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Effect of Milk Type, Pasteurization and Packaging Materials on Some Physicochemical Properties and Free Fatty Acid Profiles of Tulum Cheese

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ABSTRACT

The aim of this study was to investigate the effect of milk type, pasteurization and packaging material type on free fatty acid (FFA) content, physicochemical and sensory properties of Tulum cheese. Thus, cheese samples manufactured from pasteurized ewes' and cows' milk and ripened different material (plastic barrel, cellulose casing, cloth bag, naturel bowel) were compared with traditional Erzincan Tulum cheese during 90 days of storage. Results indicated that the ewes' milk cheeses had higher concentration of $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{18:2}$, and $C_{18:3}$ fatty acids (P<0.01). Type of packaging materials did not influence the concentrations of FFA in cheese samples. However, pasteurization significantly (P<0.01) affected the levels of $C_{6:0}$ - $C_{14:0}$ FFA. Results of a sensory panel showed that packaging materials remarkably (P<0.01) influenced the acceptability of cheese samples produced by pasteurization. Panelists' preferred experimental cheese made with ewes' milk and ripened in cellulose based packaging material.

Keywords: Tulum cheese, Free fatty acid, Packaging, Pasteurization, Ripening

Süt Tipi, Pastörizasyon ve Paketleme Materyalinin Tulum Peynirlerinin Bazı Fizikokimyasal Özellikleri ve Serbest Yağ Asidi Profili Üzerine Etkisi

ÖZ

Bu araştırmanın amacı süt çeşidi, pastörizasyon ve ambalaj materyalinin Tulum peynirinin bazı fizikokimysal nitelikleri, duyusal özellikleri ve serbest yağ asidi (SYA) içeriği üzerine etkisini belirlemektir. Bunun için 90 günlük depolanma süresince, pastörize inek ve koyun sütünden üretilerek farklı ambalaj materyallerinde (plastik kap, selüloz içerikli kılıf, bez torba ve doğal bağırsak) olgunlaştırılan tulum peynirleri, geleneksel Erzincan Tulum peyniri ile karşılaştırılmıştır. Araştırma bulgularına göre, koyun sütünden üretilen peynirlerin C_{6:0}, C_{8:0}, C_{10:0}, C_{12:0}, C_{18:2} ve C_{18:3} yağ asitleri konsantrasyonu bakımından daha zengin olduğu tespit edilmiştir (P<0.01). Farklı ambalaj materyali kullanımı peynir örneklerinin SYA içeriğini etkilememiştir. Bunun yanı sıra, pastörizasyonun C_{6:0}-C_{14:0} SYA seviyesini önemli derecede (P<0.01) etkilediği belirlenmiştir. Farklı ambalaj materyali kullanımı pastörizasyonla üretilen peynir örneklerinde genel kabul edilebilirliği önemli derecede (P<0.01) etkilemiştir. Panelistler tarafından en çok tercih edilen peynir örneği, koyun sütünden üretilerek selülozik kılıfta olgunlaştırılan örnek olmuştur.

Anahtar Kelimeler: Tulum peyniri, Serbest yağ asidi, Paketleme, Pastörizasyon, Olgunlaştırma

INTRODUCTION

Tulum cheese is traditionally made from raw sheep milk in rural areas of Eastern Anatolia [1] but sometimes goat milk, and cow milk added sheep milk probably due to the inadequacy of sheep milk. Sometimes yoghurt is added for desirable taste and texture [2]. It has a white or cream-like color, semi-hard texture, slightly sharp and piquant taste, lightly acidic, butter-like aroma, high fat content and it is easily dispersible in mouth [3]. Its name appears to be derived from the word "tulum" which means a bag-like device made of sheep or goatskin.

Tulum cheese called with different names in various regions of Turkey. Erzincan Tulum cheese is a traditional kind of cheese produced in the region shaped by plant, animal and microbial biodiversity, reflecting the rich local production skills (know-how). The cheese is produced many mountain pastures of Erzincan. It was given the Geographical Indication (GI, place of origin) registration by the Turkish Patent Institute in 2000 [4].

