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# Free fatty acid composition and sensory characteristics of Örgü cheese

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Two batch of Örgü cheese, a semi-hard, white, brine ripened Turkish traditional cheeses, from raw and pasteurized cow's milk were produced. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were used as starter culture for pasteurized milk. The main FFA observed in the raw and pasteurized milk Örgü cheeses during ripening were palmitic, oleic, myristic, steric and capric acids, representing together approximately 82% of total FFA content. Butyric (C4), caproic (C6), caprylic (C8), capric (C10) and lauric (C12) acids contents of RA and PA milk Örgü cheeses increased during ripening. However, myristic (C14), palmitic (C16), stearic (C18), oleic (C18:1) and linoleic (C18:2) acids contents of pasteurized milk cheese remained constant ( $P>0.05$ ). The results revealed that pasteurization of milk has a restricted level of lipolysis throughout ripening. Appearance and body-texture properities of PMC were not significantly inferior to that of RMC. However, PMC failed to reach the expected quality of odor and flavour level.

**Key words:** Traditional cheese, lypolysis, free fatty acids.

## INTRODUCTION

Örgü cheese is one of the traditional cheese types that is produced and consumed locally in Southeastern region of Turkey. It is a semi-hard, processed scalding the curd, and ripened in brine. It is mainly produced from ewes' milk however; it may also be produced from cows' milks or mixture of them (Akyüz et al., 1998; Turkoglu et al., 2003; Celik and Turkoglu, 2007). A few studies have been carried out on gross composition and ripening characteristics of Örgü cheese (Akyüz et al., 1998; Ozdemir et al., 1998; Turkoglu et al., 2003). In the previous study (Celik and Türkoğlu, 2007) biochemical changes during the ripening of Örgü cheese made from raw and pasteurized cow's milk, were revealed. Lipolysis is one of the major biochemical changes that contribute to flavour development during the cheese ripening, together with proteolysis (Forde and Fitzgerald, 2000). The accumulated free fatty acids (FFAs) from lipolysis, caused by enzymes from milk and microflora of raw milk, contribute to development of characteristic cheese flavor either directly or indirectly, serving as precursors for a variety of chemical compounds such as alcohols, esters, aldehydes, ketones, lactones and thioesters (Kurt, 1996; McSweeney, Sousa, 2000; Mallatou et al., 2003).

Analysis of the short and medium-chain FFA profile has been suggested as an index for characterizing cheeses over the ripening period (Woo et al, 1984; Woo and Lindsay, 1984).

Low concentrations of fatty acids in cheese indicate a young, unripened cheese. However, extensive lipolysis is considered to be undesirable for some cheese types. Especially short chain FFAs may directly affect flavour development. Excessive concentrations of some FFAs are reported to be perceived as off-flavors (Fox et al., 1995; Mallatou et al., 2003). The characteristic flavour of cheese is reported to be brought about by a very well balanced concentration of chemical compounds (Massouras et al., 2006). The level of lipolysis varies considerably among the different cheese types from low in Dutch type cheeses (Walstra et al., 1993) to extensive in the mould ripened, surface-bacterially ripened and Italian hard cheeses (Battistotti and Corradini, 1993; Gripon, 1993; Reys, 1993). Many research have been carried out into fatty acids composition and sensory characteristics of various cheese types (Partidario et al., 1998; Pavia et al., 2000; Kondyli et al., 2003; Mallatou et al., 2003; De Wit et al., 2005; Georgala et al., 2005;

Perotti et al., 2005; Poveda and Cabezas, 2006; Atasoy and Türkoğlu, 2008; 2009; Atasoy et al., 2008). Though, to the best knowledge of us, no research on fatty acids composition of Örgü cheese is available.

Traditional cheeses are generally produced from raw milk. Pasteurization of milk is important to eliminate the risks for health, but it may result in loss of its unique flavour to some extent, altering the protein structure, and reducing activity of ingenious enzymes of milk (Urbach, 1997). It is important that useful starter culture be added into pasteurized milk to develop pH, and obtain cheese with characteristic flavour and texture. *S. thermophilus* and *L. bulgaricus* are used as starter culture in some cheese milks, especially those curd of which are scalded (Atasoy et al., 2008). The purpose of the present study was to examine the changes in FFAs during ripening period of Örgü cheese made from raw and pasteurized cows' milk, using starter culture, and to compare their sensory characteristics to that made from raw milk, on 90th day of ripening period.

