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Changes of composition and free fatty acid contents of Urfa cheeses (a white-brined Turkish cheese) during ripening: Effects of heat treatments and starter cultures

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Abstract

The influences of heat treatments (at 65 °C for 20 min or 72 °C for 5 min) applied to the milk and addition of mesophilic or thermophilic starter cultures, prior to cheese-making, on the composition and free fatty acid contents of Urfa cheeses were evaluated throughout the ripening period. Sensory evaluation of cheese samples was also performed on 90th day. The basic composition of ripened cheese samples was not significantly affected by the heat treatments and starter cultures. Heat treatments adversely affected the lipolysis and sensory properties of Urfa cheeses, particularly at 72 °C. The FFA contents of cheeses made from mesophilic and thermophilic cultures were similar. Cheese made from raw milk had a higher level of lipolysis than the cheeses made from milk inoculated with mesophilic or thermophilic lactic starters ($p < 0.05$).

Keywords: Urfa cheese; Lipolysis; Heat treatment; Mesophilic and thermophilic lactic cultures

1. Introduction

Lipolysis in cheese is due to the presence of lipolytic enzymes, which cleave the ester linkage between a fatty acid and the glycerol core of the triacylglyceride, producing free fatty acids (FFA), and mono-and diacylglycerides. In cheese, lipid hydrolysis results in the formation of FFA, which may, directly contribute to cheese flavour, especially short and intermediate-chain FFA, and also serve as substrates for further reactions, producing highly flavoured catabolic end products (Collins, McSweeney, & Wilkinson, 2003).

Urfa cheese production is about 35,000–40,000 tonnes per annum (Özer, Atasoy, & Akın, 2002). Urfa cheese is a traditional semi-hard brined Turkish cheese type manufactured mainly in the southeast Anatolia region of Turkey from raw ovine milk or appropriate mixtures of ovine and caprine milk, without any starter culture. High temperatures used in the production of Urfa cheese are necessary to eliminate the pathogens which may be present in raw milk.

The main goal of milk pasteurisation for cheese-making is the elimination of pathogens, even though post-pasteurisation contamination may occur. However, pasteurisation changes the biochemistry and microbiology of ripening, as well as the flavour of the cheese (Beuvier et al., 1997; Psoni, Tzanetakis, & Litopoulou-Tzanetaki, 2006). Inactivation of indigenous milk pro-enzymes and enzymes, elimination of milk microorganisms, and modification in activity of starter bacteria are characteristics of milk, which could be changed by heat treatment (Grappin & Beuvier, 1997). Generally, raw milk cheeses mature faster and have more intense and unique flavour, but their flavour is less uniform than the flavour of cheeses produced from

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pasteurised milk (Psoni et al., 2006). Moreover, the daytime temperature in southeast Anatolia is well above the national average. As a result, the use of raw milk in the production of Urfa cheese carries a potential health risk.

Lactic acid bacteria (LAB) added as the starter culture or present as non-starter lactic acid bacteria (NSLAB) are able to transform lactic acid, citrate, lactate, proteins and fat into volatile compounds (Ortigosa, Barcenas, Arizcun, Perez-Elortondo, Albisu & Torre, 1999). In addition, the enzymes from cheese-related microorganisms are the chief factors responsible for the formation of many compounds that are essential for cheese flavour (El Soda, Madkor & Tong 2000).

Lipolysis is an important biochemical event occurring during cheese ripening and has been studied extensively in many varieties. However, lipolysis is very complex and differs from one cheese variety to another. To the best of our knowledge, no study, so far, has been carried out on the FFA content of Urfa cheese. Therefore, the objectives of this study were to investigate the effects of heat treatments (at 65 °C for 20 min or at 72 °C for 5 min), starter culture combinations (*Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris* or *Lactobacillus delbrueckii* subsp. *bulgaricus* + *Streptococcus thermophilus*), and ripening time on the lipolysis of the Urfa cheese.

2. Materials and methods

2.1. Materials

Raw ovine milk supplied from a local dairy (Sanlıurfa, Turkey) was used in the manufacture of cheese samples. The mean composition of milk used in the production of Urfa cheeses was pH 6.45 ± 0.02, total nitrogen 0.77 ± 0.23 g $100\ g^{-1}$, fat 6.75 ± 0.26 g $100\ g^{-1}$, and total solids 16.95 ± 0.18 g $100\ g^{-1}$. Rennet of animal origin was used to coagulate the milk. Freeze-dried starter cultures were obtained from Peyma-Chr. Hansen (Istanbul, Turkey). Mesophilic starter was a blend of *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* and thermophilic starter was a blend of *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* in equal proportions.

