ELSEVIER

Food Chemistry 72 (2001) 369–378

Food Chemistry

www.elsevier.com/locate/foodchem

Effect of draining temperature on the biochemical characteristics of Feta cheese

I. Kandarakis *, G. Moatsou, A.I.K. Georgala, S. Kaminarides, E. Anifantakis

Laboratory of Dairy Technology, Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

Received 15 February 2000; received in revised form 29 August 2000; accepted 29 August 2000

Abstract

Two different draining temperatures, 15 and 21°C were applied to five Feta cheese curds made with different starters, containing mesophilic or thermophilic strains or mixtures of them. After 20 h of draining, the pH of curds made with thermophilic starters ranged from 5.28 to 5.49. The draining temperature significantly affected ($P < 0.05$) the pH and the total solids of the cheeses. The inclusion of whey proteins in the cheese curd due to the insufficient draining of cheeses at 15°C, resulted in higher water-soluble nitrogen (WSN), as % of total nitrogen content. Free amino acid contents were significantly affected ($P < 0.05$) by the draining temperature and by the presence of thermophilic lactobacilli in the starter mixture. Draining temperature also significantly affected ($P < 0.05$) residual α_s- and β-casein and the RP-HPLC profiles of the WSN. The $C_{2:0}$ to $C_{8:0}$ free fatty acids, hardness (kg) and fracturability (kg), as well as the total organoleptic scores, were significantly ($P < 0.05$) higher in feta drained at 21°C.

Keywords: Feta; Draining temperature; Biochemical characteristics

1. Introduction

Feta is a semi-soft white brined cheese traditionally made from ewes' or from mixtures of ewes' and goats' milks. Its typical flavour is mildly rancid, slightly acid and salty. It has a rather firm and smooth texture, which makes the cheese sliceable. No gas holes should be present, but irregular small mechanical openings are desirable (Abd El-Salam, Alichanidis & Zerfiridis, 1993). In the past, Feta cheese was made without starters. During the last decade, yogurt or starters have been used, but usually without control of the draining temperature. Although the use of thermophilic starters is not in accordance with the temperatures used for the manufacture of this cheese variety, the use of yogurt as a starter is a common practice for traditional Feta cheese. Therefore, textural, sensory and microbiological defects, correlated with inadequate acidification of Feta cheese curd, are common.

Draining is the critical point in feta cheese manufacture. It is controlled by the acid development in the curd and by dry salting because no pressing is involved. During the draining, the greatest part of Feta proteolysis and lipolysis occur (Alichanidis, Anifantakis, Polychroniadou & Nanou, 1984; Vafopoulou, Alichanidis, & Zerfiridis, 1989). The aim of the present work was to study the influence of the draining temperature on the characteristics of Feta cheese made with different starter cultures, including mesophilic and/or thermophilic strains. Cheeses were analyzed at 60 days, which is the minimum ripening period before feta consumption (Codex Alimentarius, 1988).

2. Materials and methods

2.1. Cheese manufacture

Five cheesemaking trials were conducted at a cheese pilot plant. Each day, 300 kg of standardized (casein to fat ratio: 0.68–0.72) pasteurized (72°C/20 s), ewes' milk were equally divided in five cheesevats (A, B, C, D, E).

* Corresponding author. Tel. +301-5294675; fax: +301-5294672.
E-mail address: ykan@aua.gr (I. Kandarakis).

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00245-4

Deep frozen (−80°C) mesophilic and thermophilic strains obtained from Agricultural University of Athens-Dairy Collection and isolated from Feta cheese curd, were used as starter cultures. Mother and intermediate cultures grew in reconstituted sterilized non-fat milk powder and the final inoculum in ewes' milk heated at 95°C for 5 min. After the addition of $CaCl_2$ (10g/100kg milk), at 35°C, the starters were inoculated at 1% rate as follows: A. *Lactococcus lactis* subsp. *lactis* ACA-DC 0051 + *Lactococcus lactis* subsp. *lactis* ACA-DC 0049 (1:1); B. *Lactococcus lactis* subsp. *lactis* ACA-DC 0051 + *Lactococcus lactis* subsp. *lactis* ACA-DC 0049 + *Streptococcus thermophilus* ACA-DC 0022 (1:1:2); C. *Lactococcus lactis* subsp. *lactis* ACA-DC 0051 + *Lactococcus lactis* subsp. *lactis* ACA-DC 0049 + *Lactobacillus delbrueckii* subsp. *bulgaricus* ACA-DC 0105 (1:1:2); D. *Lactococcus lactis* subsp. *lactis* ACA-DC 0051 + *Lactococcus lactis* subsp. *lactis* ACA-DC 0049 + *Streptococcus thermophilus* ACA-DC 0022 + *Lactobacillus delbrueckii* subsp. *bulgaricus* ACA-DC 0105 (1:1:1:1) and E. *Streptococcus thermophilus* ACA-DC 0022 + *Lactobacillus delbrueckii* subsp. *bulgaricus* 0105 (1:1).

After 15 min, 3.5 g rennet powder (HALA, Chr. Hansen's, Denmark), were added per 100 kg milk. The mean clotting time was 8.2 min at 35°C. Cheese curd was cut into 20 mm cubes, 45 min after the rennet addition. After 10 min, the fresh cheese curds, marked as the respective cultures, were put into feta moulds. Each cheese curd was divided in two batches, intended to drain at 15 or 21°C. Dry salt was added and after 4 h the fresh cheeseblocks were turned and dry-salted again. After 20 h of draining they were cut into pieces, dry-salted and left in containers until the pH reached 4.80–4.60. Then, the cheese pieces were washed and kept with dry salt in sealed tins for a further 10 days in the respective room (15 or 21°C). Finally, the cheeses were washed and packaged into tins filled with 7.5% brine, sealed and stored at 4°C.

Cheese samples 60-days-old, from each treatment (symbolized as A, B, C, D and E, according to the cultures used) and from each ripening temperature, were analyzed.

