Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 98 (2006) 658–663
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of a commercial adjunct culture on organic acid contents of low-fat Feta-type cheese

P. Manolaki [a], M.C. Katsiari [b], E. Alichanidis [a,*]

[a] Laboratory of Dairy Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[b] National Agricultural Research Foundation, Dairy Research Institute, 45221 Katsikas, Ioannina, Greece

Received 13 December 2004; received in revised form 15 June 2005; accepted 15 June 2005

Abstract

The effect of a commercial adjunct culture (CR-213, containing *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* susp. *lactis* and added at the level of 0.6 g kg^{-1} or 0.9 g kg^{-1} cheese milk) on the organic acid (OA) content of low-fat Feta-type cheese was studied. Full-fat (~220 g kg^{-1}) and a low-fat (~70 g kg^{-1}) cheeses were used as controls. The main OA of all cheeses throughout ripening were lactic, citric and acetic acids. The effect of ripening time was significant ($P < 0.05$) for all OA but treatments did not affect acetic, succinic and uric acids. Cheeses with lower fat content were found to contain significantly ($P < 0.05$) more lactic and citric but less butyric acid than the full-fat control. The addition of the adjunct culture had a positive effect on butyric acid, propionic acid and acetoin content. The use of the adjunct culture could enhance the production of OA in low-fat Feta-type cheeses with eventual positive effect on their sensory properties.

Keywords: Low-fat cheese; Adjunct culture; Organic acids; Feta cheese

1. Introduction

Organic acids (OA) occur in dairy products as a result of normal animal metabolism and breakdown of milk proteins, fat, lactose and citrate during manufacture and storage (Marsili, Ostapenko, Simmons, & Green, 1981). They play an important role in the flavour of dairy products and many authors use the level of some OA to monitor starter activity and bacterial growth during cheese ripening. Additionally, OA can reflect the kind of fermentation and indicate deviations of the expected course of maturation, potentially leading to defects (Careri, Spagnoli, Panari, Zannoni, & Barbieri, 1996; de Llano, Rodriguez, & Cuesta, 1996). Finally, many authors have tried to correlate the age/ripeness of cheese with the level of OA (Akalin, Gönc, & Akbas, 2002; Bevilacqua & Califano, 1992; Careri et al., 1996; Fedio, Ozimek, & Wolfe, 1994; Hough et al., 1996; Lues & Bekker, 2002).

The OA profile was found to differ among cheese varieties and some OA are of importance for the typical flavour of some cheeses. For example, acetic acid is a major contributor to the flavour of Feta cheese (Abd El-Salam & Alichanidis, 2004), as is propionic acid for Emmental cheese (Frohlich-Wyder & Bachmann, 2004). The level of individual OA was found to vary according to the processing procedure, the ripening temperature and duration, the production season and bacterial counts. Also, it is affected by the kind of starter, adjunct starter and non-starter lactic acid bacteria (Careri et al., 1996; de Llano et al., 1996; Skeie, Lindberg, & Narvhus, 2001).

In a previous study of Katsiari, Voutsinas, Kondyli, and Alichanidis (2002), it was found that it is possible to produce a low-fat (~70 g kg^{-1}) Feta-type cheese

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

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.06.031

having a flavour intensity similar to that of the full-fat (~220 g kg^{-1}) Feta by adding the commercially available adjunct culture CR-213 to the cheese milk. It was also found (Michaelidou, Katsiari, Kondyli, Voutsinas, & Alichanidis, 2003) that the same adjunct culture could produce elevated levels of small peptides and free amino acids, which can serve as flavour precursors in the low-fat Feta-type cheeses. The objective of the present study was to investigate the effect of the adjunct culture CR-213 on the production of some OA during cheese ripening.

2. Materials and methods

2.1. Starter and adjunct cultures

A freeze-dried lactic culture containing (1:1) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Dri-vac no CH-1, Chr. Hansen's Laboratorium, Copenhagen, Denmark) was used as starter culture. It was reactivated and grown at 43 °C in 100 g kg^{-1} of sterilized reconstituted low heat-treated skim milk powder and added to the milk of all cheeses at a level of 7.5 g kg^{-1}.

A commercially available mesophilic culture was used as an adjunct culture (CR-213, Chr. Hansen's Laboratorium, Copenhagen, Denmark). The CR-213 culture contains three *Lactococcus lactis* strains: two of them are subsp. *lactis* and the third is subsp. *cremoris*.