2.2. Methods

2.2.1. Cheese-making and sampling

Five Urfa cheese-making trials denoted O_0 (raw milk cheese), O_1 (heat treated at 65 °C for 20 min, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* added milk cheese), O_2 (heat treated at 65 °C for 20 min, *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added milk cheese), O_3 (heat treated at 72 °C for 5 min, *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* added milk cheese), and O_4 (heat treated at 72 °C for 5 min, *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added milk cheese). Urfa cheeses were made from either raw or heat-treated milk, using starter culture, with three experiments each repeated three times on different days. In the first experiment, milk (50 kg) (control cheese) was warmed to 32 °C. Then raw milk was coagulated by adding 9 ml of animal rennet (strength 1/10 000) per 50 kg milk for 90 min. After curdling, the curd was cut into small cubes, approximately 1 cm^3, and left to rest (15 min). The whey separation was achieved without pressing by leaving curd hanging in special moulds of triangular shape cheese cloths called "parzin", for about 18 h at room temperature. After whey separation was complete, the cheese blocks were scalded in their own whey at 90 °C for 3 min. The cheese blocks were cooled down to room temperature and then placed into plastic containers aseptically. Cheeses were brine-salted at 4 °C for 90 days in 14% (w/v) brine concentration.

In the second and third experiments, milk (each part 100 kg) was heated at 65 °C for 20 min and 72 °C for 5 min in the vat, respectively, and cooled to 32 °C. After cooling, $CaCl_2$ was added at rate of 0.02%. Each part was divided into two separate batches. The first batch of each experiment was inoculated with mesophilic culture at a rate of 1.0% (w/w) and second batch of each experiment was inoculated with thermophilic culture at a rate of 0.5% (w/w). Milk was rested at 32 °C for 30 min to allow growth of starter bacteria before renneting. The rest of the manufacturing procedure was identical to the control cheese.

From each batch, 1, 15, 30, 60 and 90 day-old cheeses were taken. Each sample from each batch consisted of four cheese blocks (each block contained approximately 250 g of cheese). At the laboratory, the cheese blocks were ground and kept in airtight containers at −40 °C until they were analysed.

2.2.2. Cheese analyses

2.2.2.1. Chemical analyses. Total solids, titratable acidity and salt were determined according to Turkish Standards (Turkish Standards (TS), 1989). The pH was determined by using a pH meter (Orion 420). The fat content was determined by the Gerber method (Turkish Standards (TS), 1978). The total nitrogen (TN) was measured by Kjeldahl method (International Dairy Federation (IDF), 1993). All analyses were performed in duplicate.

2.2.2.2. Free fatty acid analysis. Fatty acids were isolated as outlined by Garcia-Lopez, Echeverria, Tsui, and Balch (1994). Cheese (10 g) was ground and fatty acids were extracted with a mixture of methanol and methylene chloride. Fat samples were methylated according to the procedure of Sukhija and Palmquist (1988).

Fatty acid methyl esters were analysed using a gas chromatograph (Shimadzu GC-17 AAF, V3, 230 V series; Shimadzu Corporation, Kyoto, Japan) equipped with flame ionisation detector (FID), and fitted with a fused silica capillary column (SP-2380, 100 m × 0.25 mm; Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas. Injector and detector temperature was 250 °C. The initial oven

temperature was 40 °C for 1.0 min, then increased to 240 °C at 5 °C/min. The final temperature was maintained for 10 min. The internal standard used was nonanoic acid. A standard fatty acid mixture containing 50 fatty acids and purified known individual fatty acids were used to provide standard retention times. Fatty acids were identified by comparing their retention times with those of fatty acids in standard samples.

2.2.2.3. Sensory analyses. The sensory evaluations of the 90 day-old cheeses were carried out with three replications by 10 trained panellists who were members of the Division of the Dairy Technology Department of Food Engineering, Harran University. The attributes of cheese were organised into flavour (odour and taste), body and texture, colour and appearance, and saltiness categories. Flavour attributes were scored between 0 (the worst) and 10 (the best quality). Body and texture, colour and appearance attributes were assessed on a 0–5 scale, scoring 0 for the worst and five for the best quality. Panellists were asked to evaluate the saltiness (0 = extremely salty, 5 = not salty). The total score was obtained by adding the scores for the four attributes. An excellent cheese would receive a total score of 25.

2.2.2.4. Statistical analyses. Results were processed by analysis of variance using the general linear models procedure of SAS system (1990) software, Version 6 (SAS Institute Inc., Cory, NJ). Duncan's multiple comparison tests were applied to the data. Evaluations were based on a significance level of $p < 0.05$.

3. Results and discussion

3.1. Cheese compositions

The chemical compositions of the cheese samples are shown in Table 1. The heat treatments applied to the milk did not affect ($p > 0.05$) the total solids, pH, titratable acidity, total nitrogen, fat (dry weight) and salt (dry weight) of 90 day-old cheeses. Moreover, the addition of mesophilic and thermophilic cultures did not change the chemical compositions of the ripened cheese. At the end of storage, no differences in basic composition levels were observed between mesophilic (O_1 and O_3), and thermophilic (O_2 and O_4) culture-added milk cheeses, nor between heat treated at 65 °C (O_1 and O_2) and heat treated at 72 °C (O_3 and O_4) milk cheeses, except for titratable acidity and fat (dry weight) ($p < 0.05$).

