards the end of ripening (Table 2). At this time, 2-butanol found to be at the highest abundance was the predominant VC and alcohol for PB and GS cheeses, respectively. The odd numbered secondary alcohols such as 2-pentanol and 2-heptanol are obtained from the reduction of their corresponding methyl ketones. These alcohols showed tendency to increasing towards the end of ripening.

Ethanol and 2-butanol could not be the most important for aroma due to their high detection thresholds but 3-methyl-1-butanol, 2-pentanol and 2-heptanol, with lower detection threshold, could play an important role in aroma of Tulum cheese as in raw sheep milk cheeses such as Gorgonzola and Grana Padano (Curioni and Bosset, 2002).

Esters

Esters, responsible for fruity flavor in cheese, are formed from either the enzymatic esterification reaction between short-chain free fatty acids and alcohols or produced by alcoholysis from alcohols and acylglycerols (Liu et al., 2003). The ester biosynthesis by lactic acid bacteria and micrococcaceae is of great importance in cheeses (Curioni and Bosset, 2002). We detected 17 esters, all of which were not found at all stages of ripening (Table 2). Ammonium benzoate as the first time was found only in curd. Esters such as 1-methyl propyl acetate, 2-methyl propyl butanoate, butyric acid pentanoate and heptyl acetate appeared to be at the end of ripening only. At this time, esters were the first and second most abundant chemical group, accounting for 29% and 20% of total volatiles identified in PB and GS cheeses, respectively (Figure 1A-B.). This could be related to the increase in micrococcaceae, mould and yeast count or activity, as reported by Ozturkoglu-Budak et al. (2016). Ethyl esters including ethyl acetate, ethyl butanoate, ethyl hexanoate and ethyl octanoate were the principal esters identified in the cheeses. Similar results were previously observed in Tulum cheese (Cakir et al., 2016; Ozturkoglu-Budak et al., 2016) and other sheep cheeses such as La Serena (Barron et al., 2007). Phenyl ethyl acetate being higher in PB cheese was found to be remarkable levels in the cheeses at days 90 and 180. This aromatic ester formed by the metabolism of aromatic amino acid by yeasts is reported as an important flavor compound in Camambert cheese (Molimard and Spinnler, 1996). Its presence could be related to the abundance of precursor molecule, 2-phenyl ethanol.

Ketones

Ketones are important in flavor of Manchego and Roncal, raw sheep milk cheeses (Centeno et al., 2004; Munoz et al., 2003), and Blue, in mold-ripened cheese (Hayaloglu et al., 2007a; Molimard and Spinnler, 1996). Significant ($P<0.05$) differences in ketone abundance were observed both between cheeses and ripening periods (Table 2). Among the ketones identified, 2-propanone (acetone), 2-butanone, 2-pentanone, 2-heptanone and 2-nonanone were the predominant 2-alkanones (Table 2). The present study shows that 2-propanone is the major compound in the volatile fraction of milk. It decreased in curd (0 day) and disappeared in the cheeses at day 90, thereafter significantly ($P<0.001$) increased. Acetoin and 2-butanone, the predominant volatiles in the curd, were frequently detected in the cheeses during ripening, but after day 0 (curd) acetoin, responsible for the aroma of fresh cheese, was found minimal. According to Urbach (1997), 2-propanone is a normal compound found in milk and cheese, whereas 2-butanone and acetoin are produced during the lactose and citrate metabolism by the microorganisms, in particular nonstarter lactic acid bacteria, coming from raw milk and the cheese processing environment.

Methyl ketones such as 2-pentanone, 2-heptanone, 2-nonanone and 2-undecanone are the secondary lipolytic metabolites since fatty acids can be oxidized to β-ketoacids, subsequent decarboxylated to corresponding methyl ketones, with one carbon less (Urbach, 1997). In the present study, methyl ketones that increased significantly ($P<0.0001$) during ripening were found to be the more abundance in GS cheese with the domination of 2-heptanone than PB one. This could be attributed to the oxygen permeability of goat's skin bag or the mold growth since the biosynthesis of methyl ketones is mainly due to mold metabolism. According to trained panelists used to assess Tulum cheeses, a musty flavor was perceived for GS cheese at the end of ripening

