Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 104 (2007) 800–807
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of multiple-species starters on peptide profile and free amino acids in low-fat Kefalograviera-type cheese

A. Michaelidou [a], M.C. Katsiari [b], L.P. Voutsinas [b], A. Polychroniadou [a], E. Alichanidis [a,*]

[a] *Laboratory of Dairy Technology, School of Agriculture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*
[b] *National Agricultural Research Foundation, Dairy Research Institute, 452 21 Katsikas, Ioannina, Greece*

Received 29 May 2006; received in revised form 7 November 2006; accepted 18 December 2006

Abstract

Two commercially available starter culture systems, Alp DIP and a mixture of Alp DIP D and Yoghurt V1, were compared with one commercial yoghurt starter culture, CH-1, for their effect on proteolysis in low-fat (9.5%) high-moisture (49.6%) Kefalograviera-type cheese. A full-fat control cheese (30.8% fat, 37.7% moisture) was also made with the CH-1 culture using a modified scalding procedure. The effect of the multiple-species cultures on primary casein breakdown in low-fat cheeses, as assessed by polyacrylamide gel electrophoresis, was small but secondary proteolysis, as assessed by reversed-phase HPLC determination of the total free amino acid content of the cheeses, was significantly ($p < 0.05$) enhanced. Experimental cheeses had a richer peptide profile and a generally higher free amino acid content than the controls. Cheeses were clearly separated according to the starter culture and to the scalding temperature using principal component analysis of the results from reverse-phase HPLC chromatograms of the water extracts and from individual free amino acid content.

Keywords: Low-fat cheese; Kefalograviera cheese; Multiple-species starter; Peptides; Amino acids; Chemometrics

1. Introduction

Cheese is a traditional and well-established basic food, which can take on many forms, can be consumed in many different occasions and is capable of generating staple nutrition and pleasant sensory properties. However, cheese does not have the healthy image it once had, because of the customers' tendency to reduce calories intake and to avoid food relatively rich in saturated fat. Since the turnover of any industry is driven by the customers demand, there has been a considerable interest in manufacturing reduced-fat (RF) and low-fat (LF) cheeses but without scarifying the organoleptic properties of the original full-fat (FF) product.

Fat reduction in cheese milk results in a major shift in the compositional balance of the various components of cheese compared with FF counterpart and makes the role of other constituents (e.g., moisture and protein) more important for the development of good textural and sensory qualities (Mistry, 2001).

Several interventions, such as modification of conventional manufacturing procedures or use of enzymes, additives (stabilizers and fat replacers), adjunct cultures, specially designed starters, etc., were applied aiming to improve the flavour and texture of RF or LF cheeses (Fenelon & Guinee, 2000; Mistry, 2001). As these products have a proportionally higher amount of casein compared to the respective FF cheese, an increased casein (especially α_{s1}-CN) breakdown is needed for the development of an attractive, smooth cheese body (Ardö, 1997). Moreover, the use of starter cultures exerting high peptidolytic activity would help towards the increase in concentration of small peptides and amino acids, which directly contribute to the flavour or can act as precursors of flavour compounds (Ardö, 1997; Engels & Visser, 1994).

* Corresponding author. Tel.: +30 2310 998761; fax: +30 2310 998789.
E-mail address: sali@agro.auth.gr (E. Alichanidis).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.12.041

Katsiari, Voutsinas, and Kondyli (2002a) found that it is possible to make a low-fat (95 g/kg fat) Kefalograviera-type cheese, with sensory qualities similar to those of full-fat (308 g/kg fat) cheese, by using two commercial multiple-species starter cultures (Alp DIP and Alp DIP D). The present article deals with the effect of the above-mentioned cultures on the proteolysis in low-fat Kefalograviera-type cheese.

2. Materials and methods

2.1. Starter cultures

The starter cultures used for cheesemaking in this study were the following:

1. CH-1: a regular yogurt culture, used as control (Chr. Hansen's Laboratorium, Copenhagen, Denmark), This culture is used commercially for the production of full-fat Kefalograviera cheese.
2. Alp DIP: a specially defined multiple-species (*Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *Streptococcus thermophilus, Lactobacillus helveticus* and *L. lactis*) starter culture (Wiesby GmbH & Co. KG, Niebull, Germany).
3. Alp DIP D: a special starter culture containing Alp DIP culture and *L. lactis* subsp. *lactis* biovar *diacetylactis.* It was used along with Yoghurt V1 culture, a thermophilic, undefined multiple-species culture, consisting of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* (Wiesby GmbH & Co. KG, Niebull, Germany).

Details on the starter cultures used were given in Katsiari et al. (2002a). The cultures were an offer from Hansen and Wiesby companies.

