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Evolution of lipolysis during the ripening of traditional Feta cheese

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Abstract

Lipolysis was studied during ripening of traditional Feta cheese produced in two small dairies, A and B. The cheeses were made from a thermized mixture of ewes'/goats' milk by using yoghurt as starter and artisanal rennet from lambs' and kids' abomasa (cheese A) or mixed artisanal rennet with calf rennet (cheese B).

The acid degree value and the free fatty acids (FFA) contents in both cheeses increased sharply up to 18 d (pre-ripening period at 15 °C) and continued to increase throughout ripening. In both mature cheeses, acetic acid was found at high levels (13–18% of the total FFAs). However, except for this, all FFA contents differed significantly ($P < 0.05$) between the two cheeses throughout ripening. The levels of individual and total C2:0–C8:0, C10:0–C14:0 and C16:0–C18:2 fatty acids were significantly higher ($P < 0.05$) in cheese A than in cheese B. Presumably the difference, especially in the C2:0–C8:0 content, was due mainly to the type of the rennet used. Butyric acid was the dominant FFA in cheese A (20% of the total FFAs at 120 d), while the most abundant FFAs in cheese B were capric (18%) and lauric acid (18%). In general, the lipolysis degree of the two cheeses was higher than those reported for the industrially-made Feta cheese.

In organoleptic evaluation, cheese A had a piquant taste that was attributed to its high content of butyric acid and showed a significantly ($P < 0.05$) higher total score than cheese B.

Keywords: Feta cheese; Free fatty acids; Lipolysis; Artisanal rennet

1. Introduction

Lipolysis is one of the major biochemical changes that occur during cheese ripening. The free fatty acids (FFA) released during lipolysis contribute, together with the volatile compounds and the proteolysis products, directly to cheese flavour (McSweeney & Sousa, 2000; Urbach, 1993). The level of lipolysis varies considerably among the different cheese types from low, in Dutch-type cheeses (Walstra, Noomen, & Geurts, 1993), to extensive in the mould ripened, surface-bacterially ripened and Italian hard cheeses (Battistotti & Corradini, 1993; Gripon, 1993; Reps, 1993). Furthermore, analysis of the short and medium-chain FFA profile has been suggested as an index for characterizing cheeses over the ripening period (Woo, Kollodge, & Lindsay, 1984; Woo & Lindsay, 1984).

The lipolytic agents in cheese are lipolytic enzymes found naturally in milk (milk lipase), rennet (pregastric esterases) (PGE) and microflora (Collins, McSweeney, & Wilkinson, 2003; Fox, Law, McSweeney, & Wallace, 1993). The contribution of milk lipase to cheese lipolysis depends on the heating of cheese milk, since pasteurisation reduces its activity. The contribution of rennet depends on the rennet type. Commercial calf and bovine rennets, that are commonly used in the majority of cheese varieties, are normally free from lipolytic activity. On the other hand, traditional rennets made from

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lambs' and kids' abomasa, such as the Italian rennet paste, have high lipolytic activities due to their contents of PGE (Battistotti & Corradini, 1993). PGE, found in the traditional rennets made from abomasa of small ruminants, preferentially hydrolyse the short chain acids esterified at the sn-3 position, releasing the FFA C4:0–C10:0 (Ha & Lindsay, 1993; Nelson, Jensen, & Pitas, 1977). Thus, the characteristic 'piquant' or 'pungent' flavour found in the Italian cheeses, Provolone and Pecorino Romano, have been attributed to the use of this type of rennet (Barzaghi, Davoli, Rampilli, & Contarini, 1997; Battistotti & Corradini, 1993). The contribution of cheese microflora to lipolysis occurs via the esterase/lipase systems of lactic and propionic acid bacteria, NSLAB, surface microorganisms, yeasts and moulds (McSweeney & Sousa, 2000).

Feta cheese, a P.D.O. white pickled cheese made from ewes' or a mixture of ewes' and goats' milks, is manufactured in modern dairies from pasteurised milk with mesophilic starter cultures and commercial calf or bovine rennet and is ripened in tins filled with brine. On the other hand, in small dairies, Feta cheese is still manufactured in the traditional way from thermized milk, using yoghurt as starter culture, and artisanal rennet from lambs' and kids' abomasa and is ripened in wooden barrels without brine addition (Abd El-Salam, Alichanidis, & Zerfiridis, 1993; Anifantakis, 1991a, 1991b; Moatsou, Massouras, Kandarakis, & Anifantakis, 2002). Yoghurt and rennet are produced locally by the cheesemakers.