In this study, contribution of thermophilic lactic acid bacteria to FFAs composition of Örgü cheese as compared to raw milk was investigated.

## MATERIALS AND METHODS

### Cheese making

Örgü cheese was produced as outlined in a prior study (Celik and Türkoğlu, 2007). Typical manufacturing details of Örgü cheese involve the following practices. The milk was filtered through a cloth, and divided into two parts. Raw milk cheese (RMC) was produced from the first part. The other part was pasteurized ( $72\pm 2^\circ\text{C}$ ,  $15\pm 3$  s) and Thermophilic DVS culture (EZAL TM081) composed of *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* was inoculated as starter culture to produce pasteurized milk cheese (PMC). Both milk bulks were coagulated at milking temperature, with commercial liquid rennet.

The curds that formed within 45 to 60 min were cut, and transferred into cloth-lined metal molds. Whey was drained and left for fermentation to below pH 4.9, which was necessary to get the convenient texture during scalding the curd at 72 to 76°C for 5 to 6 min in a brine of 5% salt. The curd was then processed into strings of 1 cm in diameter, and each three strings were knitted. They were kept in 14% salt for 6 h at ambient temperature, and ripened at  $8\pm 2^\circ\text{C}$  for 3 months.

The cheese samples were analyzed for FFAs on 1st, 15th, 30th, 60th and 90th days. Sensory analysis was carried out on 90th day only.

### Free fatty acids analysis

Fat was extracted from cheese samples as described by Garcia-Lopez et al. (1994), and methylated according to the procedure of Sukhija and Palmquist (1988). Fatty acids methyl esters were analyzed using GC (Thermo Quest) equipped with flame ionization detector (FID), and fitted with a fused silica capillary column (SP-2380, 30 m, 0.25 mm, Supelco Inc., Bellefonte, PA). Injector and detector temperature was 250°C. The initial oven temperature was 40°C for 1.0 min, and then increased to 240°C at 5°C/min. The final temperature was maintained for 10 min. Nonanoic acid was used

as internal standard. A standard fatty acid mixture containing 37 fatty acids (Sigma-Aldrich Chemicals 189-19) was used to provide standard retention times. Fatty acids were identified by comparing their retention times with those of fatty acids in standard samples. An autosystem Thermoquest GC-MS, equipped with flame ionization detector (FID) was used to analyse FFAs of cheese samples. The carrier gas was helium at 2 ml min<sup>-1</sup>. Injection of 1 µL sample was applied with a split ratio of 1:30 into the injector.

### Sensory analyses

The sensory analysis of Örgü cheese samples was carried out at 90th day of ripening period, at Department of Food Engineering, Harran University by a panel of 10 cheese graders, 4 female and 6 male, familiar with Örgü cheese, in duplicate. All the panelists were between the ages of 22 and 55. The panelists completed 1 h training session before the actual testing was carried out. Since Örgü cheese is ripened in brine, the samples were assessed according to the scala given by Turkish Standard-591 for white-pickled cheese (Anon, 1995). The Örgü cheese samples were graded on a 100-point hedonic scale as follows: 20 points maximum for appearance, 35 points maximum for body and texture, 10 points maximum for odour and 35 points maximum for flavour. The cheeses were cut into (2.5 x 2.5 x 2.5 cm) pieces and placed on plates. They were coded with three-digit numbers randomly, and presented to assessors in a random order. Water was provided for rinsing mouth between samples.