Terpenes

Terpenes can naturally present in milk. Their abundance and diversity are related to animal feed or pasture (Kilcawley et al., 2018). With the domination of δ-carene (monoterpene), α-pinene (monoterpene) and α-copeane (sesqueterpene), terpenes were the second most abundant group (approximately 13% of total volatiles) in the volatile fraction of milk (Table 2). This could be attributed to the natural feed of the animals in the Karaman district. As far as we know, *m*-cimene, *alpha*-cubebene, *trans*-caryophylene and δ-cadinene were identified for the first time in sheep's milk. These terpenes may be 'fingerprint' of Karaman grassland on which sheeps graze, as suggested by Cornu et al. (2005). A larger diversity but a lower concentration of terpenes was observed during the cheese production and ripening. This could be attributed to the ability of lactic acid bacteria, yeasts and fungi to modify and biosynthesize terpenes, as reported earlier (Degenhardt et al., 2009). It is thought that terpenes may be metabolized generating other hydrocarbon derivatives.

In the cheeses, α-copeane, *m*-cimene and *trans*-caryophylene were more abundant than the other terpenes. The type of ripening container (goat's skin or plastic barrel) had a significant ($P<0.001$) effect on terpene abundance but not on the diversity. Terpenes, lipophilic volatile compounds (Borge et al., 2016), were slightly higher in GS cheese with the high fat content.

Hydrocarbons

Hydrocarbons are breakdown products of fatty acids and chlorophyll from plants that animals ingest. Most hydrocarbons in cheese do not contribute to aroma due to their high perception threshold values (Delgado et al., 2010). In total, we identified 11 hydrocarbon compounds in raw milk and the cheeses (Table 2). The level of hydrocarbons was higher in GS cheese than that in PB cheese. This could be due to the high fat content of GS cheese. As reported by Hayaloglu et al. (2007b), among hydrocarbons toluene was the most abundant in particular milk and frequently found in the cheeses. O'Callaghan et al. (2016) have reported that toluene, a product of β-carotene degradation, is more likely to be present in pasture-derived milk and products. Pentadecene, hexadecane, octadecene and in particular isomers of heptadecene were found in the cheeses at trace levels. Similar hydrocarbons have been previously detected in Manchego cheese as minor volatile compounds (Barron, 2005).

Aldehydes

The content of aldehydes was the least among the volatile groups identified in both cheeses and milk (Figure 1A-B). Our results support thesis, that aldehydes are transitory compounds in cheese, which are reduced to their corresponding alcohols or oxidized to their corresponding carboxylic acids (Yvon and Rijnen, 2001). Accordingly to literature data, low level of aldehydes indicated an optimal cheese maturation (Delgado et al., 2010). We identified 2 aliphatic aldehydes (pentanal and heptanal) in milk and one aromatic aldehyde (benzaldehyde) in the cheeses only (Table 2). Benzaldehyde that has a bitter and almond flavor, is commonly found in the most cheeses (Molimard and Spinnler, 1996). In the present study, benzaldehyde showed a tendency decreasing during cheese ripening and its level was significantly higher in GS cheese than that in PB one. Yvon and Rijnen (2001) have reported that benzaldehyde is produced mainly through spontaneous chemical oxidation of tryptophan or phenylalanine derived-keto acids. This finding is accordance with the results of our previous study (Tekin and Guler, 2019), in where the contents of tryptophan and phenylalanine were significantly lower in Tulum cheese ripened in goats' skin than that in cheese ripened in plastic barrel.