Regarding the FFA profile of traditional Feta cheese throughout ripening, there is lack of information. The literature that refers to FFA composition of Feta or other pickled cheeses mainly concerns cheeses made with modern technology or cheeses of unknown origin (Abd El-Salam et al., 1993; Akin, Aydemir, Kocak, & Yildiz, 2003; Efthymiou, 1967; Georgala, Kandarakis, Kaminarides, & Anifntakis, 1999; Horwood, Lloyd, & Stark, 1981; Katsiari, Voutsinas, Alichanidis, & Roussis, 2000; Kondyli, Katsiari, Massouras, & Voutsinas, 2002; Mallatou, Pappa, & Massouras, 2003). The purpose of this study was to evaluate the lipolysis process during ripening of Feta cheese made traditionally in two different small cheese plants.

2. Materials and methods

2.1. Cheesemaking

Four cheese making trials were carried out in each of two different small Feta cheese plants, A and B, located in the Argos region of Peloponnese, according to the technology described by Moatsou et al. (2002). The cheese milk was a mixture of ewes' and goats' milk at a 4:1 ratio. About 20% of the total amount of the cheese milk was defatted and the skim milk was mixed with the rest of the full fat milk in the cheese vat. In cheese plant A, the cheese milk was heated at 67 °C for 15 s on a plate heat exchanger while, in cheese plant B, the cheese milk was heated at 66 °C for 6 min in an open vat. Yoghurt, prepared by the cheesemakers themselves in each cheese plant, was added as a starter culture. In cheese A, liquid artisanal rennet from mixed lambs' and kids' abomasa was used while, in cheese B, a mixture of 60% traditional and 40% commercial calf rennet (HALA, Chr. Hansen, Horsholm, Denmark) was used. The traditional rennets were prepared by cutting, mincing and extracting (with NaCl solution for 24 h) mixed dried and salted abomasa of lambs and kids slaughtered before weaning. The final extract (rennet) was filtered through cheesecloth and kept in a refrigerator. The lipolytic activities of the traditional rennet A and the mixed rennet B were determined by the titration method 'pH Stat' according to Barzaghi and Rampilli (1996) and expressed as LU (μeq NaOH/min/ml).

Cheese samples were taken after 3 days from the day of cheesemaking (the end of pre-packaging period of cheese), 18 days (the end of the pre-ripening period at 15 °C), 60 days (end of ripening at 5 °C) and 120 days.

2.2. Physicochemical analyses

The cheese samples were analysed in triplicate for moisture by heating at 102 ± 2 °C to constant weight according to the IDF method (1982), fat according to the BSI method (1955) and pH (Metrohm, model 632 pH-meter, Switzerland).

2.3. Acid degree value

The acid degree value (ADV) was determined according to the method described by Deeth and Fitz-Gerald (1976).

2.4. Free fatty acid analysis

FFAs were extracted from cheese and determined by gas-chromatography according to the method described by Nieuwenhof and Hup (1971). The FFA (C2:0–C18:0) were separated with a 1.50 m long by 3.175 mm outer diameter glass column packed with 5% carbowax 20 M-terephthalic acid terminated (Hewlett–Packard) on 60–80 mesh Chromosorb W-AW-DMCS support using a Hewlett–Packard model 5700 A gas chromatograph. The gas chromatograph was equipped with a flame ionization detector (FID) and was connected to a Varian recorder. The injector and detector temperatures were 250 °C, the helium gas flow rate 30 ml/min, the initial column temperature 65 °C, the temperature programme rate 4 °C/min and the final temperature, 240 °C was maintained for 32 min.

2.5. Sensory evaluation

Cheese samples 60 and 120 days old were subjected to sensory evaluation by a panel of 16 members familiar with Feta cheese grading. Cheeses were scored for colour (scale 0–10), body and texture (scale 0–30), flavour (scale 0–55) and piquant taste (scale 0–5), while the hardness was evaluated as soft or semi-hard.

2.6. Statistical analysis

Experimental data were statistically tested by multifactor analysis of variance using the LSD test ($P < 0.05$), to test the influence of the cheese plant manufacturing conditions and the ripening stage on free fatty acid concentration. The software STATGRAPHICS plus for Windows v. 5.2 (1995, Manugistics Inc., Rockville, Maryland 20852, USA) was used.