2.2. Cheese manufacture

Bulk ewes' milk was obtained from the herd of the Agricultural Research Station of Ioannina and standardized to 60 g kg^{-1} fat, for the full-fat cheese, and 15 g kg^{-1} fat, for the low-fat cheeses, by mixing skim milk and whole milk. Four vats of cheese were made in 1 d: a full-fat control cheese (A, treated with normal starter only), a low-fat control cheese (B, treated with normal starter only) and two low-fat experimental cheeses made by adding, along with normal starter, the adjunct culture CR-213 at the level of 0.6 g kg^{-1} (cheese C) or at 0.9 g kg^{-1} (cheese D).

The cheesemaking procedure was essentially as described in a previous paper (Katsiari & Voutsinas, 1994). In brief, the milk in each double-walled stainless-steel vat was pasteurized, separately, at 63 °C for 30 min, cooled to 35 °C, inoculated with starters and allowed to ripen for 30 min. $CaCl_2$ solution, 400 g l^{-1}, was added at levels of 50 ml 100 kg^{-1} milk for the 60 g kg^{-1} fat cheese milk, and 25 ml 100 kg^{-1} milk for the 15 g kg^{-1} fat cheese milk; powdered calf rennet (HA-LA, Chr. Hansen's Laboratorium, Copenhagen, Denmark), dissolved in cold water, was then added. Coagulation was achieved in about 45 min at 35 °C. After coagulation, the curd was cut into 2 cm cubes, which were allowed to rest for 10 min. The curds were then transferred into rectangular moulds for draining. The moulds were then turned three times during the first 3 h of draining and then left at 16–18 °C to allow complete draining. The next morning, the curd of each mould was cut into blocks, each weighing about 1.5 kg. The cheese blocks were placed into individual cans, and granular recrystallized NaCl, equivalent to 2.5% of the weight of the cheese, was added. After 1 d, the salty aqueous phase was removed and replaced by a 70 g l^{-1} NaCl solution, added at a level which gave a ratio of brine volume to cheese weight of 1:5. The cans were sealed and left in the ripening room (16–18 °C) until the pH of cheese dropped to a value of ~4.6, a process typically requiring 20 d. Subsequently, the cans were transferred to the storage room (3–4 °C) and remained there for up to 60 days. Three cheesemaking trials were carried out.

Samples from each cheese were taken at the day of preparation (0 d), and at 2, 20 and 60 d after manufacture, for assessment of OA changes during maturation. The values reported are means of the three cheesemaking trials.

2.3. Reagents

Analytical grade OA were used as standards. Orotate, pyruvate, lactate, and butyrate were purchased from Sigma (Sigma–Aldrich, Taufkirchen, Germany), citrate and propionate from Merck (Darmstadt, Germany), acetate from Panreac Quimica SA (Barcelona, Spain), urate from Serva (Heidelberg, Germany), and succinate from Riedel-de Haen (Seelze, Germany). Acetoin was obtained from Fluka (Sigma–Aldrich, Taufkirchen, Germany). The mobile phase used was prepared by diluting reagent grade sulphuric acid with HPLC water filtered through 0.45-μm membrane filter (Alltech Associates Inc., Deerfield, IL, USA).

2.4. Equipment and operating conditions

Analyses were conducted using an HPLC system (LKB, Bromma, Sweden), fitted with an HC-75 ion-exclusion gel-type poly(styrene-divinylbenzene)copolymer column (7.5% crosslinking) in the hydrogen form (305 × 7.8 mm; particle size 10 μm; Hamilton Company, Reno, NV, USA) and a guard column (20 × 4.6 mm) thermostatted at 62 °C. Samples were applied using a Rheodyne injector (model 7125; Rheodyne Inc., Cotati, CA, USA) equipped with a 50-μl injection loop. The absorbance of the eluate was monitored at 210 and 280 nm, using a variable wavelength ultraviolet/visible detector (Fasma 525, Linear Instruments, Reno, NV, USA), which was linked to a data acquisition and processing system (Nelson Analytical Inc., Paramus, NJ,

USA). Analyses were performed isocratically at a flow rate of 0.6 ml min^{-1}, using a mobile phase of 0.014 N sulphuric acid.

2.5. Calibration and calculations

Five aqueous calibration standards, covering a broad concentration range, were prepared for each of the OA analyzed. Duplicate injections were made. The resulting peak area counts were determined; then, the best-fit standard curve was prepared for each OA using linear regression. Analyzing samples at two different wavelengths permitted quantitation of acetoin and of propionic acid, despite their co-elution. The calculations described by Marsili et al. (1981) were applied to determine the peak areas of these two analytes.