2.2. Biochemical analyses

Total solids (TS) were determined in triplicate according to IDF (1982). Ash was determined in triplicate at, 550°C to constant weight. Total nitrogen (TN), was determined in triplicate by the Kjeldahl method. Water-soluble nitrogen (WSN) was obtained by homogenizing of 1 g cheese with 5 ml H_2O according to 'Method I' for WSN extraction, cited in 'AIR-FLORA Laboratory Manual' (1997) and nitrogen content was determined in duplicate by the Kjeldahl method. Free amino acids (N-NH_2) content was determined in 100 μl WSN in triplicate, by the Cd-ninhydrin method (Folkertsma & Fox, 1992).

Cheese samples and their water soluble extracts (WSN) were analyzed in duplicate by urea-PAGE, using the method of Andrews (1983) with direct staining using Coomassie Brilliant Blue G-250 (Blakesley & Boezi, 1977). Electrophoresis was carried out on a vertical slab unit (LKB vertical electrophoresis unit 2001, Bromma, Sweden) in slabs 140×160 mm with thickness 1.5 mm. After destaining with water, gel slabs were digitized by a scanner (Hewlett Packard, ScanJet 4c/T) linked to an image processing system (GelCompar v. 4.0, Applied Maths, Belgium). A suspension of 240 mg cheese in 10 ml stacking gel buffer, containing 6 M urea, 0.1 M β-mercaptoethanol and 0.4 ml tracking dye solution, was kept at 40°C for 15 min, and then centrifuged at 3000 ***g*** for 15 min at 4°C. The solidified fat layer was discarded and 10 μl of the supernatant were used for electrophoresis. Equal volumes of WSN fraction and stacking gel buffer, containing 9 M urea, 0.1 M β-mercaptoethanol and tracking dye, were mixed and 50 μl of this sample were used for electrophoresis.

Analyses of 50 μl water-soluble extracts were performed using an automated HPLC system of WATERS (WATERS, 34 Marple Street, Milford, MA 01757, USA), consisting of one pump to mix four solvents (WATERS 600), a diode array UV/Vis detector (WATERS 996), a helium degasser and a Rheodyne injector (model 7125, Rheodyne Inc., Cotati, California, USA). The data acquisition and processing were performed by the MILLENIUM v. 2.15 software (1994, WATERS Corp.). A RP C_{18} Nucleosil wide pore column (5um, 30nm, 250X4.0 mm, Macherey-Nagel, Duren, Germany) with a guard column (30×4.0 mm) was used. Chromatographic conditions were: Solvent A, 1 ml/l trifluoroacetic acid (TFA) in water; solvent B, a mixture of 600 ml/l acetonitrile, 399 ml/l water and 1 ml/l TFA; flow rate 0.75 ml min^{-1}. The method used for sample elution is described by Moatsou, Kandarakis, Georgala, Alichanidis and Anifantakis (1999). The absorbance of the eluate was monitored at 220 nm. The solvents and the samples were filtered through 0.45 μm Nylon 66 or cellulose acetate filters respectively (Lida Manufacturing Corp, Kenosha WI 53143-6615, USA).

Free fatty acids (FFA) were extracted from cheese and determined by gas chromatography, according to the method described by Nieuwenhof and Hup (1971). The FFA ($C_{2:0}$-$C_{12:0}$) were separated with a 1.50 m × 3.175 mm outer diameter glass column packed with 5% Carbowax 20M-terephthalic acid on Chromosorb W-AW-DMCS (60–80 mesh), using a Hewlett-Packard (model 5700A) gas chromatograph. The gas chromatograph was equipped with a flame ionization detector (FID) and was connected to Varian recorder (model 4270). The GC conditions were: 250°C injector and detector temperature and a helium gas flow rate of 30 ml/min, initial column temperature 65°C, temperature programme rate 4°C/min, final temperature 240°C.

2.3. Rheological analyses

A Shimadzu Testing Instrument, model AGS-500NG (Shimadzu Corp., Japan) equipped with a 50 kg load cell was used for objective analysis of textural properties of cheese. A plunger (diameter 50 mm) was attached to the moving crosshead. The speed of the crosshead was 30 mm/min in a downward direction. Feta cheese samples (25×25×25 mm) were compressed at 20°C to 80% of their original height. From the compression curves, hardness (the force at 80% compression), fracturability (the force at the point of fracture) and% compression at fracturability point were calculated. The textural characteristics of cheeses from the five starters and two draining temperatures were evaluated simultaneously. Five replicate measurements were made on each cheese sample.

2.4. Sensory analyses

All cheeses were tested at 60 days by a panel of 15 persons familiar to cheese grading, in a random order. They scored cheeses for colour (0–15 points), body and texture (0–30 points) and flavour (0–55 points). They also recorded flavour and textural defects.

2.5. Statistical analysis

Multifactor analysis of variance was used to test the influence of the draining temperature, of the starters and of the interaction between them. Further testing was carried out by a multiple range test procedure using the LSD test ($P < 0.05$). Relationship between different variables was determined by Regression Analysis. The software STATGRAPHICS Plus for Windows v. 5.2 (1995, Manugistics, Inc., Rockville, Maryland 20852, USA) was used for data manipulation.

3. Results and discussion

3.1. Cheese milk characteristics

The mean pH of cheese milk was 6.64. The mean chemical composition of raw milk, as determined by Milkoscan apparatus (model 255 A/B, type 25700, Fosselectric, Denmark) was: fat 6.19%, total protein 5.52%, lactose 4.74% and total solids 17.74%. The mean somatic cell counts, determined by Fossomatic apparatus (model 250, type 25800, Fosselectric, Denmark) were 1.6×10^6 cells/ml. Mesophilic aerobic flora, enumerated on Plate Count Agar (Biokar, France) after incubation at 30°C for 3 days, was 2×10^7 cfu/ml. After the pasteurization, the somatic cell counts were decreased to 8×10^5 cells/ml, due to the presence of a centrifugal clarifier in the pasteurization line, and mesophilic aerobic flora was 65×10^3 cfu/ml.

The high mesophilic aerobic flora counts of raw ewe's cheese milk are attributed to the mountainous character and the climate of Greece, which make the collection and the fast cooling of milk rather difficult.