2.2. Cheese manufacture

Bulk ewes' milk was obtained from the herd of the Agricultural Research Station of Ioannina and used for cheese manufacture as described in Katsiari et al. (2002a). The cheeses were coded as follows: cheese A (low-fat made with culture Alp DIP), cheese B (low-fat made with culture Alp DIP D supplemented with culture Yoghurt V1), cheese C (low-fat control made with culture CH-1), and cheese D (full-fat control made with culture CH-1).

To achieve comparable values of moisture in non-fat substances (MNFS) in full-fat and low-fat cheeses, the following modifications in the cooking and pressing steps were applied:

(a) The curd temperature of the full-fat control cheese (D) was raised from 38 °C to 47 °C in 20 min; in contrast, the temperature of the curds of low-fat cheeses (A–C) was raised from 38 °C to 41 °C in 5 min.
(b) The curd of cheese D was stirred after cooking for 5 min but there was no holding period for cheeses A–C after scalding temperature was reached.
(c) The curd of cheese D was pressed after moulding for 80 min but the curds of the low-fat cheeses were pressed in moulds for only 20 min.

Three replicate cheesemakings were performed. Cheese samples for analyses were taken from each batch at 5, 25, 60, 90, and 180 d after manufacture. The values reported are the means of the three replicates, except if otherwise stated.

2.3. Cheese analyses

Cheese samples were analysed for moisture, protein, fat, salt and pH, as described previously (Katsiari, Voutsinas, & Kondyli, 2002b). Water-soluble nitrogen (WSN), nitrogen soluble in 12% trichloroacetic acid (TCA-SN) and nitrogen soluble in 5% phosphotungstic acid (PTA-SN) were determined as described by Katsiari, Alichanidis, Voutsinas, and Roussis (2001). All analyses were carried out in duplicate.

Free amino acids (FAA) were determined using the method proposed by Krause, Bockhardt, Neckermann, Henle, and Klostermeyer (1995), as described by Michaelidou, Katsiari, Voutsinas, Kondyli, and Alichanidis (2003).

Nitrogenous material in the water extracts was examined by means of reversed-phase HPLC (RP-HPLC) as described by Michaelidou et al. (2003). Prior to injection, the water extracts were standardized for nitrogen content (0.2% w/w) by diluting with HPLC water.

Cheese samples and their water extracts were analysed by urea-PAGE according to the method of Andrews (1983), the gels were stained by the method of Blakesley and Boesi (1977), and the optical density of the gel bands was recorded at 590 nm, as described earlier (Michaelidou et al., 2003). From the densitograms, the levels of residual α_{s1}- and β-caseins in the cheeses through ripening were calculated and expressed as percentages of the corresponding level in the 1 d-old cheese. The results from densitometry are presented as observations and supportive data but were not statistically analysed.

2.4. Statistical analysis

The experiment was conducted to evaluate the effect of the four treatments (A–D, as described in Materials and Methods) on the variables of proteolysis during ripening. There were three replicate trials for each treatment. A two-factor (5 times × 4 treatments) factorial experiment was employed. The analysis of variance (ANOVA) was performed using the SPSS, version 12 program (SPSS Inc., Chicago, IL, USA). Differences between the means were compared at the 5% level of significance using the least significant difference (LSD) test.

The RP-HPLC chromatograms of water extracts were analysed by multivariate statistical techniques to evaluate the effects of the four treatments. Peaks for multivariate statistical analysis were selected visually and by the retention

Table 1
Compositional and proteolysis data of 90 d-old Kefalograviera-type cheeses

Cheese code[2]	Moisture %	Fat %	MNFS[3] %	Protein %	pH	WSN%TN[4]	TCA-SN%TN[4]	PTA-SN%TN[4]	TFAA[4] (μmole/g)
A	49.79 ± 1.01^{b}	9.50 ± 0.14^{a}	55.02 ± 1.13^{a}	32.26 ± 1.25^{b}	5.33 ± 0.08^{a}	21.37 ± 0.84^{b}	11.13 ± 0.38^{a}	4.72 ± 0.29^{ab}	78.82 ± 0.23^{a}
B	50.65 ± 0.67^{b}	9.17 ± 0.22^{a}	55.77 ± 0.74^{a}	31.24 ± 0.73^{b}	5.28 ± 0.09^{a}	21.45 ± 0.74^{b}	11.22 ± 0.66^{a}	4.77 ± 0.29^{ab}	80.85 ± 0.23^{b}
C	48.41 ± 1.02^{b}	9.75 ± 0.72^{a}	53.63 ± 0.81^{a}	33.39 ± 0.42^{b}	5.41 ± 0.05^{a}	18.00 ± 0.72^{a}	10.17 ± 0.89^{a}	3.70 ± 0.19^{a}	74.33 ± 0.34^{c}
D	37.70 ± 0.61^{a}	30.83 ± 0.96^{b}	54.47 ± 0.37^{a}	26.08 ± 0.53^{a}	5.49 ± 0.03^{a}	19.85 ± 0.62^{ab}	11.06 ± 0.94^{a}	5.95 ± 0.51^{b}	67.74 ± 0.35^{d}