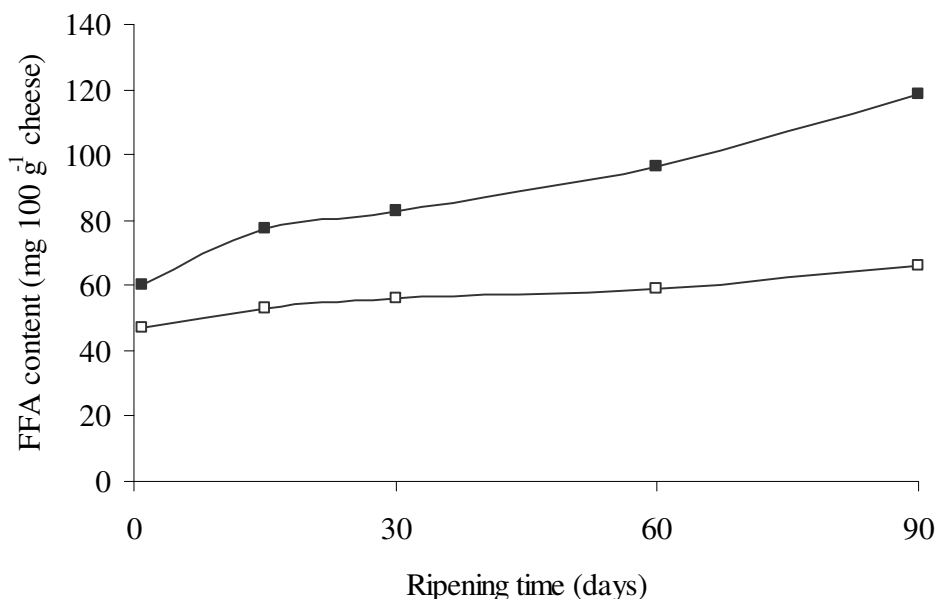
### Statistical analyses

The study was arranged as a randomized complete block, 2 x 2 x 5 factorial experimental design with pasteurization as blocks and ripening periods as factors. Variance analysis was applied to the data obtained from tests on RMC and PMC. Duncan's multiple range test was applied to the significant means using STATISTICA (ver. 5.0, 1995) package software.

## RESULTS AND DISCUSSION

The total FFA contents of experimental cheeses during ripening period are presented in Figure 1. The total FFA content of Örgü cheese made from raw milk (RMC) remained constant until the day 30, but then it increased significantly ( $P<0.05$ ). However, there was no significant increase in total FFA content during ripening in pasteurized milk cheese (PMC). Similar results were observed by Buffa et al. (2001). RMC had a statistically similar total FFA content to pasteurized ones at 1st, 15th and 30th d. However, during rest of the storage period the total FFA content of RMC was significantly ( $P<0.05$ ) higher than that of PMC. This result could be due to natural microflora of raw milk from which PMC was produced. The importance of the nonstarter microflora in raw milk cheese on ripening and developing strong flavor has been demonstrated in Cheddar cheese (McSweeney et al., 1993). Moreover, pasteurization affects the structure of indigenous enzymes of milk, and reduces their effect (Urbach, 1997). It is indigenous lipase that mainly causes significantly lipolysis in raw milk cheese.

The contribution of milk lipase to cheese lipolysis depends on the heating of cheese milk during



**Figure 1.** Total free fatty acids content of cheeses made from raw (■) and pasteurized (□) milk.

**Table 1.** Concentration of individual FFAs (100 g<sup>-1</sup> cheese) of Örgü cheese during storages<sup>a</sup>.

Cheeses	Age (days)	SCFA as % of total FFAs	MCFA as % of total FFAs	LCFA as % of total FFAs
RMC	1	4.75	24.64	70.61
	15	6.09	24.98	68.93
	30	7.56	25.60	66.84
	60	9.49	25.84	64.67
	90	10.23	27.37	62.40
PMC	1	4.51	23.72	71.76
	15	5.76	24.25	69.99
	30	7.10	24.28	68.63
	60	9.19	25.31	65.50
	90	9.85	26.09	64.06

<sup>a</sup> Means with different minuscule and capital letters within each column were significantly different ( $P < 0.05$ ). RMC: Raw milk Örgü cheese PMC: Pasteurized milk Örgü cheese.