3. Results and discussion

3.1. Rennet lipolytic activity

The lipolytic activity of traditional rennet (rennet A) used in cheese A was higher than that of mixed rennet (rennet B) used in cheese B with values 1.38 and 0.78 LU, respectively. The lipolytic activity of rennet A was similar to those reported by Barzaghi and Rampilli (1997) for Italian commercial rennet pastes.

3.2. Physicochemical characteristics

pH and contents of moisture and fat of the two series of Feta cheese throughout ripening are shown in Table 1. The pH values of both matured cheeses were from 4.43 to 4.54 and such a pH value is necessary for a mature Feta cheese to maintain its good quality during storage (Anifantakis, 1991b). In addition, the moisture and fat in dry matter (FDM) contents of both matured cheeses met the Greek legal specifications of a maximum of 56% and a minimum of 43%, respectively, for 'first quality' Feta cheese (Codex Alimentarius, 2003). These results are similar to those reported for industrially made Feta cheese (Katsiari, Voutsinas, Alichanidis, & Roussis, 1997) and indicate, together with the fact that cheeses A and B did not significantly differ ($P > 0.05$) in pH, moisture or fat in dry matter content throughout ripening, that the traditional technology does not affect the fat or moisture contents of Feta cheese.

3.3. Acid degree value

The extent of lipolysis of cheeses A and B, expressed as ADV, is shown in Table 1. The ADV of both cheeses increased continuously during the ripening period, but cheese A had significantly higher ($P < 0.05$) ADV, than cheese B at any stage of ripening. At 60 d, the ADVs were 8.36 meq-KOH/100 g fat and 7.02 meq-KOH/100 g fat for cheese A and cheese B, respectively. Similar ADVs have been reported by Georgala et al. (1999) for piquant mature Feta cheese samples of unknown origin. On the other hand, significantly lower ADVs have been reported for experimental industrial Feta or Teleme cheeses made from mixtures of ewes/goat milk (Katsiari et al., 2000; Kondyli et al., 2002; Litopoulou-Tzanetaki, Tzanetakis, & Vafopoulou-Mastrojiannaki, 1993; Mallatou et al., 2003; Vafopoulou, Alichanidis, & Zerfiridis, 1989).

In general, the high levels of ADV found in traditional Feta cheese indicated substantial lipolysis and this was in accordance with the total C4:0–C18:2 FFAs (Table 1).

3.4. FFA profile

The concentration of acetic acid and total C4:0–C18:2 FFA in both cheeses, A and B, increased throughout ripening, showing the significant effect of the ripening stage on cheese lipolysis (Table 1). The increase of acetic acid and total C4:0–C18:2 FFA during ripening was better described by the hyperbolic equations $y = 4162x^{0.22}$ ($r^2 = 0.991$) for cheese A and $y = 2637x^{0.23}$ ($r^2 = 0.996$) for cheese B (Fig. 1). Total FFA, including acetic acid, increased by 2 times from the end of the pre-packaging period (3 d) up to the end of

Table 1
Changes in pH[A], moisture (%), fat in dry matter (%), acid degree value (ADV) (meq KOH/100 g fat), total C4:0–C18:2 FFAs and acetic acid (mg kg^{-1}) during ripening and storage of traditional Feta cheese made in two small dairies A and B

Age (days)	pH		Moisture (%)		Fat in dry matter (%)		ADV (meq KOH/100g fat)		Acetic acid (mg kg^{-1})		Total C4:0–C18:2 FFA (mg kg^{-1})	
	A	B	A	B	A	B	A	B	A	B	A	B
3	5.16^{a}	5.04^{a}	56.27^{a}	56.39^{a}	52.14^{a}	53.73^{a}	5.21^{a}	4.18^{b}	230^{a}	200^{a}	4994^{a}	3195^{b}
18	4.65^{a}	4.63^{a}	54.58^{a}	54.37^{a}	55.31^{a}	56.08^{a}	7.45^{a}	6.14^{b}	1121^{a}	1014^{a}	6961^{a}	4186^{b}
60	4.54^{a}	4.51^{a}	55.63^{a}	55.91^{a}	55.24^{a}	55.45^{a}	8.36^{a}	7.02^{b}	1312^{a}	1062^{a}	8411^{a}	5499^{b}
120	4.43^{a}	4.43^{a}	54.64^{a}	54.36^{a}	57.23^{a}	56.20^{a}	8.67^{a}	7.51^{b}	1572^{a}	1518^{a}	10372^{a}	6592^{b}

[a,b] Means of pairs in the same row with different superscript are significantly different ($P < 0.05$).
[A] Mean values of four cheese-making trials.