Values presented are the means ± standard error of three replicate trials[1].
[1] Means within a column with a common superscript did not differ ($p > 0.05$).
[2] A = Low-fat made with starter culture Alp DIP; B = Low-fat made with starter cultures Alp DIP D and Joghurt V1; C = Low-fat control made with starter culture CH-1; D = Full-fat control made with starter culture CH-1.
[3] MNFS = Moisture in the non-fat substance.
[4] WSN%TN = Water soluble N (% total N); TCA-SN%TN = N soluble in 12% TCA (% total N); PTA-SN%TN = N soluble in 5% phosphotungstic acid (% total N); TFAA = Total free amino acids.

time in the chromatograms (homologous peaks); peak heights were used as variables. The concentrations of individual FAA were expressed as percentages of total FAA and the resulted (relative) values were used as variables.

Principal component analysis (PCA) was performed by standardizing the variables to zero mean and using the covariance matrix. Hierarchical cluster analysis was performed using squared Euclidean distance and mean linkage without standardizing the variables. Statistical analysis was performed using Minitab for Windows 98, release 13 (Minitab Inc., State College, PA, USA). Figures based on the statistical analysis were prepared using Grapher for Windows, version 1.32 (Golden Software Inc., Golden, CO, USA).

3. Results and discussion

3.1. Cheese composition

The gross composition and the level of nitrogen fractions of the four cheeses had been described in detail by Katsiari et al. (2002a). Table 1 shows the mean composition of the 90 d-old cheeses. It is obvious that, irrespective

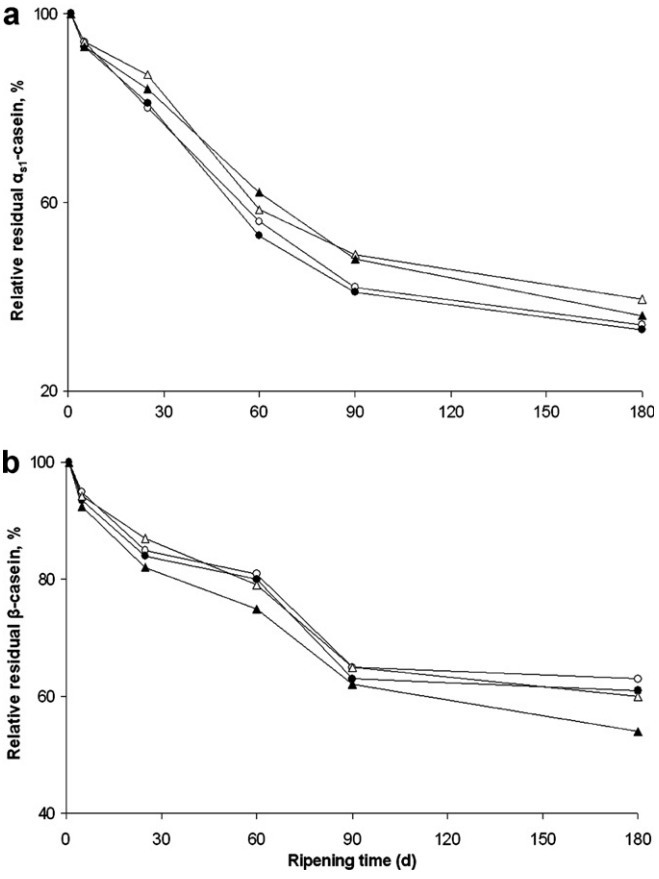

Fig. 1. Values of residual α_{s1}- (a) and β-caseins (b) expressed as percentages of the corresponding values in the 1 d-old Kefalograviera-type cheeses. (○), low-fat cheese made with starter culture Alp DIP (cheese A); (●), low-fat cheese made with starter cultures Alp DIP D and Joghurt V1 (cheese B); (▲), low-fat control cheese made with starter culture CH-1 (cheese C); (△), full-fat control cheese made with starter culture CH-1 (cheese D). Values presented are means of three replicate trials.