process. It may also contribute to lipolysis in pasteurized milk cheese (Mallatou et al., 2003), since, it is well known that lipoprotein lipase is relatively heat-labile, which may be completely inactivated by heating at  $\geq 78^\circ\text{C}$  for 10 s (Driessen, 1989). The amounts of all individual FFAs increased at different pattern during ripening period (Table 1), so the FFA composition of cheese samples varied considerably over the ripening period of 90 days. The increase of FFAs in RMC was significantly higher than in PMC, due to the activity of lipase from natural microflora. The increase in PMC was due to starter culture and also to indigenous lipases that survived pasteurization process. The main FFA observed in the

raw and pasteurized milk Örgü cheeses during ripening were palmitic, oleic, myristic, steric and capric acids, representing together approximately 82% of total FFA content. Butyric (C4), caproic (C6), caprylic (C8), capric (C10) and lauric (C12) acids contents of both RMC and PMC increased during ripening. However, myristic (C14), palmitic (C16), stearic (C18), oleic (C18:1) and linoleic (C18:2) acids contents of RMC remained constant ( $P > 0.05$ ) during storage. At 90th d, the butyric acid levels in RMC and PMC were 4.728 and 2.554 mg 100 g<sup>-1</sup> cheese, respectively, corresponding to percentages of 3.99% and 3.85%. It was lower in both cheeses than that reported by Georgala et al. (2005). He reported that

**Table 2.** Relative concentrations of short, medium and long chain FFA (% of total) in Örgü cheese during storage.

Cheese	Days	Free fatty acids (mg 100 g <sup>-1</sup> cheese)									
		C4	C6	C8	C10	C12	C14	C16	C18	C18:1	C18:2
RMC	1	1.135a <sup>A</sup>	1.026a <sup>A</sup>	0.705a <sup>A</sup>	3.078a <sup>A</sup>	2.015 a <sup>A</sup>	9.789 a <sup>A</sup>	19.003 a <sup>A</sup>	7.468 a <sup>A</sup>	14.662 a <sup>A</sup>	1.507 a <sup>A</sup>
	15	1.834b <sup>A</sup>	1.736b <sup>A</sup>	1.161b <sup>A</sup>	4.355b <sup>A</sup>	2.510 a <sup>A</sup>	12.539 b <sup>A</sup>	23.693 ab <sup>A</sup>	9.270 b <sup>A</sup>	18.214 b <sup>A</sup>	2.359 b <sup>A</sup>
	30	2.433b <sup>A</sup>	2.078b <sup>A</sup>	1.742c <sup>A</sup>	4.499b <sup>A</sup>	3.492 b <sup>A</sup>	13.192 b <sup>A</sup>	24.213 ab <sup>A</sup>	10.351ab <sup>A</sup>	18.435 b <sup>A</sup>	2.300 b <sup>A</sup>
	60	3.869c <sup>A</sup>	3.430c <sup>A</sup>	1.868c <sup>A</sup>	5.540c <sup>A</sup>	4.541 c <sup>A</sup>	14.876 c <sup>A</sup>	26.872 ab <sup>A</sup>	12.663bc <sup>A</sup>	20.277 c <sup>A</sup>	2.657 c <sup>A</sup>
	90	4.728c <sup>A</sup>	4.525d <sup>A</sup>	2.856d <sup>A</sup>	7.078d <sup>A</sup>	6.033 d <sup>A</sup>	19.298 d <sup>A</sup>	29.777 b <sup>A</sup>	16.670dc <sup>A</sup>	23.963 d <sup>A</sup>	3.460 d <sup>A</sup>
PMC	1	0.846a <sup>A</sup>	0.765a <sup>A</sup>	0.526a <sup>A</sup>	2.294 a <sup>A</sup>	1.635 a <sup>A</sup>	7.293 a <sup>A</sup>	14.534 a <sup>A</sup>	5.714 ab <sup>A</sup>	12.598 a <sup>A</sup>	1.112 a <sup>A</sup>
	15	1.178ab <sup>B</sup>	1.115ab <sup>A</sup>	0.745ab <sup>A</sup>	2.797ab <sup>B</sup>	1.946 ab <sup>A</sup>	8.049 ab <sup>A</sup>	15.650 a <sup>A</sup>	6.125 bc <sup>A</sup>	13.738 ab <sup>A</sup>	1.406 b <sup>A</sup>
	30	1.546b <sup>B</sup>	1.477bc <sup>A</sup>	0.970b <sup>B</sup>	2.944ab <sup>B</sup>	2.345 c <sup>B</sup>	8.369 ab <sup>A</sup>	16.224 a <sup>A</sup>	7.038bc <sup>A</sup>	13.936 b <sup>A</sup>	1.414 bc <sup>A</sup>
	60	2.289c <sup>B</sup>	2.029cd <sup>B</sup>	1.105b <sup>B</sup>	3.276bc <sup>B</sup>	2.865 cd <sup>B</sup>	8.795 bc <sup>B</sup>	15.457 a <sup>B</sup>	7.691 cd <sup>B</sup>	14.041 bc <sup>A</sup>	1.458 bc <sup>A</sup>
	90	2.554c <sup>B</sup>	2.444d <sup>B</sup>	1.543c <sup>B</sup>	3.823c <sup>B</sup>	3.465 d <sup>B</sup>	10.037 c <sup>B</sup>	16.551 a <sup>B</sup>	8.936 d <sup>B</sup>	15.369 c <sup>A</sup>	1.684 c <sup>B</sup>