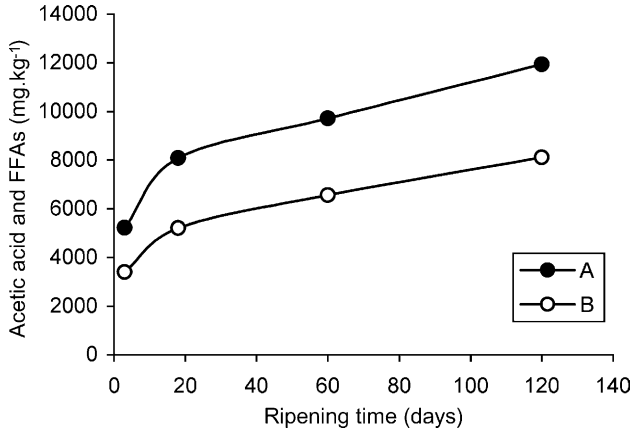

Fig. 1. Evolution of the total free fatty acids, including acetic acid, content (mg kg^{-1}) during ripening of traditional Feta cheese made in two small dairies A and B. Values correspond to the means of four cheese-making trials

ripening (60 d) and by 2.5 times up to 120 d. At 3 d, cheese A contained 4944 mg kg^{-1} of total C4:0–C18:2 FFA and cheese B 3195 mg kg^{-1} while, at 120 d, the respective values were 10372 and 6592 mg kg^{-1}. The high rate of FFA liberation up to the end of the pre-ripening period (18 d) was attributed to the enzymic activity of cheese microflora which was enhanced by the relatively high temperature (~15 °C) at which both cheeses, A and B, were kept in the wooden barrels. Manolopoulou et al. (2003) studied the microbial populations of traditional Feta cheese during ripening and found that microorganisms with high lipolytic activity, such as yeasts, NSLAB, enterococci and micrococci, reached their highest levels during the first 16 days. In addition, Kandarakis, Moatsou, Georgala, Kaminarides, and Anifantakis (2001) found that the high temperature during the pre-ripening period of Feta cheese favours C2:0–C12:0 FFA liberation.

Although the technologies used in the manufacture of cheeses A and B were similar, the total FFA contents in cheese A differed significantly ($P < 0.05$) from those in cheese B throughout ripening. This may be due to the higher lipolytic activity of the artisanal rennet used in cheese A, since the rennet is one of the major lipolytic agents in cheese ripening (Collins et al., 2003). In general, the total FFA content found in the cheeses of the present study was significantly higher than those reported for industrial type Feta cheese or Teleme cheese from mixture of ewes/goats milk (Katsiari et al., 2000; Kondyli et al., 2002; Mallatou et al., 2003; Vafopoulou-Mastrojiannaki, Litopoulou-Tzanetaki, & Tzanetakis, 1990). Probably, the high lipolysis degree, expressed here as total FFA content, of traditional Feta cheese, is due mainly to three parameters of the technology process: first, to the indigenous milk lipase, which might be present in both cheeses, since the cheese milk was heated at temperatures below 78 °C for 10 s that is required for complete inactivation of milk lipase (Driessen, 1989); second to the starter (yoghurt) and third to the artisanal rennet used. It has been found that 60 d old industrial type Feta cheese, made with certain strains of *S. thermophilus* and *L. bulgaricus* (the two constituent microorganisms of yoghurt) at a ratio 1:1, contained significantly higher ($P < 0.05$) total C2:0–C12:0 FFA than those made with combined mesophilic and thermophilic LAB (Kandarakis et al., 2001). Furthermore, the Greek traditional rennet, although a liquid, has substantial lipolytic activity, comparable to the Italian rennet paste (Moschopoulou, 2003). Moatsou et al. (2004) found higher C4:0–C10:0 FFA content in mature industrial type Feta cheese made with artisanal rennet than in cheeses made with calf rennet. The same observation has also been reported for ovine or caprine cheeses (different from those pickled and made with rennet paste) (Calandrelli et al., 1997; Larrayoz, Martinez, Barron, Torre, & Barcina, 1999; Virto et al., 2003).