Miscellaneous compounds

As shown in Table 2., we identified 14 miscellaneous compounds and their level was higher in GS cheese than PB one (Figure 1A-B). The content of miscellaneous compounds showed a tendency decreasing in the both Tulum cheeses during ripening. Miscellaneous compounds such as *delta*-decalactone, dimethyl disulfide, dimethyl trisulfide, allyl methyl sulfide, indole and 6-methyl-2-phenyl indole were found only in the ripened cheeses in a low level. Phenolic compounds such as phenol, *m*-cresol and *p*-cresol were found starting from raw milk. These phenolic compounds are considerably high in milk obtained from the animals fed pasture incorporating clover due to the metabolism of isoflavones and aromatic amino acids in the animal rumen (Kilcawley et al., 2018). The phenolic component cresol, which is formed by the breakdown of the tyrosine amino acid, has been detected at relatively high concentrations in some cheeses (Curioni and Bosset, 2002). Sulfur compounds except for dimethyl sulfanon were routinely detected in the cheeses at the trace levels. Due to low threshold values, sulfur compounds contribute to garlic and putrid aroma of mold-ripened cheeses (Molimard and Spinnler, 1996). Indoles, nitrogen-containing compounds, can be formed by destruction of tryptophan amino acid. The presence of δ-decalactone is the important in flavor of mold-ripened cheeses because of its strong fruity note and low perception threshold. In the present study, 16-oxasalutaridine, alkaloid, was found at high level (6%) in raw milk but seen to be disappear in the both Tulum cheeses at the end of ripening. As far as we know, 16-oxasalutaridine was found for the first time in raw sheep milk and Tulum cheeses.

Effect of ripening stages and different packaging materials on cheese volatile profile

Discriminant analysis was applied to whole data obtained from volatile compound analyses. This multivariate statistical technique allows clustering and grouping of observations with a similar properties group. The first 2 discriminant functions were explained 94.3% of total variation in the volatile profile of the analyzed cheeses.

Curd (0 day) and 90-day cheeses lied in the left side of score plot (Figure 2), whereas 90-day cheeses with the high alcohol content clearly differentiated from curd (0 day) with the high terpene and hydrocarbon. Cheeses at day 270 positioned at the right side of score plot but GS cheese with the high ketone content was completely separated from PB with the high ester. Cheeses at day 180, have the high carboxylic acid content, were near the axis origin and clearly separated from the other cheeses. According to discriminant analysis results, no clear effects of the ripening containers were possible between cheeses at day 90 or day 180, but a clear effect was observed for long-term ripening (over 180).

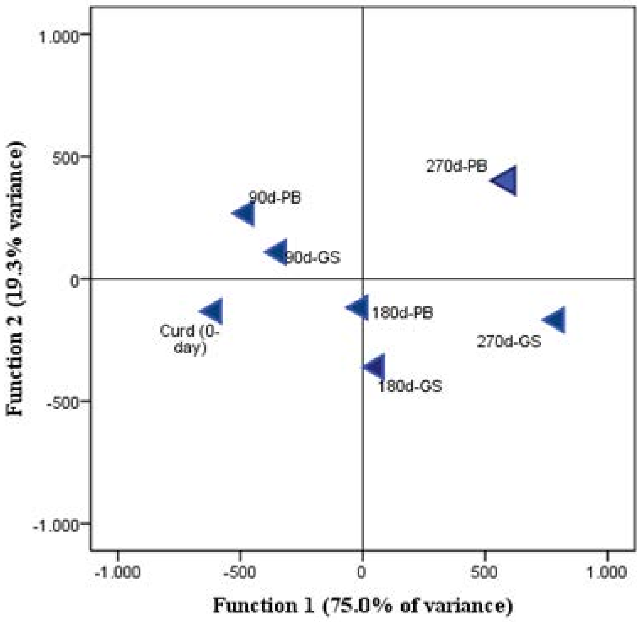

Figure 2. Discriminant analysis of the percentage compositions of volatile compounds (VCs) in curd (0d-cheese), 90d-, 180d- and 270d-old-goat skin bag Tulum cheeses (90d-GS, 180d- GS and 270d- GS, respectively), and in 90d- 180d- and 270-day plastic barrel container Tulum cheeses 90d-PB, 180d- PB and 270d- PB, respectively)

Color assessment

Color is an important criterion used to evaluate the quality of the cheese, which can actually change consumers' flavor perception. White index (*WI*) indicates the degree of whiteness. Chroma (*C*), quantitative attribute, represents color saturation degree and intensity. It is a function of changes in a^* and b^* values. Hue angle ($h°$) is to qualitatively assess color. As shown in Table 3, GS cheese at each time of ripening showed significantly (P<0.01) higher *chroma* and lower WI values than PB one. This is probably due to the evaporation of water from GS cheese and the high fat content.