3.2. Acidification rate

The mean pH and temperature values of the Feta curds during the first 20 h of draining are shown in Table 1. After 8 h of draining, pH of A, B and C cheeses ranged within pH 5.0–5.2, that is necessary for proper draining of Feta cheese (Abd El-Salam et al., 1993). In cheese D — in which the ratio of mesophilic starters was lower than that of A, B and C cheeses — and in cheese E, made without mesophilic starters, the acidification rate was slower. After 20 h, the pH values of cheeses made with mesophilic starters (A, B, C and D) were within the required pH 4.80–5.00. In cheese E there was no further acidification from 8 to 20 h. When curd temperature had been equalized with the respective draining room temperature (after 20 h), the pH of curds drained at 21°C was lower than that of their pairs at 15°C, especially between the curds made with thermophilic starters (E).

The prolonged draining resulted in a high moisture final product, with bad texture and excessive gas production during the packaging in the tins. The moulds, yeasts and coliforms had proliferated, due to the slow acidification. In order to minimize these problems, cheeses with low acidification rate had to be washed and dry-salted many times during the draining, so the final product had a high salt in moisture content. The absence of mesophilic strains from the culture E and the insufficient synergistic growth of the two thermophilic strains at the temperatures used, seemed to be the reasons for the low acidification of curd E.

Table 1
Mean[a] pH and temperature values of Feta cheese curds during the first 20 h of draining

Draining (h)	Room T[b]	Curd T[b]	pH				
			A[c]	B[c]	C[c]	D[c]	E[c]
2	15	28.9	6.17	6.00	6.07	6.27	6.28
	21	30.2	6.06	6.02	6.05	6.19	6.14
4	15	26.7	5.89	5.65	5.72	5.77	5.91
	21	27.5	5.78	5.42	5.64	5.60	5.63
8	15	21.8	5.32	5.08	5.16	5.35	5.45
	21	25.5	5.17	4.95	5.10	5.37	5.46
20	15	15.8	4.95	4.94	4.91	4.95	5.49
	21	21.4	4.81	4.86	4.83	4.80	5.28

[a] Means of five cheesemaking trials.
[b] Temperature (°C).
[c] See Section 2.1.

3.3. Biochemical characteristics

The physicochemical characteristics of 60 day-old Feta cheeses are shown in Table 2. Both factors, temperature of draining and starter culture, had a significant effect on 60-day-old Feta pH ($P = 0.006$ and $P = 0.001$, respectively). The pH of cheeses made with thermophilic starters (E) was significantly higher ($P < 0.05$) than that of cheeses A, B, C and D, as reported by Litopoulou-Tzanetaki, Tzanetakis and Vafopoulou-Mastrojiannaki, (1993) for Feta made with different starters. In all cheeses drained at 21°C, the pH value at 60 days was lower than that of cheeses drained at 15°C, as also happened with the acidification rate data (Table 1).

Total solids were significantly affected by the temperature of draining ($P = 0.000$) and by the starter ($P = 0.000$). The total solids percentage of cheeses drained at 21°C was significantly higher than that of cheeses drained at 15°C, due to the intense draining caused by the higher acid development at 21°C. As pH decreases the cheese whey removal and the loss of calcium phosphate from the casein micelles increase (Lawrence, Creamer & Gilles, 1987). Therefore, the ash contents of Feta cheeses drained at 15°C were higher. Also, cheese made with thermophilic starters (E) had significantly lower total solids and higher ash contents than A, B, C and D cheeses. Total solids of cheeses A, B, C and D drained at 15°C were at the level of 44%, which is the limit for Feta cheese according to Codex Alimentarius (1988).

The draining temperature and the starter mixtures had significant effect ($P < 0.05$) on WSN, as % of TN fraction of cheeses. In cheeses E drained at both temperatures, this fraction was significantly higher ($P < 0.05$) than that of cheeses A, B, C and D. The lower acidification of the cheeses drained at 15°C and of the cheeses E, resulted in higher retention of whey proteins in the cheese curd, which are included in the WSN. In general, the N content of this fraction was lower than that of NCN, as % of TN fraction reported for 40–80 d-old Feta, which range between 16.6-29.8% (Alichanidis et al., 1984; Litopoulou-Tzanetaki et al., 1993; Mallatou, Pappas & Voutsinas, 1994; Vafopoulou et al., 1989; Vafopoulou-Mastrojiannaki, Litopoulou-Tzanetaki & Tzanetakis, 1990), probably due to the higher salt in moisture content of the cheeses analyzed in the present study.

Draining temperature significantly affected ($P = 0.024$) the N-NH_2 content of cheeses. The same also happened with the starter mixture ($P = 0.038$). The N-NH_2 contents of the cheeses made with starters, including thermophilic strains (B, C, D and E) and drained at 21°C, were higher than the respective values of the cheeses drained at 15°C. It has been reported, for different cheeses, that the increase of ripening temperature promotes the increase of the fraction soluble at 5% PTA (Aston, Gilles, Durward & Dulley, 1985; Nuñez, Garcia-Acer, Rodriguez-Marin, Medina & Gaya, 1986; Gaya, Medina, Rodriguez-Marin & Nuñez, 1990; Freitas & Malcata, 1998), which includes mainly amino acids and peptides with molecular mass lower than 600 Da (Jarret, Aston & Dulley, 1982) more intensively than the other nitrogenous fractions. The N-NH_2 values of cheeses C, D and E, containing the strain of thermophilic lactobacilli and ripened at 21°C, were higher than that of the other cheeses. Cheese C, drained at 21°C and