Table 1
The chemical composition of Urfa cheese samples at 1, 15, 30, 60 and 90 days[A]

Cheese samples	Ripening period (days)	Total solids (g 100 g^{-1})	pH	Titratable acidity (g 100 g^{-1} a)	Total nitrogen (g 100 g^{-1})	Fat as dry matter (g 100 g^{-1})	Salt as dry matter (g 100 g^{-1})
O_0	1	54.7 ± 0.52^{a1}	5.08 ± 0.11^{ab1}	1.07 ± 0.08^{a2}	3.39 ± 0.02^{a12}	48.8 ± 1.00^{a}	7.30 ± 0.66^{d}
	15	52.9 ± 0.52^{b1}	5.04 ± 0.15^{b12}	0.84 ± 0.01^{c3}	3.27 ± 0.03^{a1}	46.2 ± 0.75^{b12}	9.82 ± 0.05^{c}
	30	52.7 ± 0.85^{b1}	5.12 ± 0.13^{ab12}	0.94 ± 0.09^{abc}	3.10 ± 0.01^{b1}	47.1 ± 1.18^{b12}	10.6 ± 0.10^{bc1}
	60	50.8 ± 0.50^{c1}	5.13 ± 0.12^{ab12}	1.02 ± 0.04^{ab2}	2.69 ± 0.06^{c}	46.4 ± 0.48^{b12}	11.5 ± 0.43^{a2}
	90	49.3 ± 0.28^{d1}	5.15 ± 0.09^{a}	0.90 ± 0.05^{bc12}	2.62 ± 0.03^{c}	45.8 ± 0.27^{b12}	11.0 ± 0.30^{ab}
O_1	1	53.8 ± 0.42^{a12}	4.73 ± 0.04^{c2}	1.26 ± 0.01^{a1}	3.37 ± 0.04^{a12}	47.6 ± 1.52	7.07 ± 0.81^{c}
	15	51.5 ± 1.25^{b123}	4.69 ± 0.06^{c3}	0.87 ± 0.03^{c23}	3.14 ± 0.07^{b12}	48.2 ± 1.09^{1}	9.74 ± 0.47^{b}
	30	50.8 ± 1.39^{bc12}	4.95 ± 0.04^{b2}	0.96 ± 0.10^{bc}	2.98 ± 0.04^{c2}	46.9 ± 1.03^{12}	10.4 ± 0.09^{b12}
	60	49.9 ± 1.70^{c12}	5.04 ± 0.02^{ab2}	1.06 ± 0.02^{b12}	2.80 ± 0.07^{d}	47.9 ± 1.15^{1}	11.6 ± 0.57^{a2}
	90	48.3 ± 1.26^{d12}	5.09 ± 0.02^{a}	0.87 ± 0.06^{c2}	2.67 ± 0.09^{d}	48.6 ± 0.40^{1}	11.9 ± 0.89^{a}
O_2	1	53.3 ± 0.36^{a12}	5.07 ± 0.03^{b1}	1.19 ± 0.07^{a12}	3.35 ± 0.15^{a12}	46.6 ± 1.40^{b}	7.02 ± 0.03^{c}
	15	52.4 ± 0.43^{ab12}	5.05 ± 0.09^{b1}	0.92 ± 0.01^{b12}	3.09 ± 0.08^{b2}	46.6 ± 1.14^{b12}	9.73 ± 0.21^{b}
	30	51.3 ± 1.07^{bc12}	5.18 ± 0.06^{a1}	1.05 ± 0.01^{ab}	2.88 ± 0.04^{c2}	48.9 ± 1.53^{a1}	10.1 ± 0.04^{b2}
	60	50.3 ± 0.77^{c12}	5.23 ± 0.05^{a1}	1.05 ± 0.04^{ab12}	2.78 ± 0.05^{c}	48.1 ± 0.91^{ab1}	11.3 ± 0.08^{a2}
	90	48.8 ± 0.49^{d12}	5.19 ± 0.07^{a}	0.96 ± 0.08^{b12}	2.60 ± 0.06^{d}	47.0 ± 0.89^{b12}	11.5 ± 0.58^{a}
O_3	1	51.5 ± 0.47^{a3}	4.99 ± 0.03^{bc1}	1.23 ± 0.07^{a12}	3.30 ± 0.04^{a2}	45.8 ± 1.61^{a}	8.14 ± 0.28^{c}
	15	49.6 ± 0.10^{b3}	4.77 ± 0.06^{c23}	0.94 ± 0.03^{b12}	3.12 ± 0.01^{b12}	44.0 ± 0.42^{b2}	10.2 ± 0.06^{b}
	30	49.7 ± 0.16^{b2}	4.95 ± 0.06^{d2}	0.98 ± 0.04^{b}	2.97 ± 0.03^{c2}	44.5 ± 0.28^{ab2}	10.7 ± 0.10^{b1}
	60	47.8 ± 0.15^{c2}	5.08 ± 0.04^{ab12}	1.16 ± 0.09^{a12}	2.64 ± 0.05^{d}	43.8 ± 0.49^{b3}	12.8 ± 0.07^{a1}
	90	46.4 ± 0.07^{d2}	5.17 ± 0.02^{a}	1.08 ± 0.04^{ab1}	2.54 ± 0.05^{d}	44.9 ± 0.31^{ab2}	12.3 ± 0.33^{a}
O_4	1	52.7 ± 0.90^{a23}	4.89 ± 0.07^{cd12}	1.17 ± 0.04^{ab12}	3.57 ± 0.01^{a1}	44.6 ± 2.06	7.82 ± 0.71^{c}
	15	50.5 ± 0.23^{b23}	4.83 ± 0.04^{d123}	0.96 ± 0.03^{c1}	3.19 ± 0.01^{b12}	44.1 ± 0.48^{2}	10.4 ± 0.07^{b}
	30	49.8 ± 0.41^{b2}	4.97 ± 0.01^{bc12}	1.12 ± 0.01^{abc}	2.96 ± 0.04^{c2}	45.5 ± 0.77^{2}	10.7 ± 0.22^{b1}
	60	48.5 ± 0.61^{c12}	5.05 ± 0.02^{ab12}	1.20 ± 0.02^{a1}	2.81 ± 0.04^{d}	45.2 ± 0.29^{23}	12.0 ± 0.07^{a12}
	90	48.4 ± 1.36^{c12}	5.08 ± 0.05^{a}	1.02 ± 0.04^{bc12}	2.59 ± 0.11^{e}	44.9 ± 2.28^{2}	11.9 ± 0.71^{a}