The content of short chain FFAs have a significant impact on the development of the characteristic aroma of Feta cheese. The percentage of this FFA group, including acetic acid (C2:0–C8:0) increased significantly from 3 to 18 d of ripening, reaching almost its final level during the pre-ripening period of both cheeses (Fig. 2). The short chain FFAs represented approximately 44% and 33% of the TFFA content in cheeses A and B, respectively, at 120 d. The percentages of C2:0–C8:0 FFA reported by other authors are contradictory, ranging from 34% (Alichanidis, Anifantakis, Polychroniadou, & Nanou, 1984; Vafopoulou et al., 1989) up to 58% (Katsiari et al., 2000; Kondyli et al., 2002). The percentage of medium chain FFAs (C10:0–C14:0) decreased from 3 to 18 d representing approximately 31% and 39% of total FFAs (including acetic acid) in cheeses A and B, respectively, at 120 d. The percentages

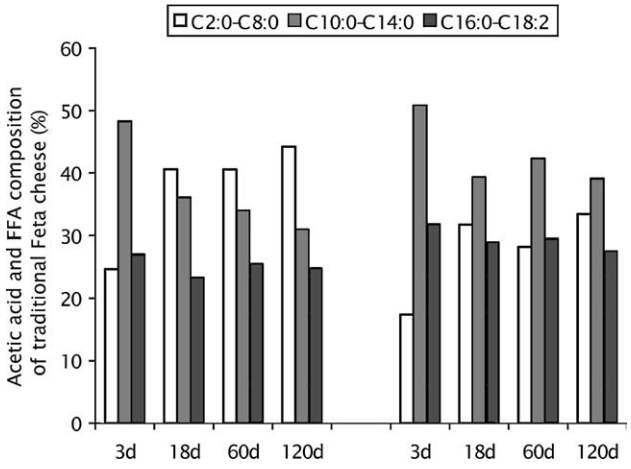

Fig. 2. Percentages of acetic and short (C2:0–C8:0), medium (C10:0–C14:0) and long (C16:0–C18:2) chain FFA of traditional Feta cheese made in two small dairies A and B after 3, 18, 60 and 120 days of ripening. Values correspond to the means of four cheese-making trials.

Table 2
Changes in acetic acid and individual free fatty acids (mg kg^{-1})[A] during ripening and storage of traditional Feta cheeses made in two small dairies A and B

	3 d		18 d		60 d		120 d	
	A	B	A	B	A	B	A	B
C2:0	230 ± 12^{a}	200 ± 10^{a}	1121 ± 22^{a}	1014 ± 24^{a}	1312 ± 20^{a}	1062 ± 15^{a}	1572 ± 23^{a}	1518 ± 25^{a}
C4:0	688 ± 19^{a}	197 ± 11^{b}	1428 ± 28^{a}	348 ± 14^{b}	1685 ± 33^{a}	409 ± 12^{b}	2385 ± 40^{c}	663 ± 12^{d}
C6:0	215 ± 9^{a}	99 ± 5^{b}	514 ± 15^{a}	114 ± 7^{b}	653 ± 21^{a}	161 ± 9^{b}	872 ± 28^{c}	279 ± 4^{d}
C8:0	154 ± 3^{a}	94 ± 1^{b}	221 ± 4^{a}	174 ± 5^{b}	294 ± 5^{a}	221 ± 2^{b}	454 ± 13^{c}	250 ± 5^{d}
Total C2:0–C8:0	1287 ± 9^{a}	590 ± 10^{b}	3284 ± 15^{a}	1650 ± 16^{b}	3944 ± 18^{a}	1853 ± 14^{b}	5283 ± 24^{c}	2710 ± 22^{d}
C10:0	1116 ± 17^{a}	759 ± 10^{b}	1326 ± 20^{a}	925 ± 15^{b}	1526 ± 31^{a}	1312 ± 22^{b}	1770 ± 32^{a}	1496 ± 28^{b}
C12:0	1271 ± 20^{a}	860 ± 12^{b}	1404 ± 22^{a}	979 ± 19^{b}	1538 ± 30^{c}	1289 ± 23^{d}	1581 ± 27^{a}	1464 ± 30^{a}
C14:0	137 ± 2^{a}	107 ± 1^{b}	185 ± 5^{a}	142 ± 3^{b}	239 ± 7^{a}	175 ± 6^{b}	347 ± 12^{a}	210 ± 9^{b}
Total C10:0–C14:0	2524 ± 13^{a}	1726 ± 11^{b}	2915 ± 14^{a}	2046 ± 12^{b}	3303 ± 16^{a}	2776 ± 12^{b}	3698 ± 18^{a}	3170 ± 13^{b}
C16:0	399 ± 8^{a}	257 ± 5^{b}	538 ± 14^{a}	417 ± 11^{b}	645 ± 10^{a}	537 ± 9^{b}	975 ± 19^{a}	549 ± 11^{b}
C18:0	527 ± 9^{a}	471 ± 7^{d}	709 ± 13^{a}	624 ± 10^{b}	996 ± 14^{a}	760 ± 12^{b}	1001 ± 20^{a}	870 ± 10^{b}
C18:1	442 ± 11^{a}	321 15^{d}	578 ± 15^{a}	423 ± 9^{b}	759 ± 15^{a}	577 ± 10^{b}	897 ± 18^{a}	736 ± 9^{b}
C18:2	45 ± 2^{a}	30 ± 1^{d}	58 ± 3^{a}	40 ± 2^{b}	76 ± 3^{a}	58 ± 2^{b}	90 ± 4^{a}	75 ± 2^{b}
Total C16:0–C18:2	1413 ± 10^{a}	1079 ± 8^{d}	1883 ± 13^{a}	1504 ± 12^{b}	2476 ± 16^{a}	1932 ± 14^{b}	2963 ± 20^{a}	2230 ± 17^{b}