SCFA: short chain fatty acids (C<sub>4</sub> to C<sub>8</sub>); MCFA: medium chain fatty acids (C<sub>10</sub> to C<sub>14</sub>); LCFA: long chain fatty acids (C<sub>16</sub> to C<sub>18:2</sub>); RMC: Raw milk Örgü cheese; PMC: pasteurized milk Örgü cheese.

higher butyric acid in Feta cheese was due to pregastric enzyme present in the artisanal rennet used.

Caproic acid (C6:0) concentration in RMC was also significantly ( $P < 0.05$ ) higher than in PMC throughout ripening period. At 90 th d, caproic acid content in RMC was 4.525 mg 100 g<sup>-1</sup> cheese, (3.82 % of the TFFA), compared to 2.444 mg 100 g<sup>-1</sup> cheese (3.68 % of the TFFA) in PMC. Caprylic acid (C8:0) concentration in RMC was also significantly ( $P < 0.05$ ) higher than that in cheese PMC throughout ripening. In RMC, the content of Caprylic acid was 2.856 mg 100 g<sup>-1</sup> cheese (2.412% of the TFFA) and 1.543 mg 100 g<sup>-1</sup> cheese (2.32 % of the TFFA) at the last period or ripening. Lauric acid (C12:0) and myristic acid (C14:0) contents showed the same tendency as short chain free fatty acids (SCFFAs) in both cheeses, throughout ripening period. RMC had higher amounts of Lauric acid and Myristic acid than PMC, but their percentage in TFFA were similar. Lauric acid contents of RMC and PMC at 90th d 5.10 and 5.22 % of the TFFA. Myristic acid was 16.30 and 15.12 of the TFFA in RMC and PMC respectively. The percentages of palmitic acid (C16:0) (C18:1) in RMC and PMC at 90th d were approximately 25.15 and 24.92%, and oleic acid were 20.24 and 23.14%, respectively.

Alichanidis et al. (1984) and Vafopoulou et al. (1989) reported that palmitic acid and stearic acid were the major FFAs in experimental industrial type Feta cheese. Also, Mallatou et al. (2003) found this group of FFA to be the most abundant throughout ripening of Teleme cheese made from a mixture of ewes/goats milk. Mulet et al. (1999) reported that decanoic acid was the most abundant volatile for fresh and ripened Mahon cheese. They also reported that the behaviours of tetradecanoic and dodecanoic acids were similar and their concentrations could be considered constant within the ripening period. Kaminarides et al. (2007) revealed that palmitic and oleic acid, which do not intrinsically contribute to

cheese flavour quite as much as short-chain FFAs do, since they have higher perception thresholds, dominated among the saturated and unsaturated long-chain FFAs of Halloumi cheese, which is processed with high thermal kneading of the curd after pressing, and kept in brine like Örgü cheese. Ziino et al. (2005) reported that hexanoic acid showed the highest area value in "Provola dei Nebrodi", a typical Sicilian cheese, and during all ripening stages; octanoic, butanoic and decanoic acids followed in decreasing order. During the ripening period, the area values of most FFAs had an increasing trend, excluding pentanoic acid.