[a–e]Means in each column with different letters were significantly affected by storage periods ($p < 0.05$); [1–3]means in each column with different numbers were significantly different between cheese samples at a similar ripening period ($p < 0.05$); O_0:raw milk cheese; O_1: heat treated at 65 °C for 20 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. c*remoris* added; O_2: heat treated at 65 °C for 20 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added; O_3: heat treated at 72 °C for 5 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. c*remoris* added; O_4: heat treated at 72 °C for 5 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added.

[A] Mean of three replicates.

In general, the total solids and total nitrogen content of all cheese samples decreased during storage. The decrease in total solids may be due mainly to breaking of peptide bonds and releasing new ionic groups, and increasing water-binding capacity of proteins (Creamer & Olson, 1982). The reduction in total nitrogen could be due to hydrolysis of proteins to water-soluble nitrogenous compounds and to the diffusion of these products into the brine (Abd El-Salam, Alichanidis, & Zerfiridis, 1993). Atasoy, Türkoglu, and Yetişmeyen (2006) reported that nitrogenous compounds in brine increased during ripening of Urfa cheese. The continuous decrease in total solids and nitrogen content of Urfa cheese throughout cold storage has been reported previously (Özer et al., 2002).

The pHs of cheese samples increased after 15 days of ripening. In parallel with the variation in pH values, the titratable acidity of cheese samples decreased until the 15 day old cheese, then fluctuated during the rest of storage. However, there were no significant differences in the titratable acidity between 15 and 90 day old cheeses for all samples.

The fat (as dry matter) content of raw milk cheese sample significantly decreased ($p < 0.05$) during ripening, however, heat-treated milk cheeses did not change at the end of storage. The salt content (as dry matter) of all cheese samples increased until day 60 and remained unchanged after this day. This means that the salt in the moisture in cheese and the salt concentration in brine reached an equilibrium state in 60 day old cheese. Salt is driven into cheese by the concentration gradient between cheese blocks and brine (Azarnia, Ehsani & Mirhadi, 1997).

3.2. The concentrations of free fatty acids

The concentrations of FFA in the cheese samples at different stages of storage are shown in Tables 2 and 3. There were no significant differences in the butyric, caproic and caprylic acid contents between raw milk cheese (O_0) and cheeses heat treated at 65 °C (O_1 and O_2) at the beginning of ripening. These results were also observed between raw milk cheese (O_0) and cheeses heat treated at 72 °C (O_3 and O_4), except for caproic acid ($p < 0.05$). However, in fresh cheese, there were differences ($p < 0.001$) in the capric acids concentration between raw milk cheese and low and high heat treated milk cheeses. At the end of storage, the volatile FFA ($C_{4:0}$–$C_{10:0}$) content of raw milk cheese was significantly ($p < 0.01$) higher than cheeses heat treated at 65 °C and 72 °C. Moreover, differences ($p < 0.05$) in butyric, caproic, caprylic and capric acid levels were observed between 90 day-old low and high heat treated cheeses.