[a,b] Means of pairs in the same row with different superscripts are significantly different ($P < 0.05$).
[A] Mean values (±SD) of four cheese-making trials.

of long chain FFAs (C16:0–C18:2) in both cheeses remained almost constant throughout ripening. At 120 d, long chain FFAs represented approximately 25–28% of TFFA in both cheeses. However, the levels of short, medium and long chain FFAs in cheese A were significantly ($P < 0.05$) higher than in cheese B (Table 2).

The mean concentration of acetic acid and individual FFAs of cheeses A and B increased throughout ripening (Fig. 3). Acetic acid contributes greatly to the final flavour of Feta cheese and is the major volatile acid extracted with FFAs. It is not produced from lipolysis by lipases but from several biochemical pathways. It is formed during the early stages of ripening and is probably a product of citrate or lactate fermentation or of amino acid catabolism by bacteria (Abd El-Salam et al., 1993; McSweeney & Sousa, 2000). In both cheeses, acetic acid increased by 4–6 times from 3 to 18 d, was almost constant up to 60 d and increased about 1.5 times at 120 d. The increase of acetic acid up to 120 d may be due to lactate fermentation, since lactose can be present even in mature cheese (Abd El-Salam et al., 1993). Acetic acid concentration did not differ significantly ($P > 0.05$) between the two cheeses, A and B, ranging from 1062 to 1312 mg kg^{-1} at 60 d and 1518–1572 mg kg^{-1} at 120 d (Table 2). Similar values have been reported for 60 d old Feta cheese made with different rennet types (Alichanidis et al., 1984; Georgala et al., 1999; Kandarakis et al., 2001), while lower values have been reported by Efthymiou (1967), Horwood et al. (1981), Katsiari et al. (1997), Kondyli et al. (2002) and Vafopoulou et al. (1989). Acetic acid in Feta cheese usually represents from 30% to 50% of the volatile fatty acids and from 11.6% to 19.1% of the total FFA content (Alichanidis et al., 1984; Efthymiou, 1967; Vafopoulou et al., 1989). However, values of acetic acid that reach 80–92% of the volatile fatty acids have been reported for Feta cheese of non-Greek origin (Efthymiou, 1967; Horwood et al., 1981). In the mature traditional Feta cheese of this study, acetic acid made up from 30% to 57% of the volatile fatty acids and from 13% to 19% of the total FFA contents.

Butyric acid (C4:0) is also an important component of Feta cheese, which contributes greatly to its flavour and piquant taste. In both cheeses, A and B, butyric acid increased by 2 times from 3 to 18 d and continued to increase up to 120 d. However, cheese A contained significantly ($P < 0.05$) more butyric acid than cheese B throughout ripening. In addition, although the dominant acid in Feta cheese is acetic (Abd El-Salam et al., 1993; Efthymiou, 1967), the dominant acid in traditional cheese A was butyric acid. At 60 and 120 d, the butyric acid levels in cheese A were 1685 and 2385 mg kg^{-1}, respectively, corresponding to percentages 17–20% of TFFA while, in cheese B, the respective values were 409 and 663 mg kg^{-1}, corresponding to percentages of 6% and 8%. The percentages found in cheese A were significantly higher, compared to the values 0.85% and 5.5% reported by Katsiari et al. (2000) and Kondyli et al. (2002) for industrially made Feta cheese. According to Efthymiou (1967), high percentages of free butyric acid (13–19% of the TFFA) in Feta cheese indicate selective lipolytic activity. In our opinion, the high level of butyric acid in cheese A is due to PGE present in the artisanal rennet used.