Table 3. Color parameters of Tulum cheeses in goats' skin bag (GS) or in plastic barrel container(PB)

Color parameter	Curd	Cheese	Ripening Time (day)			R. effect	R*C
			90	180	270		
WI	79.78 ± 0.78^{C}	GS	$73.64\pm0.85^{a.A}$	$77.91\pm0.95^{b.C}$	$77.10\pm1.03^{b.C}$	**	**
		PB	$76.64\pm0.97^{a.B}$	82.60 ± 0.78^{b}D	$81.74\pm0.82^{b.D}$	***	
		^{1}P	*	**	**		
Chroma	18.61 ± 0.18^{B}	GS	$20.60\pm0.32^{b.C}$	19.64 ± 0.45^{a}B	$19.93\pm0.57^{a.B}$	*	**
		PB	$18.59\pm0.35^{b.B}$	$15.41\pm0.41^{a.A}$	$15.51\pm0.37^{a.A}$	**	
		P	**	**	**		
h°	96.38±0.05	GS	97.19±0.05	95.79±0.15	96.10±0.09	NS	NS
		PB	98.35±0.14	96.97±0.15	97.60±0.09	NS	
		P	NS	NS	NS		

WI: Whitness index; *h°*: hua angle GS and PB indicate Tulum cheese ripened in a goat's skin bag and in a in plastic barrel, respectively. R*C; interaction between ripening time and ripening container. ^{1}P; Differences between cheeses at each ripening day. *P <0.05; **P< 0.01; ***P < 0.001 significant levels.. NS: not significant. Different lower case superscript letters at the same row indicate the effects of ripening period. Different upper lower case superscript letters indicate the differences between all cheeses during ripening starting from the curd, regardless of ripening material

Hue angle values of the cheeses are corresponding to the second quadrant of the three-dimensional diagram of colors between 90° (yellow hue) and 180° (green hue). Ripening period and packaging material did not a significant effect on hue values, but GS cheese compared to PB was slightly more yellow. To our knowledge, none of the previous studies carried out on color of Tulum cheeses.

Sensory analysis

Sensory attributes and overall acceptability scores are shown in Figure 3A-B. As the 'animal like', 'fruity' and 'milky' flavor notes are not perceived by the panelists, they are not shown in Figure 3A. With respect to appearance attribute, the panelists evaluated cheese GS as more yellow than PB. This was in agreement with the whiteness index and *chroma* values. An unhomogeneous appearance was observed only in cheese GS at the end of ripening. As texture attributes, 'firmness' and 'crumbly' scores were significantly (P<0.01) greater in GS cheese than PB one. This result may be due to the high dry-matter content of GS. In terms of flavor attributes, 'salty' and 'musty' flavor notes were perceived only in GS cheese until day 180 and at day 270, respectively.

(A)

(B)

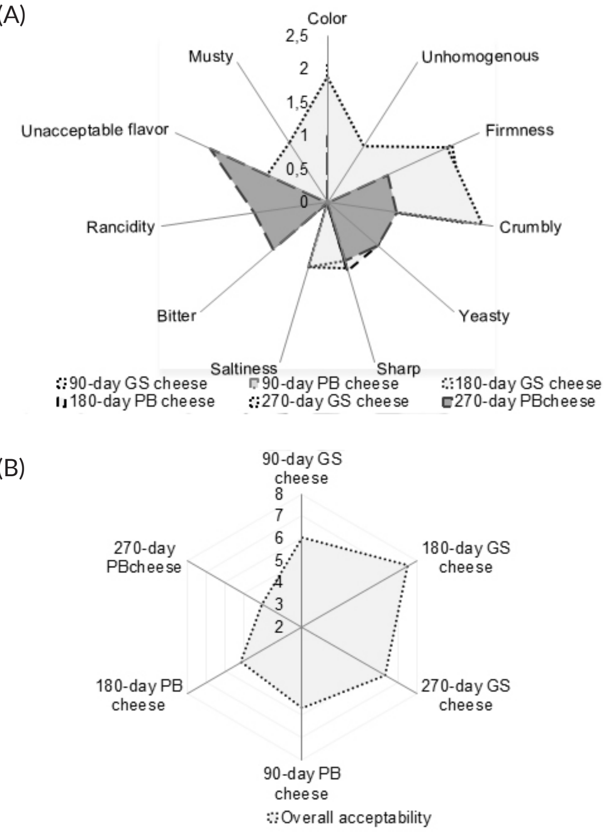

Figure 3. Sensory characteristics (a) and overall acceptability (b) scores of Tulum cheeses ripened in a goat skin bag (GS) and ripened in a plastic barrel container (PB) for 90, 180 and 270 days