Table 2
Volatile free fatty acids (mg 100 g^{-1} cheese) of Urfa cheese samples at different stages of storages[A]

Cheese samples	Ripening period (days)	Butyric acid ($C_{4:0}$)	Caproic acid ($C_{6:0}$)	Caprylic acid ($C_{8:0}$)	Capric acid ($C_{10:0}$)
O_0	1	0.81 ± 0.08^{d1}	0.70 ± 0.06^{e1}	0.40 ± 0.05^{e12}	2.44 ± 0.04^{d1}
	15	1.64 ± 0.05^{c1}	1.03 ± 0.05^{d1}	0.59 ± 0.10^{d}	2.50 ± 0.06^{d1}
	30	1.63 ± 0.07^{c1}	1.42 ± 0.08^{c1}	1.22 ± 0.06^{c1}	3.11 ± 0.08^{c1}
	60	2.05 ± 0.07^{b12}	1.99 ± 0.06^{b1}	1.37 ± 0.02^{b1}	3.78 ± 0.07^{b1}
	90	2.67 ± 0.04^{a1}	2.32 ± 0.08^{a1}	1.60 ± 0.05^{a1}	3.95 ± 0.04^{a1}
O_1	1	0.80 ± 0.03^{d1}	0.40 ± 0.05^{e2}	0.30 ± 0.04^{d2}	1.81 ± 0.09^{e2}
	15	1.64 ± 0.06^{b1}	0.88 ± 0.02^{d2}	0.66 ± 0.06^{c}	2.00 ± 0.08^{d2}
	30	1.38 ± 0.07^{c2}	1.21 ± 0.02^{c2}	0.95 ± 0.05^{b2}	2.38 ± 0.08^{c3}
	60	1.82 ± 0.09^{b23}	1.83 ± 0.06^{b1}	1.25 ± 0.02^{a12}	2.71 ± 0.02^{b3}
	90	2.25 ± 0.05^{a2}	2.01 ± 0.04^{a2}	1.34 ± 0.03^{a23}	3.17 ± 0.03^{a2}
O_2	1	0.82 ± 0.08^{c1}	0.72 ± 0.03^{d1}	0.39 ± 0.03^{d12}	1.73 ± 0.04^{d2}
	15	0.73 ± 0.10^{c3}	0.78 ± 0.04^{d23}	0.49 ± 0.01^{d}	1.95 ± 0.05^{c2}
	30	1.39 ± 0.08^{b2}	1.04 ± 0.05^{c3}	0.87 ± 0.06^{c23}	2.57 ± 0.06^{b23}
	60	2.14 ± 0.06^{a1}	1.60 ± 0.03^{b2}	1.23 ± 0.06^{b2}	2.66 ± 0.06^{b3}
	90	2.11 ± 0.08^{a23}	1.92 ± 0.02^{a23}	1.41 ± 0.05^{a2}	2.83 ± 0.08^{a3}
O_3	1	0.93 ± 0.03^{d1}	0.54 ± 0.13^{d12}	0.37 ± 0.02^{d2}	1.76 ± 0.06^{c2}
	15	1.28 ± 0.04^{c2}	0.69 ± 0.06^{cd3}	0.51 ± 0.04^{c}	1.88 ± 0.06^{c23}
	30	1.67 ± 0.07^{b1}	0.87 ± 0.04^{c4}	0.88 ± 0.08^{b23}	2.60 ± 0.08^{b2}
	60	1.84 ± 0.08^{b23}	1.55 ± 0.05^{b23}	1.17 ± 0.03^{a2}	2.87 ± 0.04^{a2}
	90	2.05 ± 0.06^{a3}	1.77 ± 0.04^{a3}	1.28 ± 0.05^{a23}	2.84 ± 0.04^{a3}
O_4	1	0.51 ± 0.04^{d2}	0.36 ± 0.03^{d2}	0.48 ± 0.02^{e1}	1.42 ± 0.10^{e3}
	15	1.03 ± 0.11^{c2}	0.45 ± 0.03^{d4}	0.62 ± 0.08^{b}	1.75 ± 0.07^{d3}
	30	1.61 ± 0.06^{b1}	0.76 ± 0.03^{c4}	0.74 ± 0.09^{b3}	1.92 ± 0.04^{c4}
	60	1.63 ± 0.13^{b3}	1.40 ± 0.05^{b3}	1.13 ± 0.08^{a2}	2.37 ± 0.03^{b4}
	90	2.03 ± 0.06^{a3}	1.75 ± 0.07^{a3}	1.22 ± 0.04^{a3}	2.67 ± 0.05^{a3}

[a–e]Means in each column with different letters were significantly affected by storage period ($p < 0.05$); [1–4]means with different numbers were significantly different between cheese samples at a similar ripening period ($p < 0.05$); O_0: raw milk cheese; O_1: heat treated at 65 °C for 20 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* added; O_2: heat treated at 65 °C for 20 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added; O_3: heat treated at 72 °C for 5 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* added; O_4: heat treated at 72 °C for 5 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added.

[A] Mean of three replicates.