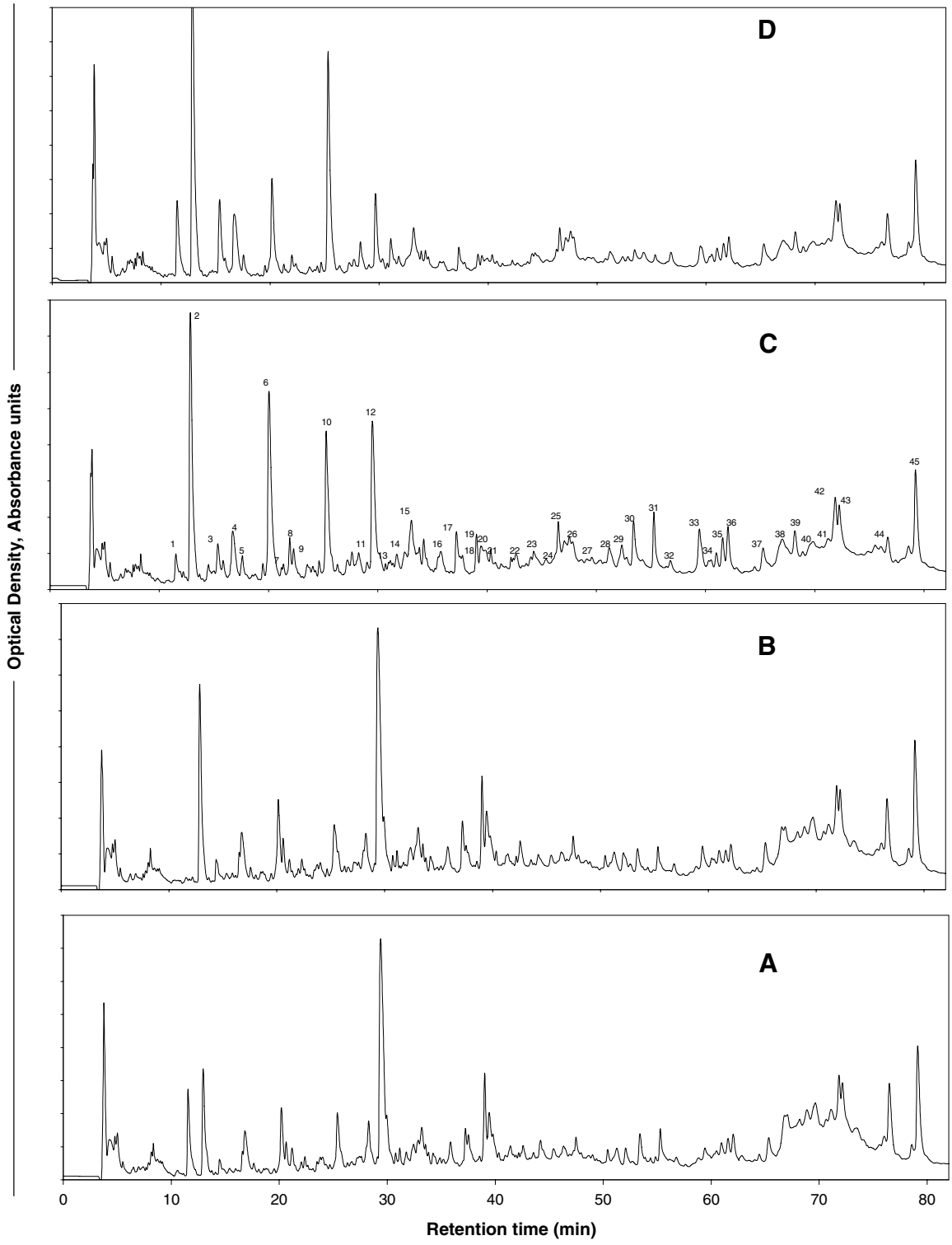

Fig. 2. Reverse-phase HPLC profiles of the water extracts from 60 d-old Kefalograviera-type cheeses. Cheese code (A, B, C, D): see Fig. 1.

of the differences in moisture and fat of the cheeses, the modifications applied in cheese-making resulted in similar MNFS and pH, thus similar environmental conditions for the cultures to act.

The levels of the N fractions expressed as percentages of the total nitrogen did not exhibit large differences between the low-fat control C and the experimental cheeses A and B (Table 1), although these differences were significant ($p < 0.05$) for WSN. However, the total free amino acid content of the cheeses did significantly differ ($p < 0.05$). It is probable that the relatively low cooking temperature applied to the curds of all these cheeses had favoured the

growth of mesophilic lactococci in cheeses A and B with a concomitant increase in proteolysis. Ardö (1997) reported similar results. Also, part of the differences may be attributed to the presence of lactobacilli in cheeses A and B, especially *Lb. helveticus*, which exerts relatively high peptidolytic activity (Fröhlich-Wyder & Bachmann, 2004).

Urea-PAGE electrophoretograms of the four cheeses were similar. Although the level of rennet retained in the curd of the full-fat cheese (D) was expected to be higher than in the other cheeses, degradation of α_{s1}-casein was comparable in all cheeses. This was probably due to the higher cooking temperature applied to the full-fat control (D) exerting a negative effect on rennet activity. The rate of degradation of α_{s1}- and β-casein in the experimental cheeses (A and B) was marginally higher as compared to the controls (C and D); this may be attributed to the proteinases of the adjunct culture (Fig. 1). Additionally, the slightly higher degradation of β-casein in cheese D might be due to the higher cooking temperature of this cheese resulting in a small enhancement of the plasmin activity.