'Bitter' and 'yeasty' flavor notes were not observed in GS cheese, but PB cheese had a 'yeasty' flavor note at all stages of ripening and 'bitter' flavor (1 point out of 3) at days 180 and 270. 'Unacceptable' flavor for PB cheese at day 270 reached a maximum score (1.9 point). In accordance with the pH values, both cheeses had a similar 'sharp' flavor score (1 point out of 3) during ripening. In general, GS cheese compared to PB was characterized by a yellow color, a firm and crumbly texture, an unhomogenous appearance, and a salty and musty flavor, the latter was described as having bitter, yeasty and unacceptable flavor.

Regarding overall acceptability, a significant (P<0.001) decrease in scores was observed for PB cheese from day 90 to 270 (Figure 3B). This may be due to the accumulation of esters. Overall acceptability score for GS cheese increased significantly (P<0.01) from day 90 to 180, after that decreased. The highest overall acceptability scores (7.54±0.51 out of 9 point and 5.67±0.48) for GS and PB cheeses were observed at days 180 and 90, respectively. During ripening period, GS compared to PB cheese was preferred by the panelists. Actually, the increases in ester and ketone abundances together with the decreases in fatty acid and alcohol abundances were negatively affected overall acceptability. Guler and Uraz (2003) reported that slight rancid flavor due to short-chain fatty acids is desirable for Tulum cheese flavor, if well balanced with other taste attributes.

Overall, GS cheese more preferred by panelists had relatively high levels of carboxylic acid and methyl ketones and low levels of esters and alcohols as well as high dry matter content and chroma value compared with PB cheese. In GS cheese, lipolysis is thought to be the main pathway for the formation of volatile fatty acids and secondary lipolytic catabolities such as 2-heptanone, 2-nonanone. On the contrary, in PB cheese the formation of volatile compounds is governed by lactose, lactate and citrate metabolism.

In the present study, different terpene (m-cimene, α-copeane and trans-caryophylene) and alkaloid (16-oxasalutaridine) compounds detected in sheep milk and its cheeses may be a 'botanical marker' for Karaman grassland in spring season.

The analysis of volatile compounds (VCs) during cheese ripening seemed to be a useful tool for discriminating of ripening stages. According to discriminating analysis, effect of different ripening containers on volatile compound profile was minor at the first 180 days but was pronounced after day 180.

It could be concluded that GS and PB cheeses at the end of ripening are in fact two different products from the volatile compound abundance and overall acceptability scores point of view. The maturation extended to 270 days for Tulum cheese appears not to be essential, in terms of the overall acceptability. Ripening Tulum cheese in goat's skin bag compared to plastic barrel may advise to cheese producer due to its high overall acceptability. However, plastic barrel may use for short-term cheese ripening as 90 days. This paper can be useful for comparative purposes in future studies on raw sheep milk cheeses.

ACKNOWLEDGEMENT

This work was supported by Mustafa Kemal University, Scientific Research Projects (BAP) Commission (Project No: 8641). The authors gratefully thank Professor Dr. Park W. Young, Fort Valley State University, Fort Valley-George, for his proof reading.

REFERENCES

Avila, M., Gomez-Torresa, N., Delgado, D., Gaya, P., Gardea, S. (2017) Effect of high-pressure treatments on proteolysis, volatile compounds, texture, colour, and sensory characteristics of semi-hard raw ewe milk cheese. Food Research International, 100, 595-602. DOI: http://dx.doi.org/10.1016/j.foodres.2017.07.043

Barron, L. J. R., Redondo, Y., Flanagan, C. E., Perez-Elortondo, F. J., Albisu, M., Najera, A.I., Renobales, M., Fernandez-Garcia, E. (2005) Comparison of the volatile composition and sensory characteristics of Spanish PDO cheeses manufactured from ewes' raw milk and animal rennet. International Dairy Journal, 15, 371–382. DOI: https://doi.org/10.1016/j.idairyj.2004.08.005

Barron, L. J. R., Redondo, Y., Aramburu, M., Gil, P., Pérez-Elortondo, F. J, Albisu M, Nájera AI, de Renobales, M., Fernández- García, E. (2007) Volatile composition and sensory properties of industrially produced Idiazabal cheese. International Dairy Journal, 17, 1401–1414. DOI: https://doi.org/10.1016/j.idairyj.2007.04.001

Bodyfelt, F.W., Tobias, J., Trout, G.M. (1988) The Sensory Evaluation of Dairy Products. 1st Edit. New York: Van Nostrand Reinhold, pp. 300-375.