Table 2
Biochemical characteristics[a] of 60-day-old Feta cheeses

	Draining temperature (°C)	A[b]	B[b]	C[b]	D[b]	E[b]
pH	15	4.63a,b	4.61a*	4.58a	4.61a*	4.83b
	21	4.53a	4.44a	4.48a	4.43a	4.74b
Total Solids, %	15	44.14b	44.67b	43.95b	44.26b	40.18a
	21	48.93b*	50.31b*	49.84b*	49.31b*	46.77a*
Ash,%	15	5.25a	5.21a	5.34a	5.27a	7.44b
	21	4.62a	4.54a	4.62a	4.63a	6.85b
WSN,% of TN[c]	15	16.59a*	15.37a	16.91a*	16.58a*	24.10b*
	21	10.72a	11.39a	11.45a	10.89a	14.79b
N-NH_2 (mmol Leu)	15	0.57a	0.46a	0.71a	0.57a	0.52a
	21	0.53a	0.61a,b	0.90b	0.75a,b	0.71a,b
Residual β-CN[d]	15	27.2	27.5 *	29.5	27.5 *	28.9
($\times 10^3$)	21	19.4a,b	21.9a,b	22.9a,b	18.0a	23.4b
Residual α_s-CN[d]	15	20.3	21.1	21.1	21.4 *	24.0
($\times 10^3$)	21	18.9	19.0	18.3	17.5	19.0
Faster than	15	20.8	19.5	22.2 *	21.9 *	17.8
α_s-CN[d] ($\times 10^3$)	21	19.4a,b	21.9a,b	22.9a,b	18.0a	23.4b

[a] Means of five cheesemaking trials. Means in the same row of each cell with different letters are significantly different (LSD test, $P < 0.05$).
[b] See Section 2.1.
[c] Water-soluble nitrogen expressed as percentage of total nitrogen.
[d] Casein area determined by urea-PAGE, expressed as percentage of cheese total protein.
*Significantly different means in the same column of each cell (LSD test, $P < 0.05$).

made with starters combining mesophilic strains and the thermophilic bacilli, had the highest N-NH_2 content. Therefore, the elevated temperature of draining promoted the high aminopeptidase and dipeptidase activities reported for thermophilic lactobacilli (Pritchard & Coolbear, 1993).

The draining temperature had a significant effect on the area of the fractions moving faster than α_s-CN ($P=0.000$), on the area of the residual α_s-CN ($P=0.011$) and on the area on the residual β-CN ($P=0.000$). The starter mixture had no significant effect on these results. The profile of cheese E was very different, especially concerning the zone *i*, which was evident only in cheeses E and was more intense in cheese E ripened at 21°C (Fig. 1). The profiles of the WSN extraction tended to be in accordance with the N contents of the water soluble fraction.

Residual α_s-CN and β-CN areas were higher, although not significantly, in cheeses drained at 15°C. The residual α_s-CN was higher in cheeses with lower acidification rate (Table 1). The higher draining temperature resulted in lower cheese curd pH, which enhanced residual rennet activity and α_s-CN hydrolysis during the first stage of cheese ripening. The main proteolytic factors in Feta are expected to be the residual rennet and both the starters and the native microflora enzymes. Plasmin activity was not expected to be intense in view of the pH and salt conditions of this

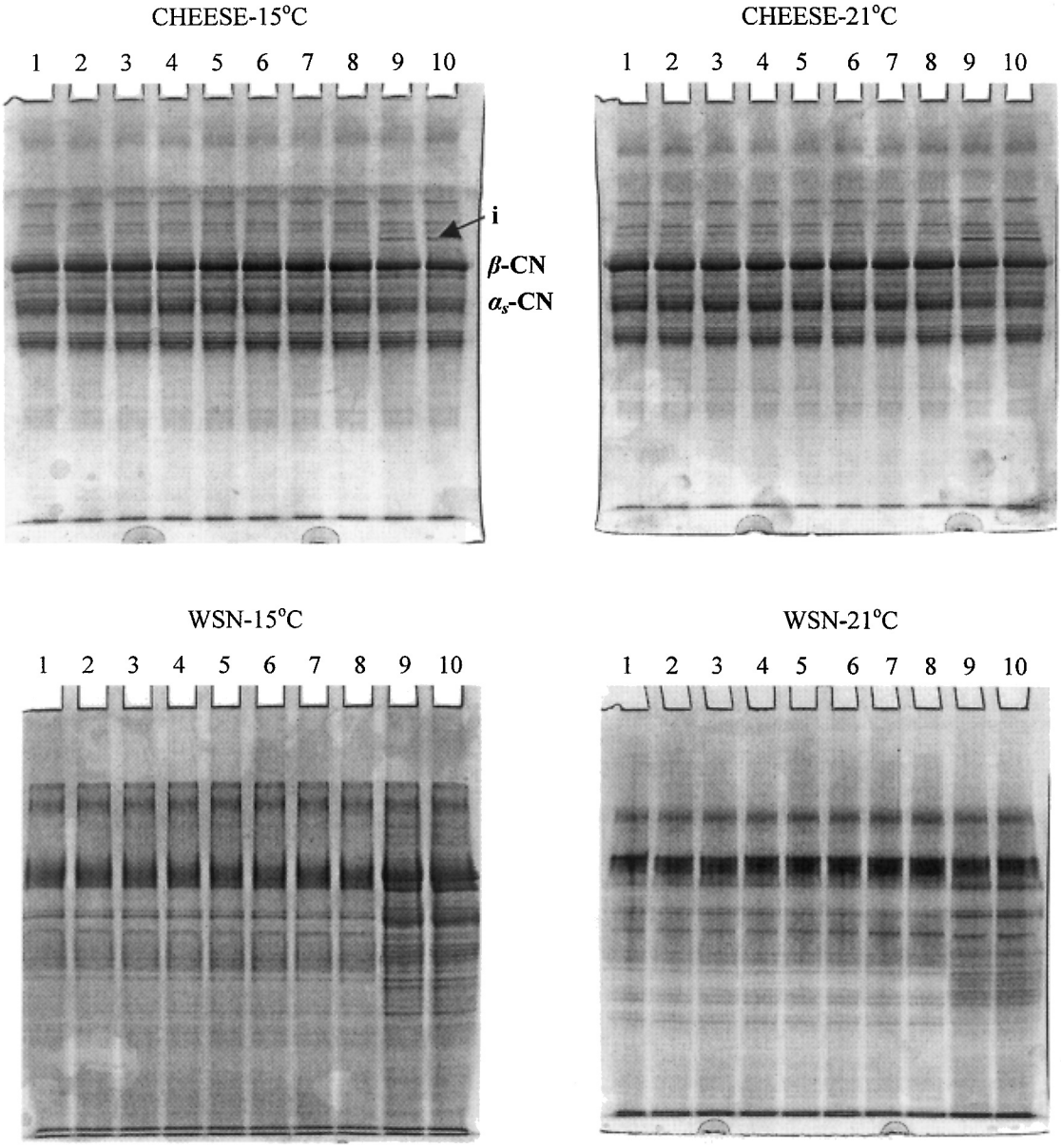

Fig. 1. Urea-PAGE profiles of 60-day-old feta cheeses and of the respective water-soluble fractions drained at 15 or at 21°C. Lanes 1, 2: Feta A; lanes 3, 4: Feta B; lanes 5, 6: Feta C; lanes 7, 8: Feta D; lanes 9, 10: Feta E (for A, B, C, D and E cheeses, see Section 2.1).