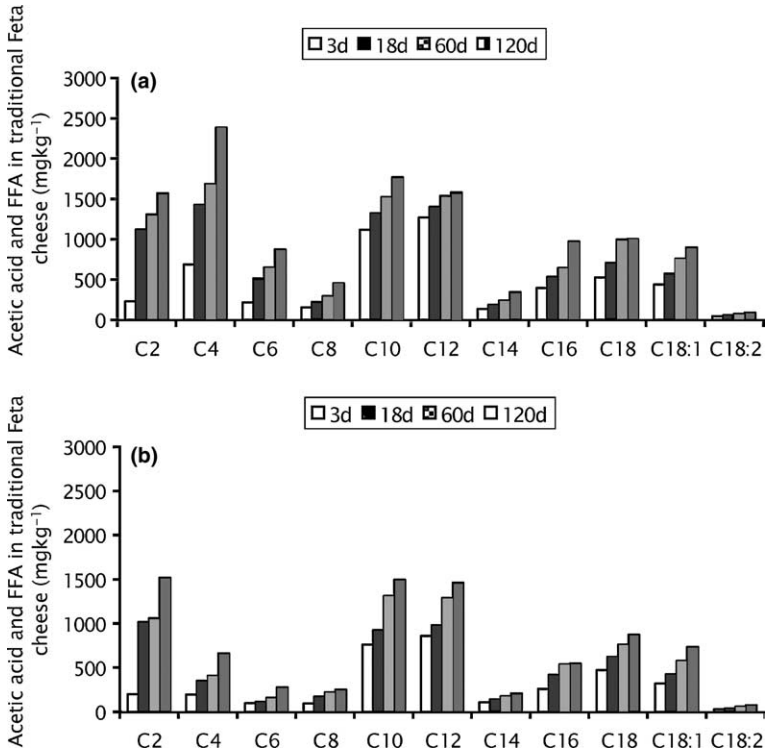

Fig. 3. Acetic acid and individual free fatty acids (FFAs) accumulated during ripening of traditional Feta cheeses made in two different small dairies A and B. Values correspond to the means of four cheese-making trials

Caproic acid (C6:0) concentration in cheese A was also significantly ($P < 0.05$) higher than in cheese B throughout ripening. At 60 d, caproic acid content in cheese A was 653 mg kg^{-1} (6.7% of the TFFA), compared to 161 mg kg^{-1} (2.5% of the TFFA) in cheese B. At 120 d, the respective contents were 872 and 279 mg kg^{-1}. Similar values have been reported by Georgala et al. (1999) for mature piquant Feta cheese samples obtained from the Athens market. On the other hand, low caproic acid contents, up to 101 mg kg^{-1}, have been reported for mature industrial-type Feta cheese (Kandarakis et al., 2001; Katsiari et al., 2000; Kondyli et al., 2002).

Caprylic acid (C8:0) concentration in cheese A was also significantly ($P < 0.05$) higher than that in cheese B throughout ripening. In cheese A, the contents were 294 mg kg^{-1} (3% of the TFFA) and 454 mg kg^{-1} (3.8% of the TFFA) at 60 and 120 d, respectively while, in cheese B, the corresponding values were 221 mg kg^{-1}(3.4% of the TFFA) and 250 mg kg^{-1} (3.0% of the TFFA). Georgala et al. (1999) found 520–2650 mg kg^{-1} of caprylic acid in mature piquant Feta cheese samples and 61–520 mg kg^{-1} in non-piquant samples, both of unknown origin. On the other hand, lower values have been reported for industrial-type Feta cheese (Kandarakis et al., 2001; Katsiari et al., 2000; Kondyli et al., 2002).