Borge, G.I.A., Sandberg, E., Øyaas, J., Abrahamsen, R.K. (2016) Variation of terpenes in milk and cultured cream from Norwegian Alp ne rangeland-fed and in-door fed cows. Food Chemistry, 199, 195–202. DOI: https://doi.org/10.1016/j.foodchem.2015.11.098

Cakir, Y., Cakmakci, S., Hayaloglu, A.A. (2016) The effect of addition of black cumin (*Nigella sativa* L) and ripening period on proteolysis, sensory properties and volatile profiles of Erzincan Tulum (Savak) cheese made from raw Akkaraman sheep's milk. Small Ruminant Research, 134, 65–73. DOI: https://doi.org/10.1016/j.smallrumres.2015.12.004

Centeno, C.A., Fernandez-Garcia, E., Gaya, P., Tomillo, J., Medina, M., Nunez, M. (2004) Volatile compounds in cheeses made from raw ewes' milk ripened with a lactic culture. Journal Dairy Research, 71, 380–384. DOI: https://doi.org/10.1017/S0022029904000214

Cornu, A., Kondjoyan, N., Martin, B., Verdier-Metz, I., Pradel, P., Berdague, J.L., Coulon, J.B. (2005) Terpene profiles in Cantal and Saint-Nectaire type cheese made from raw or pasteurised milk. Journal of the Science of Food and Agriculture, 85, 2040-2046. DOI: https://doi.org/10.1002/jsfa.2214

Curioni, P.M.G., Bosset, J.O. (2002) Key odorants in various cheese types as determinedby gas chromatography-olfactometry. International Dairy Journal, 12, 959-984. DOI: https://doi.org/10.1016/S0958-6946(02)00124-3

Degenhardt, J., Köllner, T.G., Gershenzon, J. (2009) Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry, 70, 1621–1637. DOI: https://doi.org/10.1016/j.phytochem.2009.07.030

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, P., Ramírez, R. (2010) Characterisation by SPME–GC–MS of the volatile profile of a Spanish soft cheese PDO Torta del Casar during ripening. Food Chemistry, 118, 182–189. DOI: https://doi.org/10.1016/j.foodchem.2009.04.081

Fernandez-Garcia, E., Carbonell, M., Nunez, M. (2002) Volatile fraction and sensory characteristics of Manchego cheese1 Comparison of raw and pasteurized milk cheese. Journal Dairy Research, 69, 579-593. DOI: https://doi.org/10.1017/S0022029902005794

Food Codex (2015) Turkish Food Codex, Cheese Statement, Regulation No: 2015/6 Ankara: Republic of Turkey Ministry of Food, Agriculture and Livestock Home.

Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H. (2000) Fundamentals of Cheese Science. Gaithersburg, MD: Aspen Publishers, Inc. DOI: https://doi.org/10.1007/978-1-4899-7681-9

Guler, Z., Uraz, T. (2003) Proteolytic and lipolytic composition of Tulum cheese. Milchwissensaft, 58, 502-505.

Guler, Z. (2014) Profiles of organic acid and volatile compounds in acid-type cheeses containing herbs and spices (Surk Cheese). International Journal Food Properties, 17, 1379–1392. DOI: https://doi.org/10.1080/10942912.2012.697957

Hayaloglu, A.A., Fox, P.F., Guven, M., Cakmakci, S. (2007a) Cheeses of Turkey: 1 Varieties ripened in goat-skin bags. Lait, 87, 79–95. DOI: http://dx.doi.org/10.1051/lait:2007006

Hayaloglu, A.A., Cakmakci, S., Brechany, E.Y., Deegan, K.C., McSweeney. P.L.H. (2007b) Microbiology, biochemistry, and volatile composition of Tulum Cheese ripened in goat's skin or plastic bags. Journal Dairy Science, 90, 1102-1121. DOI: https://doi.org/10.3168/jds.S0022-0302(07)71597-7

International Dairy Federation (IDF). (1982). "Standard 4A: Cheese and processed cheese. Determination of the total solids content." International Dairy Federation, Brussels, Belgium.