The relatively higher increase was viewed in the concentration of SCFFA (C4 to C8), which has a significant impact on the development of characteristic aroma of cheese, during ripening than medium chain free fatty acids (MCFFA) (C10 to C14) and long chain free fatty acids (LCFFA) (C16 to C18:2) (Table 2). This could mainly be due to specificity of milk lipoprotein lipase and starter lipase towards FFA located at the positions sn-1 and sn-3 of the triglyceride. Generally SCFFA are predominantly esterified at the outer esters bond of tri- or diacylglycerides (Juarez et al., 2003; Collins et al., 2003). Medium and long-chain FFA represented approximately 24 to 27% and 63 to 72% respectively, of all FFA in the RMC and PMC. Despite the quantitative importance of medium and long-chain FFA, they are not the main contributors to cheese flavor (Rahmat and Richter, 1996; Freitas and Malcata, 1998). Butyric acid was the main FFA in SCFFA experimental cheese samples, ranging from 4.73 mg 100 g<sup>-1</sup> cheese in RMC to 2.55 mg 100 g<sup>-1</sup> of cheese in PMC. The predominant FFAs were myristic acid in MCFFA, and palmitic acid in LCFFA in Örgü cheeses, reaching values that 29.78 and 16.55 mg 100 g<sup>-1</sup> of cheese in RMC and PMC respectively, at the end of ripening period. SCFFA, MCFFA and LCFFA contents of PMC were lower than RMC on 60 and 90th days.

**Table 3.** Sensory evaluation of 90 day-old cheese samples A.

Cheese	Days	Appearance (20)	Body-texture (35)	Odor (10)	Flavour (35)
RMC	90	18.891 <sup>a</sup>	33.945 <sup>a</sup>	9.349 <sup>a</sup>	33.447 <sup>a</sup>
PMC	90	14.900 <sup>a</sup>	30.440 <sup>a</sup>	6.534 <sup>b</sup>	23.038 <sup>b</sup>

<sup>A</sup> Means with different letters within each column were significantly different ( $P < 0.05$ ). RMC: Raw milk cheese PMC: Pasteurized milk cheese.

*Lactococcus* spp. and *Lactobacillus* spp. were reported to have lower lipolytic activity than other bacteria and moulds (Fox et al., 2000).

Generally, the TFFA content of Örgü cheese was evidently lower than that of many other cheese varieties. The lower degree of lipolysis in Örgü cheese may be due to scalding the curd in hot 5% brine at 72 to 76°C for 5 to 6 min, which inactivates the indigenous lipase (McSweeney and Sousa, 2000; Mallatou et al., 2003). Since Örgü cheese was ripened in highly concentrated (14% salt) brine, its pH and salt content (Celik and Turkoglu, 2007) are far from the optimum action values of native milk lipase (Driessen, 1989). Inhibitory effects of NaCl on lipolytic activity have been reported by some researchers (Pavia et al., 2000; Vlaemyneck, 1992). On the other hand, the low lipolysis in Örgü cheese could be associated with low storage temperature. Lipolysis in white pickled cheese stored at 5°C was also reported to be lower than cheese stored at 10 to 20°C (Abd El-Salam et al., 1993).

### Organoleptic evaluation

According to results of sensory analysis, appearance and body-texture properties of PMC were not significantly inferior to that of RMC. However, PMC failed to reach the expected quality of odor and flavour level (Table 3).

### Conclusions

The percentages of MCFFA and LCFFA were higher than SCFFA at all ages. However, the higher relative increase was determined in SCFFA. The relative increases in SCFFA of RMC were higher than that of PMC at the end of storage. Palmitic (C16:0), and oleic acids (C18:1) were the most abundant FFA in fresh and ripened Örgü cheeses. The results clearly show that pasteurization of milk prior to cheese-making has a marked influence not only on the level of lipolysis throughout ripening, but also on the relative amounts of SCFFA. It can be concluded that native lipases and/or non starter lactic acid bacteria were primarily responsible for the development of lipolysis in Örgü cheese. The results also demonstrated that restricted lipolysis occurred in PMC during ripening as compared to RMC. In order to improve organoleptic properties such as odor and flavour of PMC, future

studies should be intensified on the selection of suitable starter combination to manufacture Örgü cheese having close lipolytic characteristics to the traditional ones. Also, further studies should be dedicated to higher lipolytic level of Örgü cheese without impairing the nature of the end products.

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