Oğlak (kid of a goat) skin bags were generally used for cheese packaging because of being stronger and more flexible than sheep's skin and it has specific permeable structure to water and air [5]. Despite its superior properties, there are some microbial problems associated with goatskin, high cost and transportation problems. Nowadays, cheap and easily obtainable plastic barrels of different size and structure have been used for packaging in order to prevent the contamination by some pathogenic and saprophytic microorganisms [6]. Unfortunately, reliability of these plastic containers for storing Tulum cheese has not been tested. Thus, packaging is still an open question for storing this type of traditional cheeses.

Flavor development in cheese is the result of complex combination of microbial and biochemical activities [7] throughout the ripening period which include the breakdown of milk protein, fat, lactose and citrate and leads to the formation of a heterogeneous mixture of volatile and non-volatile compounds [8].

Milk fat is one of the main components in the quality of Tulum cheese. It is well established that milk fat is essential for the development of correct flavor in cheese during ripening [9]. The FFA released has a direct role in some cheese profiles, and many fatty acid esters are essential to characteristic flavors [10]. FFAs make significant contributions to the characteristic flavor of Tulum cheese [5, 11]; however, lipolysis can affect negatively the flavor of cheese (rancid taste) by production of an excessive amount of volatile fatty acid released by the action of lipase [9, 12, 13]. Therefore, controlled lipolysis is needed for Tulum cheese as in many internal bacterially ripened Swiss and Italian cheeses during the long ripening period [5, 14]. Recently, FFA composition and acid degree value were determined in Tulum cheeses made from raw milk, but a comparative study on FFA content in traditional Erzincan Tulum cheese made from pasteurized milk and ripened in different package has not been carried out. Therefore, the aim of this study was to investigate the effect of milk types, pasteurization and packaging materials on physicochemical, sensory properties and FFA content during storage period.

MATERIALS and METHODS

Materials

Raw ewes' milk and packing material tulum "goatskin" were obtained from a farmhouse on "Ahorik" mountain pasture in Kemah, Erzincan in traditional method. In the second method, raw ewes' milk was obtained from some farmers while raw cows' milk and fibre-reinforced cellulose casing with 60 mm diameter and inner barrier (water-vapor, aroma and oxygen barrier) was obtained from the Food Engineering Department of Ataturk University in Erzurum. Starter cultures (Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteorides subsp. cremoris and Lactococcus lactis subsp. lactis biovar diacetylactis; mesophilic aromatic strarter cultures) were obtained from Peyma-Chr. Hansen's Inc. (Istanbul, Turkey). High density polyethylene plastic vessel, were obtained from Balacan Dairy Plant in Erzincan, cloth bags and natural bowel were purchased from a local market of Erzurum

Methods

In this study, Tulum cheese samples were produced traditional method and an alternative standardized method (Table 1). Erzincan Tulum cheese samples (RE cheese) was made from raw ewe's milk according to the traditional cheese making method specified in GI registration and curds filled into goatskin [4]. All stages of production were carried out on the pasture in Erzincan. The pH, dry matter, fat and acidity values of raw milk were 6.53, 17.30, 6.5 and 0.20%.

The pH, dry matter, fat and acidity values of pasteurized cow's milk were 6.43, 10.50, 3.0 and 0.16%, respectively. The pH, dry matter, fat and acidity values of pasteurized ewe's milk used in the alternative production of 6.50, 18.51, 6.0, and 0.18%, respectively. In this method, the applications of the first method were considered as possible as, except for heat treatment, starter culture inoculation and CaCl₂ addition. After this, curds were filled into the packaging materials and ripened at 6-8°C for 90 days.

Physical and Chemical Analysis

Total solids, fat, salt, titratable acidity and ash contents of the experimental cheeses were determined according to the methods described by Kurt et al. [15]. pH was measured using a pH meter (model WTW pH-340-A, Weilheim, Germany) with a combined glass electrode. The contents of fat and salt in total solids were estimated by calculation based on the fat and salt contents of cheese samples.