International Dairy Federation (IDF). (1986). "Standard 5B: Cheese and processed cheese products Determination of fat content." Brussels: International Dairy Federation.

International Dairy Federation (IDF). (1988). "Standard 88A: Cheese and processed cheese Determination of chloride content." Brussels: International Dairy Federation.

International Dairy Federation (IDF). (1993). "Standard 20B: Milk determination of nitrogen content." Brussels: International Dairy Federation.

Kilcawley, K.N., Faulkner, H., Clarke, H.J., O'Sullivan, M.G., Kerry, J. (2018) Factors influencing the flavour of bovine milk and cheese from grass based versus non-grass based milk production systems. Foods, 7, 37-43. DOI: https://doi.org/10.3390/foods7030037

Liu, S.Q., Holland, R., Crow, V.L. (2003) Ester synthesis in an aqueous environment by Streptococcus thermophilus and other dairy lactic acid bacteria. Applied Microbiology and Biotechnology, 63, 81-88. DOI: https://doi.org/10.1007/s00253-003-1355-y

Molimard, P., Spinnler, H.E. (1996) Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. Journal Dairy Science, 79, 169–184. DOI: https://doi.org/10.3168/jds.S0022-0302(96)76348-8

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 Chemisty, 83, 329–338. DOI: https://doi.org/10.1016/S0308-8146(03)00133-X

O'Callaghan, T.F., Hennessy, D., McAuliffe, S., Kilcawley, K.N., O'Donovan, M., Dillon, P., Ross, R.P., Stanton, C. (2016) Effect of pasture versus indoor feeding systems on raw milk composition and quality over an entire lactation. Journal Dairy Science, 99, 9424–9440. DOI: https://doi.org/10.3168/jds.2016-10985

Ozturkoglu-Budak, S., Gursoy, A., Aykas, D.P., Kocak, C., Donmez, S., De-Vries, R.P., Bronll, P.A. (2016) Volatile compound profiling of Turkish Divle Cave cheese during production and ripening. Jounal Dairy Science, 99, 5120-5131. DOI: https://doi.org/10.3168/jds.2015-10828

Pallant, J. (2010) A step by step guide to data analysis using the SPSS program SPSS survival manual 4th ed., 494.

Tekin, A., Guler, Z. (2019) Glycolysis, lipolysis and proteolysis in raw sheep milk Tulum cheese during production and ripening: Effect of ripening materials. Food Chemistry, 286, 160-169. DOI: https://doi.org/10.1016/j.foodchem.2019.01.190

Urbach, G. (1997) The flavour of milk and dairy products II Cheese: contribution of volatile compounds. International Journal Dairy Technology, 50, 79–89. DOI: https://doi.org/10.1111/j.1471-0307.1997.tb01743.x

Valdivielso, I., Albisu, M., de Renobales, M., Barron, L.J.R. (2016) Changes in the volatile composition and sensory properties of cheeses made with milk from commercial sheep flocks managed indoors,part-time grazing in valley, and extensive mountain grazing. International Dairy Journal, 53, 29-36. DOI: https://doi.org/10.1016/j.idairyj.2015.09.007

Wolf, I.V., Perotti, M.C., Bernal, S.M., Zalazar, C.A. (2010) Study of the chemical composition, proteolysis, lipolysis and volatile compounds profile of commercial Reggianito Argentino cheese: Characterization of Reggianito Argentino cheese. Food Research International, 43, 1204–1211. DOI: https://doi.org/10.1016/j.foodres.2010.02.018

Wrolstad, R.E., Smith, D.E. (2010) Colour Analysis In: Nielson S.S. (ed), Food Analysis New York: Springer Science + Business Media, LLC2010, pp. 575-586.

Yvon, M., Rijnen, L. (2001) Cheese flavour formation by amino acid catabolism. International Dairy Journal, 11, 185-201. DOI: https://doi.org/10.1016/S0958-6946(01)00049-8