3.2. Peptide profiles

For further investigation of the effect of different treatments on proteolysis, the water-soluble extracts (WSE) of the cheeses were analysed by RP-HPLC. The number of peaks in the chromatograms of all cheeses increased for 25 d but it remained relatively constant throughout the rest of maturation. However, differences were observed between treatments regarding the height of the peaks. A set of typical RP-HPLC profiles of the WSE of the cheeses at 60 d of ripening is shown in Fig. 2. Peaks being recognized visually and by retention times as equivalent (homologous) in the four cheeses were numbered. Their heights were used as variables in multivariate statistical analysis.

Principal component analysis (PCA) of the peptide data sets of 25, 60, 90 and 180 d-old cheeses (mean of three batches) showed that principal component 1 (PC1) and principal component 2 (PC2) accounted for 83.8% and 10.3% of the variation, respectively (Fig. 3a). PC1 separated the controls from the experimental cheeses A and B and PC2 separated the cheeses based generally on their age. PCA was also applied to the data obtained from each particular age of cheese (not shown). At any age, the heights of peaks 12, 19, 20 and 38 were the highest in cheeses A and B, whereas they were the lowest in both control cheeses.

Hierarchical cluster analysis highlighted closely related clusters (Fig. 3b), which are indicated as ovals on the score plot. At any age, experimental cheeses A and B, prepared under similar manufacturing conditions (cooking temperature, etc.), formed a common cluster and were not separated from each other on the score plot. Thus, the additional yogurt culture used in cheese B had no major effect on its peptide profile, probably because of the low cooking temperature applied.

Three clusters were formed on PC2 (Fig. 3a): 25 d-old and 180 d-old cheeses were well separated but 60 d and 90 d-old cheeses formed a common cluster. Although the peak heights (used as variables) varied during ripening, changes were less pronounced between 60 and 90 d. This fact could explain the poor separation of the 60 and 90 d-old cheeses.

For the preparation of control cheeses C and D, the same culture (yoghurt) was used but curd-cooking temperature was higher for cheese D than for cheese C and the experimental cheeses. On PC1 the samples of cheese C were separated from those of cheese D and located at an intermediate distance between those of cheese D and the experimental cheeses A and B, indicating that the grouping of

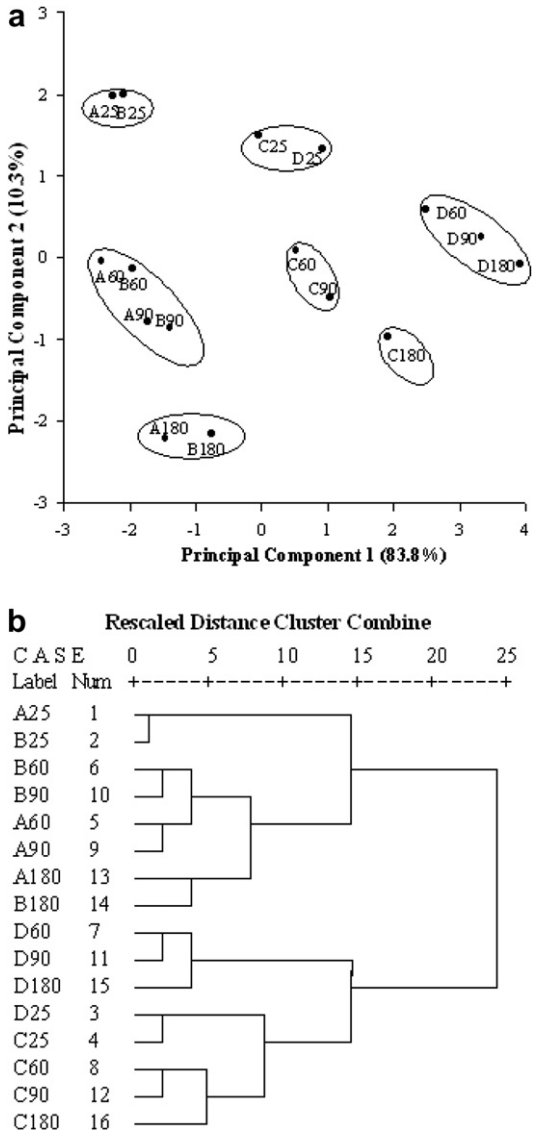

Fig. 3. Principal component (a) and hierarchical cluster (b) analyses of data from RP-HPLC profiles of the water extracts from Kefalograviera-type cheeses. Cheese code (A–D): see Fig. 1.

cheese C was influenced by the scalding temperature (Fig. 3a). The more intense cooking may positively affect the growth of thermophilic culture in cheese D (Ardö, 1997) as well as plasmin activity and, thus, change the rate of production of some peptides in this cheese (Somers & Kelly, 2002; Sousa, Ardö, & McSweeney, 2001). In respect of the peaks 2, 3, 4 and 10, cheese D exhibited at any age the greatest peak heights among the four cheeses, whereas cheeses A and B the lowest peak heights. In contrast, in respect of the peaks 6, 8, 30, 33 and 36, control cheese C showed the greatest peak heights and cheese D the lowest.