Free Fatty Acids Analysis

The cheese samples were prepared for analysis according to the procedures described by Akalin et al. [16] and methylation to fatty acid methyl esters (FAME) by Aksu and Kaya [17]. Fatty acids were analyzed by gas chromatography (GC-Agilent 6890N, USA) with a

HP-Innowax capillary column ($60m \times 0.25 \ \mu m \times 0.2 \ mm$ ID), temperature (increasing from 100°C to 200°C with rate of 5°C/min), FID detector (H2 and dry hair) at 260°C, helium gas (1 mL/min, 150 kPa) and injection block temperature of 250°C [18].

Table 1. Experimental cheeses

Cheese making method	Milk type	Packaging material	Cheese code
Traditional	RE: Raw ewes' milk	Tulum	RE (control)
P: Pasteurization	E: ewes' milk	Plastic barrel (p)	PEp
		Cellulose casing (s)	PEs
		Cloth bag (c)	PE_c
		Natural bowel (b)	PE_b
P: Pasteurization	C: cows' milk	Plastic barrel (p)	PC _p
		Cellulose casing (s)	PC _s
		Cloth bag (c)	PC_c
		Natural bowel (b)	PC _b

Sensory Analysis

The experimental cheeses were ripened for 30, 60 and 90 days and then graded by eight panelists (from the permanent staff of the Department of Food Engineering, Ataturk University, Turkey) who were familiar with Tulum Cheese. They evaluated the cheeses for appearance, body and texture, flavor (odor and taste) and total score using a 25-point scale, with 5 being the worst and 25 the best quality. The evaluation was conducted according to the Turkish Standards for Turkish Tulum cheese [19]. The raw milk cheese samples were not performed sensory evaluation.

Statistical Analysis

Cheeses were analyzed on the storage days of the 2nd, 30th, 60th and 90th in duplicates. Analysis of variance (ANOVA) was employed to establish statistical differences between physicochemical parameter values, free fatty acids analysis and sensory analysis scores by using Minitab[®] statistical software [20]. Different groups were analyzed by Duncan's Multiple Range Test.

RESULTS

Results of physical and chemical analysis of the cheeses are shown in Table 2. Total solid contents of all cheeses increased regularly during ripening due to evaporation of water from the surface of cheese or packaging material (approximately 56.51-74.22% for 90 d). Loss of moisture was observed at lower level in the cheese ripened in the plastic case. There are no significant differences in total solid contents between RE and PEs for 90 days.

The fat in total solids contents were >45% (w/w) and the cheeses could be categorized as full-fat cheese according to Codex Alimentarius [21]. A relative increase was observed in total fat level in the experimental cheeses during ripening period due to the losses of moisture content in the cheeses. Likewise, salt and ash contents increased relatively (P<0.01, P<0.05).

Milk and packaging material types had significant effect on the titratable acidity (P<0.01). Titratable acidity in the samples RE and PE $_{p}$ were higher than other samples. Moreover, the cheeses ripened in small plastic barrel (PE $_{p}$ and PC $_{p}$) had lower dry matter than that of other cheese samples. In addition, the cheeses produced from pasteurized ewes' milk had higher acidity level than cows' milk cheeses (P<0.01).

The initial pH of cheeses was approximately 5.20. The pH values decreased rapidly from 5.20 to 5.07 for 30 days of ripening. The highest mean value of pH (5.17) was found in PC $_{\rm c}$ and the lowest one (5.07) in PCs. The differences among the samples were determined statistically significant (P<0.01). The pH values increased between 30 d and 90 d of ripening. The increase in pH may be attributed partly to the use of lactic acid by yeasts and moulds, the numbers of which were high during this period. The similar results were obtained by Kondyli et al. [22] in Kefalograviera-type cheese.