cheese, while residual rennet activity was expected to be high (Van den Berg & Exterkate, 1993). Michaelidou, Alichanidis, Urlaub, Polychroniadou and Zerfiridis (1998) have reported that most of the major water-soluble peptides in 6-month-old Feta originated from the N-terminal half of α_s-CN molecule and their formation can be attributed to chymosin action.

As was shown from the ratio of residual a_s-CN to residual β-CN, the draining temperature had more influence on residual β-CN than α_s-CN. This could be ascribed to the increase of the starter activity at 21°C, since residual chymosin activity on β-CN is inhibited by high salt in moisture content, because of the conformational effect of NaCl on the molecule region that is susceptible to hydrolysis by chymosin (Fox, 1989). But in 6-month-old Feta, major water-soluble peptides originating from the C-terminal domain of β-CN are the result of the cleavage of bonds susceptible to chymosin action (Michaelidou et al., 1998).

The area of the fractions moving faster than α_s-CN was higher in cheeses drained at 15°C than in those drained at 21°C. Apparently the low molecular mass nitrogenous compounds that according to the N-NH_2 contents were higher in cheeses drained at 21°C, were not fixed on polyacrylamide gel with 12% TCA.

The ratio of hydrophobic to hydrophilic peptides (HB/HL) in RP-HPLC profiles of the WSN extractions (Fig. 2) was calculated according to Gonzalez de Llano, Polo and Ramos (1995), that is the ratio of peptides eluted from 40 to 100 min to those eluted from 10 to 40 min (Table 3). The effect of ripening temperature was significant on the 10–40 min portion ($P = 0.000$), on the 70–100 min portion ($P = 0.000$) and on HB/HL ratio ($P = 0.000$). The effect of starters was significant ($P < 0.05$) on all the portions of RP-HPLC profiles. The higher activity of starters at 21°C resulted in higher percentages of the 10–40 min portion, that includes hydrophobic and/or small peptides (Belitz & Kaiser, 1993; Kaiser, Belitz & Fritsch, 1992). In cheese E, in which the acidification was low, this percentage was lower than that of cheeses A, B, C and D.

The 40–70 min portion had the same tendency. It has been reported that amino acids and peptides with molecular mass lower than 3000 Da are usually eluted by CH_3CN concentration $< 30\%$ (Engels & Visser, 1994; Kaiser et al., 1992). The rear region (70–100 min portion of the profiles) was greater in cheeses ripened at 15°C and in cheeses E drained at both temperatures. According to Tieleman and Warthesen (1991), hydrophobic and/or large peptides and proteins are eluted in this region. Michaelidou et al. (1998) have reported that whey proteins are eluted after 80 min. The area percentage of the 70–100 min portion was linearly correlated with WSN, as % of TN fraction ($r = 0.748$, $P < 0.01$). Furthermore, the 10–40 and 40–70 min portions were negatively linearly correlated with WSN, as % of TN fraction ($r = -0.625$ and $r = -0.532$, respectively, $P < 0.01$). Therefore, the high percentages of the 70–100 min portion in these cheeses, was the result of insufficient draining. The residual rennet activity was expected to be lower than in the other cheeses, due to the higher cheese curd pH. So, the pool of large and medium-size peptides resulting from chymosin action on caseins, which are the substrate for the microflora peptidases, was expected to be lower, leading to lower accumulation of small peptides, and amino acids in the front region of the chromatogram.

As shown in Table 3, the draining temperature more intensively affected the HB/HL ratios of Feta cheeses, made with starters including thermophilic lactobacilli strains. In cheeses A and B, drained at 15°C this ratio (HB/HL) was about 22% higher than those drained at 21°C. In C, D and E cheeses this difference was greater than 30%, likely due to the increase of aminopeptidase and dipeptidase activity of thermophilic lactobacilli at the higher draining temperature. The HB/HL ratio was linearly correlated with cheese pH ($r = 0.518$, $P < 0.001$).

Table 3
Peptide areas[a] in RP-HPLC profiles of water-soluble extracts of 60-day-old Feta cheeses

RP-HPLC portion (min)	Draining temperature (°C)	A[b]	B[b]	C[b]	D[b]	E[b]
10–40	15	10.3 b	8.6 a, b	9.5 a, b	8.5 a, b	7.0 a
	21	12.3 a, b	10.4 a, b	13.3 b	11.6 a, b	9.7 a
40–70	15	44.5 c	43.3 b, c	40.5 a, b	42.5 b, c	37.8 a
	21	46.3 b	45.1 b	45.1 b	43.9 b	37.0 a
70–100	15	33.6 a	36.1 a	36.2 a *	37.9 a, b *	43.2 b
	21	28.9 a	31.7 a	29.4 a	31.0 a	39.9 b
HB/HL[c]	15	7.8 a	9.5 a, b	8.1 a *	9.6 a, b	12.4 b
	21	6.1 a, b	7.4 a, b	5.6 a	6.7 a, b	8.2 b

[a] Means of three cheesemaking trials, expressed as percentage of Chromatogram Area Units (CAU) of each portion on total chromatogram CAU. Means in the same row of each cell with different letters are significantly different (LSD test, $P < 0.05$).

[b] See Section 2.1.

[c] Ratio of peptides eluted from 40 to 100 min to those eluted from 10 to 40 min.

*Significantly different means in the same column of each cell (LSD test, $P < 0.05$).