The addition of mesophilic or thermophilic culture to the milk affected ($p < 0.001$) the volatile FFAs at the end of storage. However, there were no significantly differences in volatile FFAs, except for capric acid, between mesophilic and thermophilic culture-added ripened cheeses. The concentration of volatile FFAs content of all experimental cheese increased during ripening. These findings are in good agreement with previous studies for different brined cheese (Akın, Aydemir, Koçak, & Yıldız, 2003; Kondyli, Katsiari, Masouras, & Voutsinas, 2002). The highest contents of volatile FFAs were observed in raw milk cheese. The dominant volatile FFA was capric acid for all experimental cheese samples. A similar result was observed for ewes' milk cheeses (Perotti, Bernal, Meinardi, & Zalazar 2005; Zhang, Mustafa, Ng-Kwai-Hang, & Zhao 2006).

In the early stages of ripening, there were no statistical differences in FFAs (C_{12}–$C_{18:2}$) between raw and low heated milk cheeses, except for oleic and linoleic acids. However, the heat treatments applied to milk statistically influenced ($p < 0.001$) the FFA content of 90 day-old cheeses. At the same time, differences ($p < 0.01$) in FFA were observed between low heated (65 °C) milk cheeses and high heated (72 °C) milk cheeses, except for palmitic and linolenic acid. At the same heat treatments, cheeses made with mesophilic culture had similar FFAs content with its thermophilic counterpart, except for lauric acid, at the beginning and end of storage.

At day 90, short ($C_{4:0}$–$C_{8:0}$), medium ($C_{10:0}$–$C_{14:0}$) and long-chain FFA ($C_{16:0}$–$C_{18:2}$) represented approximately 7%–8%, 20%–23% and 70%–72%, respectively, of all FFA in all experimental cheeses. Despite the quantitative importance of medium and long-chain FFA, they are not the main contributors to cheese flavour (Freitas & Malcata, 1998; Rahmat & Richter 1996). Butyric acid was the main short chain FFA in all cheeses, ranging from 2.67 mg 100 g^{-1} of cheese in raw milk cheese to 2.03–2.25 mg 100 g^{-1} of cheese in cheeses made from heat-treated milk and added starter culture. Myristic acid was the predominant medium-chain FFA. Palmitic acid was the main long-chain FFA in the five types of cheese, reaching, after 90 days of storage, values that ranged between 22.43 and 23.77 mg 100 g^{-1} of cheese in O_1, O_2, O_3 and O_4 cheeses, and 26.93 mg 100 g^{-1} of cheese in O_0.

The total free fatty acid content of raw milk cheese was markedly higher than that of heat-treated milk cheeses. It is well known that milk lipoprotein lipase is a relatively heat-labile enzyme, which can be completely inactivated by a thermal treatment of 78 °C for 10 s (Driessen, 1989). The

Table 3
Free fatty acids (mg 100 g^{-1} cheese) of cheese samples at during ripening[A]