2.6. Sample preparation

Grated cheese samples were analyzed for pyruvate, propionate, orotate, lactate, succinate, citrate, butyrate, acetate, urate and acetoin. A 5 g grated sample was added to 25 ml of 0.014 N sulphuric acid (HPLC mobile phase), homogenized for 3 min at 7000 rpm in a mixer (Model N133/1281-0, Biospec Products, OK, USA), and centrifuged at 5000*g* at 4 °C for 15 min. The supernatant was filtered through 0.2 μm cellulose acetate filter (Alltech Associates Inc., Deerfield, IL, USA). All samples were stored at 4 °C. Duplicate analyses were performed on all samples.

2.7. Recovery study

The efficiency of the extraction procedure was evaluated by determination of recovery of the OA from a spiked Feta cheese preparation. A known amount of standard solution of OA was added to 5 g of Feta cheese and extracted as described above. Duplicate injections were performed for each sample of Feta and spiked Feta cheeses. The recovery rates of all OA were found to be >95% except for those of succinate (78%) and propionate (87%). Recovery rates were taken into account when calculating the results presented in this paper.

2.8. Statistical analysis

The experiment was conducted to evaluate the effect of the following four treatments: A, full-fat control cheese made without adjunct culture; B, low-fat control cheese made without adjunct culture; C, low-fat experimental cheese made with 0.6 g kg^{-1} of adjunct culture and D, low-fat experimental cheese made with 0.9 g kg^{-1} of adjunct culture on the level of various OA during ripening. There were three replicate trials for each treatment. A two-factor (4 times × 4 treatments) factorial experiment was employed for this study. The analysis of variance (ANOVA) was performed using the SPSS program (SPSS Inc., Chicago, IL, USA). Differences between means were compared at the 5% level of significance using the least significant difference (LSD) test.

3. Results and discussion

The main OA of all Feta and Feta-type cheeses throughout ripening were lactic, citric and acetic acids. This is consistent with findings of Papadakis and Polychroniadou (2005). The effect of treatments and of the time of ripening on the OA content of the experimental cheeses is presented in Table 1. The effect of ripening time was significant ($P < 0.05$) for all OA but treatments affected only some of them.

Lactic acid content showed an important increase from 0 to 2 d, because of the high activity of the lactic acid bacteria at the relatively high temperature (16–18 °C) of the

Table 1
Effect of treatment and ripening time on organic acid concentrations[A] (mg kg^{-1}) of full-fat Feta and low-fat Feta-type cheeses, as indicated by the analysis of variance ($n = 12$)

Factors	Lactic	Butyric	Acetic	Propionic	Pyruvic	Orotic	Succinic	Citric	Uric	Acetoin
Treatment[B]										
A	14,025^{a}	90.2^{a}	373	62^{a}	50.0^{a}	15.5	21.2	616^{a}	8.56^{a}	295^{a}
B	16,117^{b}	50.2^{b}	422	66^{a}	65.2^{b}	18.2	23.5	705^{b}	10.3^{b}	283^{a}
C	16,084^{b}	69.6^{c}	443	101^{b}	51.8^{a}	17.5	21.9	750^{b}	9.93^{ab}	458^{b}
D	16,512^{b}	75.8^{d}	526	105^{b}	49.0^{a}	15.5	23.8	704^{b}	9.93^{ab}	478^{b}
Ripening time (d)										
0	13,462^{a}	18.3^{a}	330^{a}	34^{a}	143^{a}	36.5^{a}	36.0^{a}	1009^{a}	17.6^{a}	242^{a}
2	17,366^{b}	24.3^{a}	338^{a}	40^{a}	49.6^{b}	14.4^{b}	19.3^{ab}	862^{b}	15.0^{b}	228^{a}
20	15,423^{c}	107^{b}	471^{ab}	108^{b}	12.8^{c}	7.31^{c}	19.6^{ab}	496^{c}	3.24^{c}	419^{b}
60	16,487^{b,c}	136^{c}	644^{b}	152^{c}	10.4^{c}	8.49^{c}	15.6^{b}	408^{d}	2.87^{c}	624^{c}

^{a,b,c,d} Means in the same column with different superscripts differ significantly ($P < 0.05$).

[A] Values are the means of n determinations.

[B] Cheese code: A, full-fat control; B, low-fat control; C, low-fat with 0.6 g kg^{-1} adjunct culture; D, low-fat with 0.9 g kg^{-1} adjunct culture.