The changes in FFA composition during the ripening of Erzincan Tulum cheese samples were presented in Table 3. As seen from the table, myristic (C_{14:0}), palmitic $(C_{16:0})$, stearic $(C_{18:0})$ and oleic $(C_{18:1})$ acids are the most abundant FFA in all cheeses during the storage time. In all types of cheeses the concentration of C4:0-C10:0 and C_{18:2} increased during the ripening. The concentration of FFA was compared and results showed that the ewes' milk cheeses had richer concentration of $C_{6:0}$ - $C_{12:0}$, $C_{18:2}$, and C_{18:3} acids. It was found that using different packaging material did not effect on the concentrations of FFA (C4:0-C18:3) in cheese made from same type of milk. However, milk pasteurization significantly (P<0.01) affected the levels of $C_{6:0}\text{-}C_{14:0}$ FFA. These FFA in sample RE was significantly higher (P<0.01) than that of PE and PC cheeses. This trend indicates low lipolytic activity in the PE and PC cheeses, which probably results exclusively from the starter microorganisms [7, 23-25]. Hickey et al. [26] also reported that FFA concentration in the cheese made from pasteurized milk was lower than that found in raw milk cheeses. Lipoprotein lipase activity is very important in raw milk cheeses as the enzyme is largely inactivated by

pasteurization, although 78°C , 10~s is required to inactivate this enzyme completely [7].

	Ash (%)**	(0/) IISV	4.966⁴	5.251	5.238°	6.162₃♭	6.473	4.999	4.902⁴	5.682№	6.214 ^{ab}	Ash (%)**	(6/) 115/	4.946♭	5.467₃₺	5.717 ^{ab}	6.045⁴
	Titratable	acidity (%)**	1.005₃	0.945	0.916abc	0.855abc	0.838abc	0.742bcd	0.736bcd	0.627	0.731⁴	Titratable	acidity (%)**	0.682⁵	0.798ªb	0.805ab	1.002₃
ig storage	**Ha	III	5.153ab	5.128°	5.108°	5.163	5.158ab	5.078	5.073⁴	5.170₃	5.140ab	nH**		5.196₃	5.067₺	5.104ab	5.152₃
nental cheeses durin	Salt in total	solids (%)**	6.67⁴	7.25°	6.94⊶	7.91	7.3460	7.4360	7.05⊶	7.81	7.40№	Salt in total	solids (%)**	7.12⁵	7.07⊳	7.68₃	7.62₃
Table 2. Mean values of some chemical and physical properties obtained from the experimental cheeses during storage	Salt (%) **	Salt (/0)	3.95⁴	4.12⁴	4.09⁴	4.80♭	5.24ªb	4.20⁴	4.18⁴	5.03ab	5.448	Salt (%)**	(a/) mag	4.02°	4.43♭	4.80₃	4.99₃
properties obtaine	Fat in total	solids (%)*	52.47	48.65bcd	47.40°d	47.69bcd	47.24 ^{cd}	51.54	49.56₺	49.16bod	48.41bcd	Fat in total	solids (%)	48.49	49.29	49.19	49.54
ical and physical pr	Eat (%) 1**	rat (/0)	31.06₺	27.66⁴	27.83⁴	28.84⊶	33.88	29.13°	29.41° 31.56° 35.94°		35.94₃	Eat (%)**	(0/)	27.39₺	30.76 ₃	31.47₃	32.74₃
lues of some chem	Total solids	**(%)	59.20°	56.86⁴	58.71◦	60.45⁵	71.68 ■	56.51	59.39°	64.20₽	74.22₃	Total solids	**(%)	56.50₽	62.62₃	64.09 ■	66.22₃
Table 2. Mean va	Experimental	cheeses	RE	PĒ	PE	PĘ	PĒ	PÇ	PÇ	ပိ	PÇ	Storage time	(day)	2	30	09	06