Cheese samples	Ripening period (days)	Lauric acid ($C_{12:0}$)	Myristic acids ($C_{14:0}$)	Palmitic acid ($C_{16:0}$)	Stearic acid ($C_{18:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)
O_0	1	2.28 ± 0.02^{d23}	7.98 ± 0.33^{c1}	18.4 ± 0.08^{d12}	7.27 ± 0.45^{e}	14.5 ± 0.42^{d1}	1.16 ± 0.05^{d1}
	15	2.35 ± 0.03^{d2}	8.23 ± 0.55^{c12}	19.3 ± 0.28^{cd}	8.77 ± 0.31^{d1}	14.3 ± 0.49^{d1}	1.56 ± 0.06^{a1}
	30	2.90 ± 0.03^{c1}	8.36 ± 0.51^{c2}	19.5 ± 0.45^{c}	12.2 ± 0.26^{c1}	15.5 ± 0.58^{c1}	1.30 ± 0.01^{b1}
	60	3.68 ± 0.06^{b1}	9.30 ± 0.13^{b2}	23.4 ± 0.86^{b1}	15.3 ± 0.57^{b1}	17.6 ± 0.63^{b1}	1.22 ± 0.02^{c1}
	90	4.05 ± 0.05^{a1}	12.4 ± 0.44^{a1}	26.9 ± 0.43^{a1}	17.5 ± 0.75^{a1}	19.0 ± 0.29^{a1}	1.14 ± 0.02^{d1}
O_1	1	2.36 ± 0.10^{d2}	6.82 ± 0.35^{d2}	19.0 ± 0.39^{c1}	7.14 ± 0.23^{d}	12.7 ± 0.31^{c2}	0.79 ± 0.01^{c2}
	15	2.21 ± 0.03^{e3}	7.43 ± 0.16^{c23}	19.1 ± 0.33^{c}	7.84 ± 0.21^{d2}	13.0 ± 0.23^{bc2}	0.85 ± 0.01^{b2}
	30	2.59 ± 0.02^{c3}	7.86 ± 0.09^{c23}	19.6 ± 0.45^{c}	8.71 ± 0.37^{c2}	13.6 ± 0.01^{b2}	0.92 ± 0.01^{a23}
	60	2.81 ± 0.08^{b2}	8.75 ± 0.07^{b3}	20.7 ± 0.53^{b2}	9.96 ± 0.17^{b2}	14.8 ± 0.57^{a2}	0.87 ± 0.01^{ab2}
	90	3.16 ± 0.03^{a2}	10.4 ± 0.43^{a2}	22.5 ± 0.69^{a23}	12.3 ± 0.16^{a2}	15.4 ± 0.71^{a2}	0.83 ± 0.02^{bc3}
O_2	1	2.33 ± 0.03^{d2}	8.06 ± 0.04^{d1}	19.0 ± 0.47^{d1}	7.08 ± 0.42^{d}	12.8 ± 0.32^{b2}	0.77 ± 0.02^{a23}
	15	2.60 ± 0.03^{c1}	8.70 ± 0.39^{d1}	19.5 ± 0.28^{d}	7.69 ± 0.40^{cd23}	13.2 ± 0.26^{b2}	0.83 ± 0.01^{b2}
	30	2.62 ± 0.05^{c23}	9.87 ± 0.06^{c1}	20.5 ± 0.76^{c}	8.30 ± 0.65^{c2}	13.3 ± 0.17^{b2}	0.96 ± 0.02^{a2}
	60	2.82 ± 0.09^{b2}	11.3 ± 0.02^{b1}	22.0 ± 0.23^{b12}	9.85 ± 0.26^{b2}	15.1 ± 0.26^{a2}	0.91 ± 0.01^{a2}
	90	3.07 ± 0.06^{a2}	11.8 ± 0.05^{a1}	23.8 ± 0.22^{a2}	12.0 ± 0.15^{a23}	15.5 ± 0.21^{a2}	0.83 ± 0.01^{b3}
O_3	1	2.12 ± 0.02^{b3}	7.57 ± 0.03^{d1}	18.0 ± 0.20^{c2}	6.98 ± 0.37^{d}	13.0 ± 0.20^{b2}	0.70 ± 0.01^{c3}
	15	2.19 ± 0.04^{b3}	7.79 ± 0.07^{cd123}	18.8 ± 016^{c}	6.93 ± 0.14^{d3}	13.7 ± 0.09^{b12}	0.80 ± 0.01^{b2}
	30	2.24 ± 0.03^{b4}	8.26 ± 0.21^{c23}	20.1 ± 0.43^{b}	8.20 ± 0.12^{c2}	13.2 ± 0.38^{b2}	0.86 ± 0.02^{a4}
	60	2.47 ± 0.06^{a3}	8.74 ± 0.34^{b34}	21.5 ± 0.14^{a2}	9.51 ± 0.32^{b2}	15.1 ± 0.10^{a2}	0.87 ± 0.02^{a2}
	90	2.54 ± 0.03^{a4}	10.1 ± 0.08^{a2}	22.4 ± 0.16^{a3}	10.8 ± 0.23^{a34}	14.9 ± 0.09^{a2}	0.87 ± 0.01^{a23}
O_4	1	2.83 ± 0.06^{a1}	6.37 ± 0.13^{d2}	18.8 ± 0.12^{d12}	7.05 ± 0.20^{c}	12.9 ± 0.18^{d2}	0.72 ± 0.01^{c23}
	15	2.44 ± 0.03^{b2}	6.76 ± 0.26^{d3}	19.5 ± 0.28^{cd}	7.09 ± 0.02^{c23}	13.3 ± 0.05^{cd2}	0.83 ± 0.01^{b2}
	30	2.71 ± 0.04^{a2}	7.45 ± 0.10^{c3}	20.4 ± 0.25^{c}	8.58 ± 0.40^{b2}	14.1 ± 0.10^{b2}	0.90 ± 0.01^{a34}
	60	2.83 ± 0.08^{a2}	8.23 ± 007^{b4}	21.5 ± 0.09^{b2}	9.92 ± 0.41^{a2}	13.8 ± 0.25^{bc2}	0.91 ± 0.02^{a2}
	90	2.76 ± 0.07^{a3}	9.04 ± 0.14^{a3}	22.8 ± 0.16^{a23}	10.7 ± 0.32^{a4}	15.1 ± 0.49^{a2}	0.92 ± 0.02^{a2}

[a–e]Means in each column with different letters were significantly affected by storage ($p < 0.05$); [1-4]means with different numbers were significantly different between cheese samples at a similar ripening period ($p < 0.05$); O_0: raw milk cheese; O_1: heat treated at 65 °C for 20 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. c*remoris* added; O_2: heat treated at 65 °C for 20 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added; O_3: heat treated at 72 °C for 5 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. c*remoris* added; O_4: heat treated at 72 °C for 5 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added.

[A] Mean of three replicates.