The separation of control cheeses from each other on PC1 was based mainly on the major peaks eluted at retention times lower than 30 min, where hydrophilic peptides and amino acids were expected to be eluted (McSweeney, Fox, Lucey, Jordan, & Cogan, 1993). Therefore, RP-HPLC analysis of the PTA-SN and TCA-SN fractions was also performed to determine which of the homologous peaks were included in each of them.

The results showed that all peaks of PTA-SN were eluted within the first 30 min and very few peaks with small height were eluted after this time. Also, the peptides included in TCA-SN were eluted in times shorter than 58 min. The total peak height of each chromatogram region (0–30 min and 30–58 min) was statistically analyzed against ripening time. The outcome of the calculations was that, after 25 d of ripening, the values for the peptides of full-fat cheese (D) in PTA-SN or TCA-SN were significantly ($p < 0.05$) higher or lower, respectively, than the values of all low-fat cheeses (A–C). Also, the rate of production of PTA-soluble peptides after 90 d of ripening declined in cheese D as compared to the other cheeses. The differences in PTA-soluble peptides between full-fat control D and the low-fat cheeses combined with the relatively slow rate of proteolysis in cheese D may explain the clustering of the 60, 90 and 180 d-old samples from cheese D in a common group on PC2, in a manner different than for all low-fat cheeses.

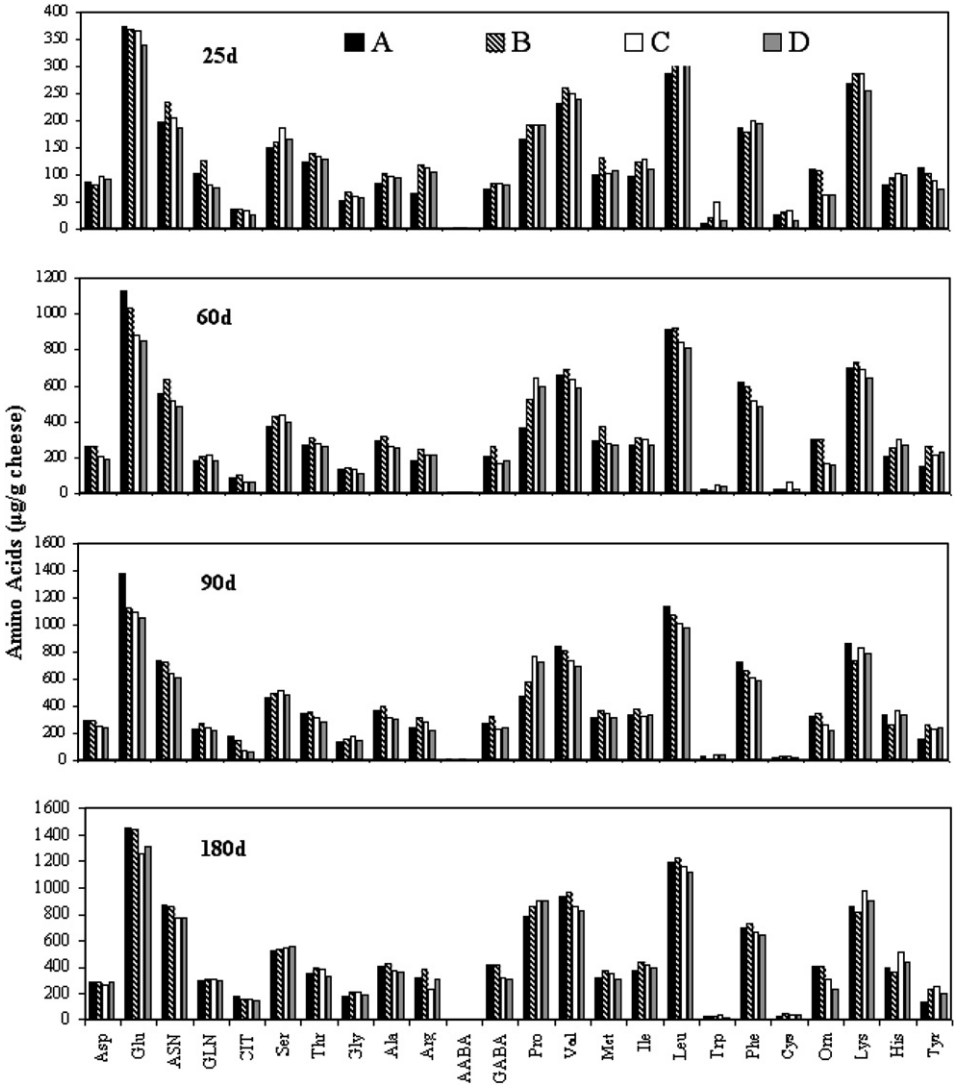

Fig. 4. Levels of individual free amino acids in Kefalograviera-type cheeses at 25, 60, 90 and 180 d of ripening. Values presented are the means of two replicate trials. Cheese code (A–D): see Fig. 1.