The draining temperature significantly affected ($P < 0.05$) the $C_{2:0}$, $C_{4:0}$, $C_{6:0}$, $C_{10:0}$ and $C_{12:0}$ free fatty acids shown in Table 4, as well as the total of $C_{2:0}$-$C_{8:0}$. The starter mixture significantly affected ($P < 0.05$) all the variables of Table 4. The FFA $C_{2:0}$ to $C_{8:0}$, especially acetic and butyric acid, are known to contribute to the organoleptic characteristics of Feta cheese. Higher concentrations of volatile acids develop in ewes' milk cheese than cow's milk cheese (Abd El Salam et al., 1993). The concentration and percentages of acetic and butyric acids, which are characteristic of feta flavour, vary considerably (Efthymiou, 1967; Alichanidis et al., 1984; Vafopoulou et al., 1989). The elevation of draining temperature enhanced the overall lipolytic activity in cheese during the early stages of ripening. The acetic acid is produced during the early stages of ripening and

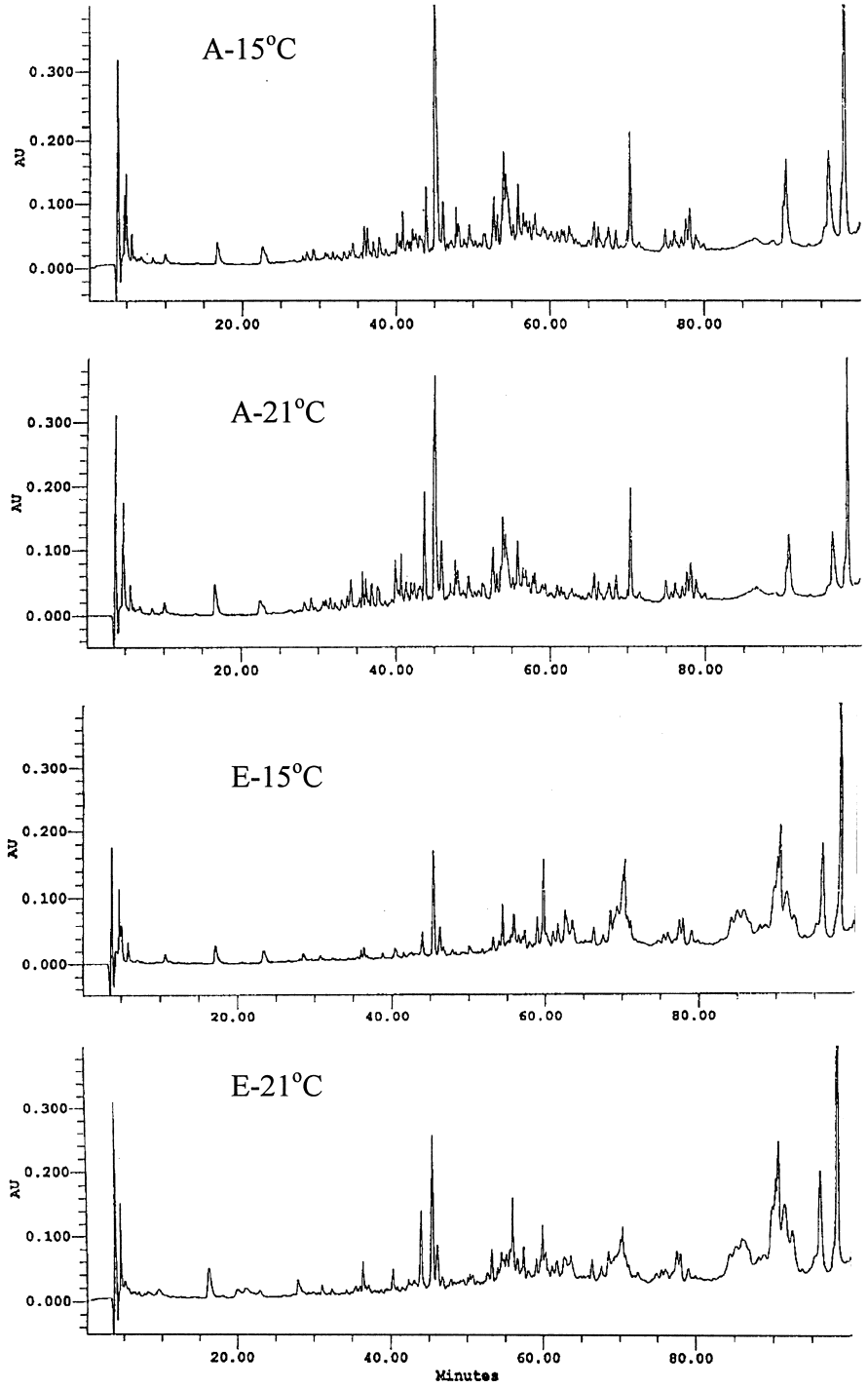

Fig. 2. RP-HPLC profiles (A_{220}) of 60-day-old feta water-soluble nitrogen, drained at 15°C and at 21°C. (A) Feta made with mesophilic starters and (E) Feta made with thermophilic starters, as described in Section 2.1.

lactose is present even in mature cheese, so it could be assumed that acetic acid is produced mainly through the fermentation of lactate (Abd El Salam et al.). The highest amount of acetic acid in cheese E, could be attributed to the growth of microbial groups including species that produce acetic acid from lactose.

3.4. Rheological characteristics

The results of the rheological examinations are shown in Table 5. There were statistically significant differences ($P < 0.05$) in the hardness and fracturability of the cheeses at both draining temperatures. The cheeses drained at 21°C exhibited higher values for hardness and fracturability than those drained at 15°C, due to their lower pH values and higher total solids and protein content (Table 2). It is well known that high acidity, protein and total solids contents make cheese harder and less easily deformed (Creamer & Olson, 1982; Fernandez del Polo, Gaya, Medina, Rodriguez-Marin & Nuñez, 1988). Starter mixtures did not significantly affect the rheological properties, although hardness and fracturability of cheeses E were lower than that of the others at both draining temperatures.