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1.535° 1.562° 1.372° 4.214 3.161 10.821 28.609 10.934° 21.921 1.659° 1.699° 1.466° 4.414 3.215 10.861 28.644 10.808° 21.864	10.830	_		1.468
1659° 1699° 1466° 4414 3215 10861 28644 10808° 21864 (10.821	_		1.473
0000	3.215 10.861 28.6	44 10.808b	21.864 3.523 ^{ab}	1.482
indicate significant differences * (P<0.05), ** (P<0.01). RE: (control) raw ewes' milk, packed in tull s' milk, packed in cellulose casing: PE; pasteurized ewes' milk, packed in cloth bag: PE; pasteurized ewes' milk packed in cloth bag: PE; pasteuriz	90 1.659° 1.699° 1.466° 4.414 3.215 10.861 28.644 10.808° Different letters indicate significant differences * (P<0.05), ** (P<0.01). RE: (control) raw ewes' milk, packed in tulum; PEs: pasteurized epasteurized ewes' milk, packed in cellulose casing; PE: pasteurized ewes' milk, packed in cellulose casing; PEs: pasteurized ewes' milk, packed in cellulose casing; PCs: pasteurized cows' milk, packed in cellulose casing; PCs: pasteurized cows' milk, packed in cloth bag;	3.6 7. p. y.	8.644 10.808b m; PEp: pasteurized ew red ewes' milk, packed ir, packed in cloth bag; F	8.644 10.808b 21.864 3.523ab 1.482 m; PEs: pasteurized ewes' milk, packed in plastic barrel; PEs: packed in cloth bag; PCs: pasteurized cows', packed in cloth bag; PCs: pasteurized cows' milk, packed in cloth bag; PCs: pasteurized cows' milk, packed in

Storage time had significant (P<0.01) effect on the levels of $C_{4:0}$, while the milk type had no effect on the amounts of this FFA. The concentration of caproic acid ($C_{6:0}$) in the sample RE was higher significantly (P<0.01) On the other hand, the results showed that the concentrations of $C_{6:0}$ - $C_{12:0}$ in the sample ewes' milk cheeses were higher (P<0.01) than those of the cows' milk cheeses during storage times.

In general, long-chain fatty acids (>12 carbon atoms) play a minor role in the flavor, given their relatively high perception thresholds. Short and moderate-chain, even numbered fatty acids (C4:0-C12:0) have much lower perception thresholds and each has characteristic note [28]. Short- and medium-chain even-numbered FFAs are more important for the flavor development of cheese than the long chain fatty acids, since they have low perception thresholds. The fatty acids ($C_{2:0}$ - $C_{10:0}$) constituted the small proportion of total volatile acids in Tulum cheese, but are major contributors to typical flavor [5]. Caprylic $(C_{8:0})$ and capric $(C_{10:0})$ acids are responsible for characteristic natural sharp and piquant flavor of Tulum cheese. It is desirable for Tulum cheese flavor, if well balanced with other taste attributes [14]. The relative increase in the concentration of short-chain FFA (C_{4:0}-C_{8:0}) during ripening was higher than those of medium-chain FFA (C_{10:0}-C_{14:0}), which in turn were higher than those of long-chain FFA (C_{16:0}-C_{18:3}). This could be explained that lipolytic enzymes exhibits preference for hydrolysis of triglycerides containing medium-chain fatty acids $(C_{6:0}\text{-}C_{12:0})$, and acts preferentially at the sn-1 and sn-3 positions of triglycerides [7, 9] and generally, short-chain fatty acids predominantly esterifies at the sn-3 position [30].

Palmitic acid ($C_{16:0}$), and oleic acid ($C_{18:1}$) contents of cheeses made from cows' milk were significantly (P<0.01) higher than those of raw and pasteurized ewes' milk cheeses. Talpur [31] reported that cows' milk

than that of PE and PC cheeses. There were no differences in cheeses made from the pasteurized ewes' and cows' milks in terms of $C_{6:0}$ concentrations. Mallatou et al. [27] reported similar results for Teleme cheese. had richer palmitic acid ($C_{16:0}$) concentration than that of ewes' milk. Sousa et al. [32] found that palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$) concentrations were higher in the cheese made from cows' milk than those found in other milk cheeses. Ruiz-Sala et al. [33] stated that cow milk fat is richer in long-chain and unsaturated triglycerides when compared with ewes' and goat's milk.

In all the experimental cheeses, as ripening progressed, the level of stearic acid ($C_{18:0}$) decreased significantly (P < 0.05). It was observed that the lowest concentration of stearic acid ($C_{18:0}$) was on day of 90 of ripening.