3.3. Free amino acids

Free amino acids (FAA) are the end products of proteolysis released from peptides by peptidase action. They accumulate during ripening and subsequently are metabolised, to varying extent by the enzymes of the cheese microflora, into compounds volatile or not, which contribute to flavour development in cheese (McSweeney & Sousa, 2000).

The FAA profiles of the cheese during ripening are shown in Fig. 4. In agreement with previous studies on Kefalograviera cheese (Katsiari et al., 2001; Michaelidou et al., 2003), the principal FAA were Leu, Glu, Val, Pro and Lys, which together with Asn, Ser and Phe summed up for over 60% of the total FAA throughout ripening. Also, the non-casein amino acids γ-aminobutyric acid and ornithine were found in all cheeses at appreciable concentrations. In contrast, the concentration of citrulline, α-aminobutyric acid, Trp, and Cys in all cheeses at all ripening times was very low accounting for only 2% of the total FAA content.

While all cheeses shared the same major or minor FAA, the relative concentration of each amino acid differed among cheeses of a certain age. The relative concentrations of all amino acids (normalization by the total FAA at each age for each cheese, percentages) after 60, 90 and 180 d of ripening were used as variables for PCA.

Following PCA of the data set of individual amino acids at 60, 90, and 180 d, PC1 was found to separate the cheeses according to their starter cultures, while for PC2 separation was based generally on the cheese age (Fig. 5a). These components accounted for 64.8% and 16.5% of the variation, respectively. Full-fat (D) and low-fat (C) control cheeses, made using the same starters, clustered together at 60 and 90 d of ripening but formed a separate group at 180 d. Hierarchical cluster analysis (Fig. 5b) verified this finding and, also, showed that the evolution of some characteristic amino acids was time dependent and not affected by the differences in cooking temperature and fat content. The control cheeses had a higher ($p < 0.05$) percentage of the amino acids Pro, Ser, His, Gly and Lys at all ages compared to both experimental cheeses. Percentages of Leu and Val did not differ ($p > 0.05$) between cheeses.

The samples of low-fat experimental cheese B, at 60 d and 90 d of age, were well separated from those of the low-fat experimental cheese A of the same ages (Fig. 5a) and formed a cluster located at an intermediate distance between the cluster of control cheeses and that of cheese A. The starter culture of cheese B was similar to that of cheese A, but supplemented with yoghurt culture strains. Thus, the composition of the starter culture system used in cheese B relates cheese B both with control cheeses (C and D) and with cheese A and may explain the position of the cluster.

Compared to control cheeses (C and D) both experimental cheeses (A and B) contained higher ($p < 0.05$) proportions of Glu, ornithine and γ-aminobutyric acid

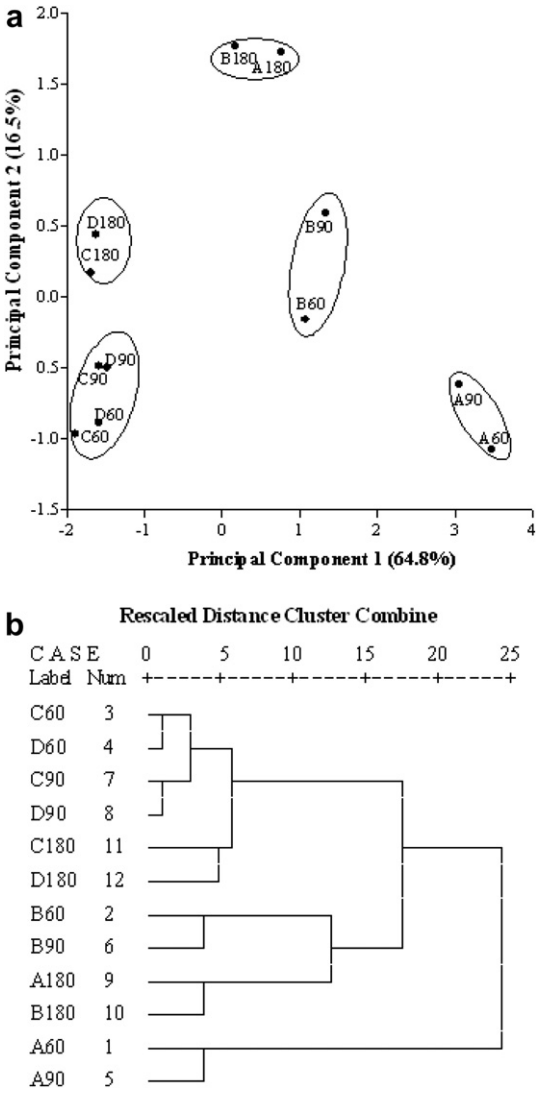

Fig. 5. Principal component (a) and hierarchical cluster (b) analyses of the data for relative concentrations of individual free amino acids of Kefalograviera-type cheeses. Cheese code (A–D): see Fig. 1.

throughout ripening. Despite the cultures used for cheesemaking, the proportion of γ-aminobutyric acid and Asn increased with ripening time, while that of Lys, Tyr and Phe decreased, possibly because a part of these three amino acids was converted to biogenic amines.