3.5. Organoleptic characteristics

The draining temperature significantly affected the body and texture of 60-day-old cheeses at $P = 0.055$ and their flavour at $P = 0.058$ (Table 6). Total organoleptic score (colour + body and texture + flavour) was affected at $P = 0.038$. The effect of starter mixture on the total organoleptic scores was significant ($P = 0.000$). Cheeses E drained at 15 or 21°C had significantly ($P < 0.05$) lower scores from those, in which mesophilic starters were included in the starter mixture. Similar results have been reported by Litopoulou-Tzanetaki et al. (1993). But, small differences in the organoleptic parameters of Feta cheese are difficult to determine, bearing in mind the mildly rancid, acid and salty taste of this cheese. As expected, the linear correlation of total organoleptic score with the 70–100 min region, the HB/HL ratio and

Table 4
Free fatty acids (FFA) of 60-day-old Feta cheese[a]

FFA (ppm)	Draining temperature (°C)	A[b]	B[b]	C[b]	D[b]	E[b]
$C_{2:0}$	15	465 a	466 a	646 b	538 a, b	1375 c
	21	1066 a *	1018 a *	1508 b *	1135 a *	1550 b *
$C_{4:0}$	15	78 a	63 a	67 a, b	83 b	129 c
	21	81 a	89 a	98 a *	104 a, b	148 c
$C_{6:0}$	15	49 a	52 a	60 a	62 a, b	73 b
	21	71 a *	68 a	62 a	67 a	93 b
$C_{8:0}$	15	168 a	102 a	143 a	100 a	304 b *
	21	214 b	135 a	181 a, b	161 a	222 b
Total $C_{2:0}$-$C_{8:0}$	15	760 a, b	682 a	916 b	782 a, b	1881 c
	21	1432 a *	1309 a *	1848 b *	1467 a *	2012 b
$C_{10:0}$	15	467 b *	431 a, b *	484 b *	330 a *	456 a, b *
	21	204 b	112 a	148 a, b	200 b	318 c
$C_{12:0}$	15	741 b *	755 b *	725 b *	351 a *	662 b *
	21	418 b	230 a	256 a	247 a	464 b
Total $C_{2:0}$-$C_{12:0}$	15	1967 b	1868 b	2125 b	1463 a	3000 c
	21	2053 b, c	1651 a	2252 c	1915 a, b *	2794 d

[a] Means of three cheesemaking trials. Means in the same row of each cell with different letters are significantly different (LSD test, $P < 0.05$).
[b] See Section 2.1.
*Significantly different means in the same column of each cell (LSD test, $P < 0.05$).

Table 5
Rheological characteristics[a] of 60-day-old Feta cheese

Rheological	Draining temperature (°C)	A[b]	B[b]	C[b]	D[b]	E[b]
Hardness (kg)	15	6.97	7.94	6.63	7.21	4.18
	21	12.75*	11.27*	12.08*	11.11*	8.24*
Fracturability (kg)	15	2.40	2.75	1.97	2.81	1.33
	21	4.56*	4.52*	4.77*	3.93*	2.75*
Compression at fracturability point (%) at	15	21.65	21.41	21.47	22.56	22.61
	21	21.11	20.18	20.17	19.78	24.78

[a] Means of five cheesemaking trials.
[b] See Section 2.1.
*Significantly different means in the same column of each cell (LSD test, $P < 0.05$).

Table 6
Organoleptic characteristics[a] of 60-day-old Feta cheeses

	Draining temperature (°C)	A[b]	B[b]	C[b]	D[b]	E[b]
Color	15	12.8b	12.9b	12.8b	12.6b	11.09a
	21	12.9	12.8	12.8	12.6	12.3
Body and texture	15	24.4b	25.4b	24.2b	23.9b	15.0a
	21	24.6b	24.9b	24.7b	25.1b	19.2a
Flavour	15	39.7b	40.7b	39.9b	38.2b	23.6a
	21	39.9b	41.8b	41.3b	41.7b	28.3a

[a] Means of five cheesemaking trials. Means in the same row of each cell with different letter are significantly different (LSD test, $P < 0.05$).
[b] See Section 2.1.

the WSN% of TN fraction was negative ($r = -0.754$, $r = -0.505$ and $r = -0.637$, respectively, $P < 0.01$) and with total solids content was positive ($r = 0.517$, $P < 0.01$).

4. Conclusions

Draining temperature significantly affected ($P < 0.05$) most of the characteristics of 60-day-old Feta cheeses. Although yogurt as starter is often used for Feta cheese manufacture, the combination of mesophilic and thermophilic strains was found be more suitable. Cheeses made with starters, including mesophilic cocci and thermophilic lactobacilli at 2:1 ratio, had the higher N-NH_2 content. The draining temperature should be between 15 and 21°C, in order to get an essential cheese yield along with high acidification rate during the first hours of draining.

The water-soluble extraction was not the proper index for the evaluation of proteolysis in Feta cheese because, apart from the casein hydrolysis products, it also includes whey proteins to an extent depending on curd pH. An index based on the medium and small molecular mass nitrogenous compounds, as they were eluted in the RP-HPLC profiles of water-soluble extracts or from the N-NH_2 content would be more appropriate for the mature cheese.

Acknowledgements

This study was a part of the project "Standardization of traditional Greek cheeses-Stabilization systems of raw material quality", funded by *Operational Program for Research and Technology II-Subprogram 1* of the Greek General Secretariat for Research and Technology (Ministry of Technology). The authors gratefully acknowledge Mrs. E. Manolopoulou for culture preparation and Mr. S. Maganaris for his assistance.

References

Abd El-Salam, M. H., Alichanidis, E., & Zerfiridis, G. K. (1993). Domiati and Feta type cheeses. In P.F. Fox, *Cheese: chemistry, physics and microbiology, major cheese groups* (2nd ed., vol. 2, pp. 316–322). London: Chapman and Hall.

AIR/FLORA. (1997). Laboratory Manual: Chemical methods for the characterization of cheese ripening; Work material, AIR-Concerned Action No 3-CT94-2039.

Alichanidis, E., Anifantakis, E. M., Polychroniadou, A., & Nanou, M. (1984). Suitability of some microbial coagulants for Feta cheese manufacture. *Journal of Dairy Research, 51*, 141–147.