Table 4
Sensory evaluation of 90 day-old cheese samples[A]

Cheese samples	Flavour	Body and texture	Color and appearance	Saltiness	Total
O_0	8.98 ± 0.21^{a}	4.37 ± 0.09^{a}	4.35 ± 0.08^{a}	3.53 ± 0.10^{a}	21.2 ± 0.46^{a}
O_1	8.53 ± 0.15^{b}	3.75 ± 0.07^{c}	3.87 ± 0.03^{b}	3.15 ± 0.07^{b}	19.3 ± 0.15^{b}
O_2	9.14 ± 0.06^{a}	4.12 ± 0.07^{b}	4.16 ± 0.07^{a}	3.29 ± 0.07^{ab}	20.7 ± 0.06^{a}
O_3	6.76 ± 0.13^{c}	3.15 ± 0.07^{d}	3.33 ± 0.16^{c}	2.88 ± 0.09^{c}	16.1 ± 0.45^{c}
O_4	7.13 ± 0.07^{c}	2.94 ± 0.06^{d}	3.72 ± 0.06^{b}	3.06 ± 0.03^{bc}	16.9 ± 0.02^{c}

[a–d]Means in the same column with different letters were significantly ($p < 0.05$); O_0: raw milk cheese; O_1: heat treated at 65 °C for 20 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* added; O_2: heat treated at 65 °C for 20 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus*; O_3: heat treated at 72 °C for 5 min and *Lc. lactis* subsp. *lactis* and *Lc. lactis* subsp. *cremoris* added; O_4: heat treated at 72 °C for 5 min and *Lb. delbrueckii* subsp. *bulgaricus* and *Str. thermophilus* added.
[A] Mean of three replicates.

total FFA contents of mesophilic and thermophilic culture-added cheeses were similar and lower than control cheese at the end of storage. *Lactococcus* spp. and *Lactobacillus* spp. have lower lipolytic activity than other bacteria and moulds (Fox, Guinee, Cogan, & McSweeney, 2000). Generally, the total FFA content of Urfa cheese was evidently lower than that of many other cheese varieties. The low degree of lipolysis in Urfa cheese may be due to scalding in its own whey at 90 °C for 3 min after whey separation, which inactivates the indigenous lipoprotein lipase (Mallatou, Pappa, & Massouras, 2003; McSweeney & Sousa, 2000). In addition, Urfa cheese was salted in 14% brine solution. Inhibitory effects of NaCl on lipolytic activity have been reported by some researchers (Azarnia et al., 1997; Freitas, Pintado, Pintado, & Malcata, 1999; Vlaemynck, 1992).

3.3. Sensory evaluation

The sensory evaluation of 90 day-old cheese samples is shown in Table 4. It is known that short-chain FFAs have a very important effect on the flavour of cheese (Freitas & Malcata, 1998; Rahmat & Richter 1996). In this study, raw milk cheese (O_0) after 90 day of ripening had higher short-chain FFA levels than that of other experimental cheeses. However, no differences were observed between O_0 and O_2 cheeses for flavour, colour and appearance, saltiness and total scores. At the low heat treatment, cheese manufactured with thermophilic culture had higher scores for organoleptic characteristics than its mesophilic counterpart, except in the case of saltiness, levels of which were similar. Regardless of the starter culture, high heat treated (72 °C) milk cheeses had lower organoleptic scores than low heat-treated milk cheeses. The highest body and texture scores were observed for O_0, followed by O_2, then O_1, and finally O_3, which were similar to O_4.

4. Conclusion

The basic composition of low and high heat treated milk cheeses did not significantly differ from raw milk-ripened cheese. In addition, no difference was observed between raw milk and mesophilic or thermophilic culture-added ripened cheeses.

On day 1, short, medium, and long-chain fatty acids represented about 3%–4%, 20%–22%, and 74%–77%, respectively, of all FFA in all types of cheese. The relative increases were more marked in short-chain than in the medium or long-chain FFA. Palmitic ($C_{16:0}$), and oleic acids ($C_{18:1}$) were the most abundant FFA in all cheeses at both sampling ages.

In the present work, heat treatment at high temperature (72 °C) adversely affected the lipolysis and sensory properties of Urfa cheeses. The adverse effects of heat treatment at a lower temperature (65 °C) on properties of the cheeses were less pronounced. The FFA content of mesophilic and thermophilic culture-added milk cheeses were similar. As the raw milk cheese had a higher level of lipolysis than the cheeses made from milk inoculated with mesophilic or thermophilic lactic starters, it can be concluded that native lipases and/or non-starter lactic acid bacteria (NSLAB) were primarily responsible for the development of lipolysis in Urfa cheese. Generally, total FFA content increased during ripening in all the experimental Urfa cheeses. However, degree of lipolysis in Urfa cheeses was very low. Future studies should be intensified on the selection of suitable starter combination(s) to manufacture Urfa cheese having similar lipolytic characteristics to the traditional ones. Also, further studies should be dedicated to obtaining a higher lipolytic level in Urfa cheese without impairing the nature of the end products.

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