4. Conclusions

The use of commercial multiple-species starter culture systems (Alp DIP or a combination of Alp DIP D and Yoghurt V1), as compared with the yoghurt starter culture CH-1, had a positive effect on secondary proteolysis in low-fat Kefalograviera-type cheeses.

Using PCA of the RP-HPLC peptide profiles, cheeses were arranged into two main groups: the control cheeses made with the yoghurt culture CH-1 were separated from the experimental cheeses made with multiple-species cultures. Within the control cheeses, the higher scalding tem-

perature and fat content of the full-fat control cheese resulted in a peptide profile with higher amounts of peptides of short retention times (soluble in PTA). In contrast, PCA failed to distinguish among experimental cheeses (at any sampling age), indicating that the effect of the additional thermophilic culture on the peptide formation in cheese B was small, probably because of the low scalding temperature applied.

While the main FAA for all cheeses of this study were Leu, Glu, Val, Pro, and Lys, the relative concentration (as percentage of the total free amino acids) of individual amino acids produced by each culture used in cheesemaking were different. Using these relative concentrations of FAA as variables, PCA distributed the cheese samples according to the starter culture, despite the differences in fat content and scalding temperature.

In conclusion, the use of multiple-species cultures could, as expected, improve secondary proteolysis in low-fat Kefalograviera-type cheese. Within the cheesemaking parameters applied, mesophilic starters were found to play the major role in peptide and FAA production

Acknowledgement

This study was performed as part of NAGREF Project No VI/7 in the framework of the Programme DEMETRA '95 funded by the National Agricultural Research Foundation of Greece.

References

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research, 50*(1), 45–55.

Ardö, Y. (1997). Flavour and texture in low-fat cheese. In B. A. Law (Ed.), *Microbiology and biochemistry of cheese and fermented milk* (pp. 207–218). London: Chapman & Hall.

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

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*(2), 127–140.

Fenelon, M. A., & Guinee, T. P. (2000). Flavour development in low-fat cheese. In T. M. Cogan, P. L. H. McSweeney, & T. P. Guinee (Eds.), *Proceedings of sixth Moorepack cheese symposium* (pp. 31–42). Dublin: Teagasc/UCC.

Fröhlich-Wyder, M. T., & Bachmann, H. P. (2004). Cheeses with propionic acid fermentation. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.). *Cheese: Chemistry, physics and microbiology* (3rd ed. Vol. 2, pp. 141–156). London: Elsevier.

Katsiari, M. C., Alichanidis, E., Voutsinas, L. P., & Roussis, I. G. (2001). Proteolysis in reduced sodium Kefalograviera cheese made by partial replacement of NaCl with KCl. *Food Chemistry, 73*(1), 31–43.

Katsiari, M. C., Voutsinas, L. P., & Kondyli, E. (2002a). Improvement of sensory quality of low-fat Kefalograviera-type cheese by using commercial special starter cultures. *Journal of Dairy Science, 85*(11), 2759–2767.

Katsiari, M. C., Voutsinas, L. P., & Kondyli, E. (2002b). Improvement of sensory quality of low-fat Kefalograviera-type cheese with commercial adjunct cultures. *International Dairy Journal, 12*(9), 757–764.

Krause, I., Bockhardt, A., Neckermann, H., Henle, T., & Klostermeyer, H. (1995). Simultaneous determination of amino acids and biogenic amines by reverse-phase high-performance liquid chromatography of the dabsyl derivatives. *Journal of Chromatography A, 715*, 67–79.

McSweeney, P. L. H., Fox, P. F., Lucey, J. A., Jordan, K. N., & Cogan, T. M. (1993). Contribution of the indigenous microflora to the maturation of Cheddar cheese. *International Dairy Journal, 3*(7), 613–634.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening. A review. *Lait, 80*(3), 293–324.

Michaelidou, A., Katsiari, M. C., Voutsinas, L. P., Kondyli, E., & Alichanidis, E. (2003). Effect of commercial adjunct cultures on proteolysis in low-fat Kefalograviera-type cheese. *International Dairy Journal, 13*(9), 743–753.

Mistry, V. V. (2001). Low fat cheese technology. *International Dairy Journal, 11*(4–7), 413–422.

Somers, J. M., & Kelly, A. L. (2002). Contribution of plasmin to primary proteolysis during ripening of cheese: effect of milk heat treatment and cheese cooking temperature. *Lait, 8*(2), 181–191.

Sousa, M. J., Ardö, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. *International Dairy Journal, 11*(4–7), 327–345.