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research, 50*, 45–55.

Aston, J. W., Gilles, J. E., Durward, I. G., & Dulley, J. R. (1985). Effect of elevated ripening temperatures on proteolysis and flavour development in Cheddar cheese. *Journal of Dairy Research, 52*, 565–572.

Belitz, H.-D., & Kaiser, K. P. (1993). Monitoring Cheddar cheese ripening by chemical indices of proteolysis. 3. Identification of several high molecular mass peptides. *Zeitschrift für Lebensmittel - Untersuchung und –Forschung, 197*, 118–122.

Blakesley, R. W., & Boezi, J. A. (1977). A new staining technique for protein in polyacrylamide gels using Coomassie Brilliant Blue G-250. *Analytical Chemistry, 82*, 580–582.

Codex Alimentarius (1988). *Official Journal of Hellenic Republic* (Vol. B, No 892, Article 83, Supplement No 3, June 1994). Athens: National Printing Office.

Creamer, L. K., & Olson, N. F. (1982). Rheological evaluations of maturing Cheddar cheese. *Journal of Food Science, 47*, 631–647.

Efthymiou, C. (1967). Major Free Fatty Acids of Feta cheese. *Journal of Dairy Science, 50*, 20–24.

Engels, W. J. M., & Visser, S. (1994). Isolation and comparative characterization of components that contribute to the flavour of different types of cheese. *Netherlands Milk and Dairy Journal, 48*, 127–140.

Fernandez del Polo, B., Gaya, P., Medina, M., Rodriguez-Marin, A., & Nuñez, M. (1988). Changes in chemical and rheological characteristics of La Serena ewes' milk cheese during ripening. *Journal of Dairy Research, 55*, 457–464.

Folkertsma, B., & Fox, P. F. (1992). Use of Cd-ninhydrin reagent to asses proteolysis in cheese during ripening. *Journal of Dairy Research, 59*, 217–224.

Fox, P. F. (1989). Proteolysis during cheese manufacturing and ripening. *Journal of Dairy Science, 72*, 1379–1400.

Freitas, A. C., & Malcata, F. X. (1998). Effects of different ripening procedures on the final characteristics of Picante cheese. *Zeitschrift für Lebensmittel -Untersuchung und-Forschung, 207*, 281–291.

Gaya, P., Medina, M., Rodriguez-Marín, M. A., & Nuñez, M. (1990). Accelarated ripening of ewes' milk Manchego cheese: the effect of elevated ripening temperatures. *Journal of Dairy Science, 73*, 26–32.

Gonzalez de Llano, D., Polo, M. C., & Ramos, M. (1995). Study of proteolysis in artisanal cheeses: High Performance Liquid Chromatography of peptides. *Journal of Dairy Science, 78*, 1018–1024.

IDF. (1982). Cheese and processed cheese. Determination of the total solids content (Standard No. 4A) Brussels: International Dairy Federation.

Jarret, W. D., Aston, J. W., & Dulley, J. R. (1982). A simple method for estimating free amino acids in Cheddar cheese. *Australian Journal of Dairy Technology, 37*, 55–58.

Kaiser, K.-P., Belitz, H.-D., & Fritsch, R. J. (1992). Monitoring Cheddar cheese ripening by chemical indices of proteolysis. 2. Peptide mapping of casein fragments by reverse-phase high-performance liquid chromatography. *Zeitschrift für Lebensmittel - Untersuchung und –Forschung, 195*, 8–14.

Lawrence, R. C., Creamer, L. K., & Gilles, J. (1987). Texture development during cheese ripening. *Journal of Dairy Science, 70*, 1748–1760.

Litopoulou-Tzanetaki, E., Tzanetakis, N., & Vafopoulou-Mastrojiannaki, A. (1993). Effect of the type of lactic starter on microbiological, chemical and sensory characteristics of Feta cheese. *Food Microbioloy, 10*, 31–41.

Mallatou, H., Pappas, C. P., & Voutsinas, L. P. (1994). Manufacture of Feta cheese from sheep's milk, goat's milk or mixture of these milks. *International Dairy Journal, 4*, 641–664.

Michaelidou, A., Alichanidis, E., Urlaub, H., Polychroniadou, A., & Zerfiridis, G. K. (1998). Isolation and identification of some water soluble peptides in Feta cheese. *Journal of Dairy Science, 81*, 3109–3116.

Moatsou, G., Kandarakis, I., Georgala, A., Alichanidis, E., & Anifantakis, E. (1999). Effect of starters on proteolysis of Graviera Kritis cheese. *Le Lait, 79*, 303–315.

Nieuwenhof, F. F. J., & Hup, G. (1971). Gas-chromatographic determination of free fatty acids in cheese. *Netherlands Milk and Dairy Journal, 25*, 175–182.

Nuñez, M., García-Acer, C., Rodriguez-Marín, M. A., Medina, M., & Gaya, P. (1986). The effect of ripening and cooking temperatures on proteolysis and lipolysis in Manchego cheese. *Food Chemistry, 21*, 115–123.

Pritchard, G. G., & Coolbear, T. (1993). The physiology and biochemistry of the proteolytic system of lactic acid bacteria. *FEMS Microbiology Reviews, 12*, 179–206.

Tieleman, A. E., & Warthesen, J. J. (1991). Comparison of three extraction procedures to characterize Cheddar cheese proteolysis. *Journal of Dairy Science, 74*, 3686–3694.

Vafopoulou, A., Alichanidis, E., & Zerfiridis, G. (1989). Accelerated ripening oF Feta cheese, with heat-shocked cultures or microbial proteinases. *Journal of Dairy Research, 56*, 285–296.

Vafopoulou-Mastrojiannaki, A., Litopoulou-Tzanetaki, E., & Tzanetakis, N. (1990). Effect of Pediococcus pentosaceus on ripening changes of Feta cheese. *Microbiologie-Aliments-Nutrition, 8*, 53–62.

Van den Berg, G., & Exterkate, F. A. (1993). Technological parameters involved in cheese ripening. *International Dairy Journal, 3*, 485–507.