Capric acid (C10:0) contents in cheese A were significantly ($P < 0.05$) higher than in cheese B throughout ripening. However, the contents in matured 120 d cheeses were high enough, 1770 mg kg^{-1} (ca. 15% of the TFFA) and 1496 mg kg^{-1} (ca. 18.5% of the TFFA) in cheeses A and B, respectively, and this is also attributed to PGL present in the rennets used. According to Ha and Lindsay (1993) pregastric lipases liberate high proportions of C10:0 from caprine and ovine milk fat and also the greatest part (>66%) of the total C4:0–C10:0 FFA released by them consists of C4:0 and C10:0. In cheeses (other than pickled) made with rennet paste, capric acid was also found in significant amounts (Calandrelli et al., 1997; Larrayoz et al., 1999).

Lauric acid (C12:0) contents showed the same tendency as capric acid in the two cheeses and were also similar to those of capric acid throughout ripening (13% and 18% of the TFFA in cheeses A and B, respectively, at 120 d). On the other hand, myristic acid (C14:0) contents in both cheeses A and B were low throughout ripening and the respective percentages at 120 d were 2.9% and 2.6%.

The long chain FFA contents in matured cheeses A and B were lower than those of the medium chain capric and lauric acids. The percentages of each of them in the TFFA of cheese A, 120 d old, were approximately 8.2% palmitic (C16:0), 8.4% stearic (C18:0), 7.5% oleic (C18:1) and 0.8% linoleic acid (C18:2) while, in cheese B, these were 6.8%, 10.7%, 9% and 0.9%, respectively. It seems that the long chain FFAs do not characterize traditional Feta cheese. On the other hand, in

Table 3
Organoleptic characteristics[A] of traditional Feta cheese made in two small dairies A and B

Cheese age (days)	Colour		Structure–texture		Flavour		Piquant taste		Total	
	A	B	A	B	A	B	A	B	A	B
60	14.0[a]	13.0[a]	26.0[a]	24.5[a]	45.0[a]	42.5[b]	5.0[a]	1.0[b]	90.0[a]	81.0[b]
120	14.5[a]	14.0[a]	26.5[a]	25.5[a]	47.5[a]	44.5[b]	5.0[a]	1.0[b]	93.5[a]	85.0[b]

[a,b] Means of pairs in the same row with different superscripts are significantly different ($P < 0.05$).
[A] Mean values of four cheese-making trials.

experimental industrial type Feta cheese palmitic and stearic were the major FFAs (Alichanidis et al., 1984; Vafopoulou et al., 1989). 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.

3.5. Organoleptic characteristics

The results from the organoleptic test showed no significant differences ($P > 0.05$) in colour and structure–texture between the cheeses A and B (Table 3). Concerning aroma and piquant taste, the values given by the judges for cheese A were significantly higher ($p < 0.05$) than those for cheese B, probably reflecting the contribution of the total short chain (C2:0–C8:0) FFA that was significantly higher ($P < 0.05$) in cheese A than in cheese B (Table 2). Specifically, the most piquant taste that was observed for cheese A at 60 and 120 d was possibly associated with the high butyric acid concentration determined in cheese A (Table 2). Georgala et al. (1999) also reported that piquant Feta cheese samples from the Athens market had a significantly ($P < 0.05$) higher amount of butyric acid than the non-piquant Feta samples examined. Furthermore, no strong rancid, peppery or soapy taste was observed in either cheese A or B. This could be attributed to the relatively low levels of the long chain free fatty acids (Efthymiou, 1967).

The total score for organoleptic characteristics of cheese A was significantly ($P < 0.05$) higher than that of cheese B. The main difference between the manufacture of cheeses A and B was in the rennet used. The traditional rennet from lambs' and kids' abomasa is considered to contribute to the development of the very pleasant aroma and peppery taste of Feta cheese (Anifantakis, 1991a, 1991b). Piquant flavour has also been observed in Parmesan cheese (Woo & Lindsay, 1984), in the Greek Kefalotyri cheese (Anifantakis, 1976) and in the Spanish cheeses Idiazabal and Majorero (Fontecha et al., 1990; Virto et al., 2003) when traditional kid or lamb rennet has been used.

4. Conclusions

The above results show that a substantial lipolysis occurs during ripening of traditional Feta cheese. The traditional technology which is applied causes the liberation of significantly higher amounts of butyric, capric and lauric acid compared to those reported for industrially-made Feta cheese. The fat hydrolysis is clearly enhanced when only artisanal rennet is used and, finally, the high lipolysis degree observed in this case is associated with a piquant taste.

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