The concentration of $C_{18:2}$ did not differ between cheeses made from raw or pasteurized ewes' milk. Using different packaging material did not effect on linoleic acid ($C_{18:2}$) rate. The level of linoleic ($C_{18:2}$) and linolenic acid ($C_{18:3}$) was higher (P<0.01) in both raw and pasteurized ewes' milk cheeses than those of cows' milk cheeses. Jahreis et al. [34] reported that ewe milk has highest conjugated linoleic acid (CLA) because ewe milk fat is the richest source of CLA. Sousa et al. [32] observed the highest level of linolenic acid ($C_{18:3}$) in cheeses manufactured from ovine milks than those of bovine and caprine milk cheeses.

Sensory properties (crosscut appearance, body, odour and taste) of cheese samples were performed on days of 30, 60 and 90 of storage (Table 4). PE_s had highest mean point (92.833) in terms of total score, while the lowest mean point (52.792) belonged to PC_b . The sensory evaluations indicated that the packaging materials and milk types influenced the crosscut appearance, body and flavour characteristics of the experimental cheeses significantly (P<0.01; P<0.05).

Table 4. Mean values of some sensory properties obtained from the experimental cheeses during storage

Experimental cheeses	Crosscut appearance*	Body**	Odour*	Taste**	Total score**
PEp	19.583 ^{abc}	19.375 ^{ab}	24.167 ^{ab}	20.208 ab	83.333 ab
PEs	22.708 a	21.875 a	25.000 a	23.250 a	92.833 a
PEc	14.167 bc	13.542 ^{ab}	20.583 abc	15.833 ^{ab}	64.125 b
PE _b	14.583 ^{abc}	12.292 b	17.708 bc	14.083 ab	58.666 b
PC_p	20.208 abc	18.542 ab	20.833 abc	15.833 ^{ab}	75.416 ab
PC _s	22.500 ab	21.875 ^a	22.917 abc	20.625 ab	87.917 ab
PC_c	13.750 °	12.083 ^b	16.875 °	12.917 ^b	55.625 b
PC _b	12.500 °	11.667 ^b	16.750 °	11.875 b	52.792 ^b
Storage time (day)	Crosscut appearance*	Body**	Odour*	Taste**	Total score**
30	17.109	16.719	21.406	17.500	72.734
60	18.438	17.109	21.562	16.797	73.906
90	16.953	15.391	18.844	16.188	51.188

Different letters indicate significant differences * (P<0.05), ** (P<0.01). RE: (control) raw ewes' milk, packed in tulum; PEp: pasteurized ewes' milk, packed in plastic barrel; PEs pasteurized ewes' milk, packed in cellulose casing; PEc: pasteurized ewes' milk, packed in cloth bag; PEb: pasteurized ewes' milk, packed in natural bowel; PCp: pasteurized cows' milk, packed in plastic barrel; PCs: pasteurized cows' milk, packed in cellulose casing; PCc: pasteurized cows' milk, packed in cloth bag; PCb: pasteurized cows' milk, packed in natural bowel.

DISCUSSION

Our results indicated that milk type, packaging materials, and storage time significantly affected (P<0.01; P<0.05) the gross chemical composition of cheese samples. According to the results obtained in this study, using different packaging materials did not affect the concentrations of FFA (C4:0-C18:3). Myristic $(C_{14:0})$, palmitic $(C_{16:0})$, stearic $(C_{18:0})$ and oleic $(C_{18:1})$ acids were the most abundant FFA in all cheeses during the storage time. FFA concentrations in the cheeses made from pasteurized milk were lower than raw milk cheeses. The FFA concentrations (C_{6:0}-C_{14:0}) of ewes' milk cheese produced using traditional method and packed in tulum was much higher than other samples. The study indicated that the concentrations of C_{6:0}, C_{8:0}, $C_{10:0}$ and $C_{12:0}$ in the ewes' milk cheeses were significantly higher than those of the cows' milk cheeses during storage. Indeed, the first three of these are responsible for the formation of the characteristic flavor of cheeses made from ewe's milk.

The results of sensory evaluation showed that the experimental cheeses made with ewes' milk and ripened in the cellulose casing (PE_s) had highest point in terms of total score. It was concluded that cellulose casing could be used as packaging material in producing of Erzincan Tulum cheese.

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