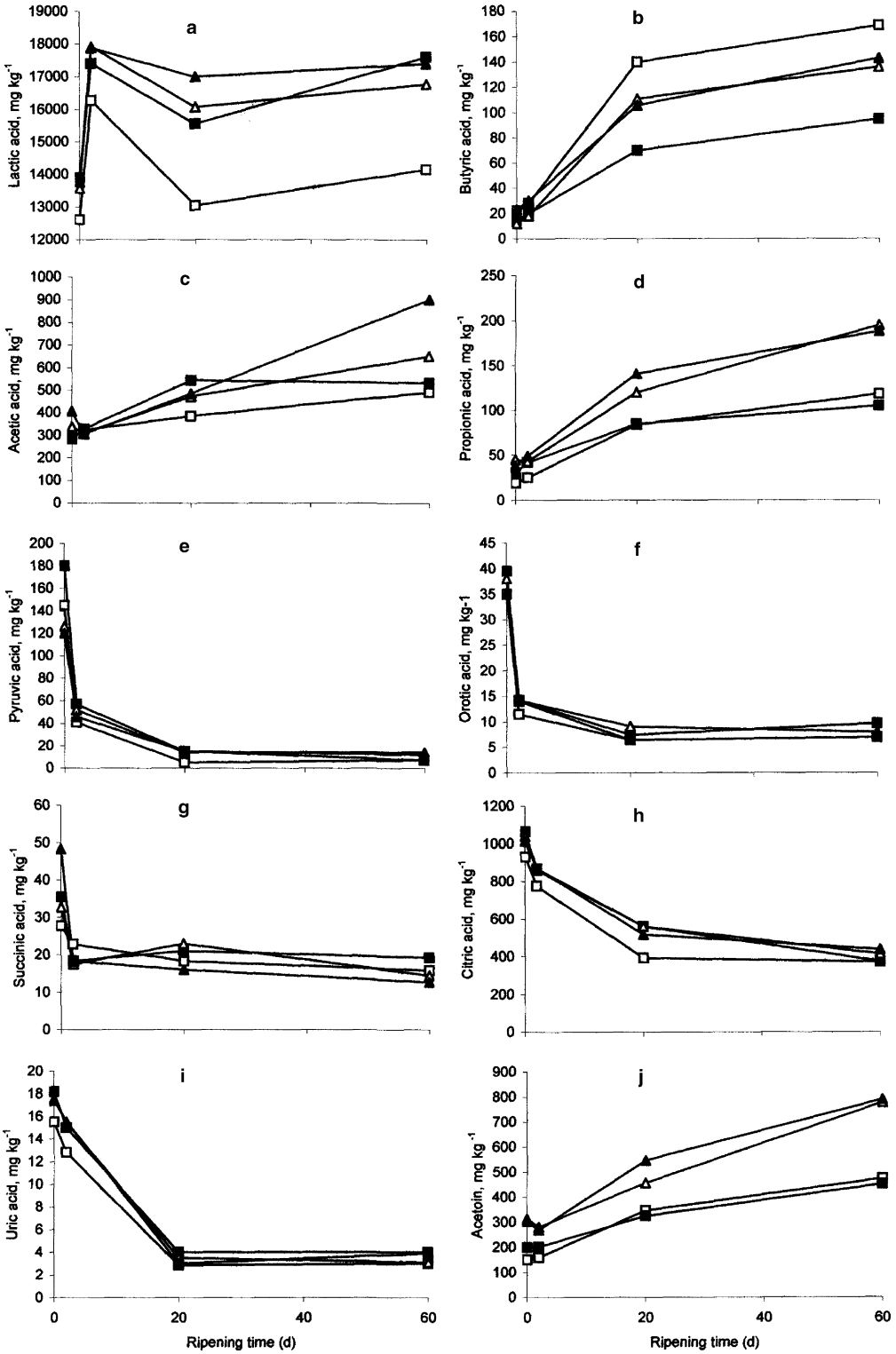

Fig. 1. Changes in organic acid concentration during ripening in: full-fat control cheese, A (□); low-fat control cheese, B (■); low-fat experimental cheese made with 0.6 g kg^{-1} adjunct culture, C (△) and low-fat experimental cheese made with 0.9 g kg^{-1} adjunct culture, D (▲). Values are means of three replicate trials.

ripening room. The acidification of the curd is essential for assuring the good quality and the proper ripening process of Feta cheese (Abd El-Salam & Alichanidis, 2004). Lactic acid content decreased slightly up to 20 d but increased from 20 to 60 d, reaching again the level of 2 d (Table 1). An analogous trend was observed during

ripening of Mozzarella and a (similar to Feta) white brined cheese (Califano & Bevilacqua, 1999; Akalin et al., 2002, respectively). Significant ($P < 0.05$) differences were also observed between full-fat (A) and low-fat cheeses (B, C, and D) which had generally higher lactic acid contents (Fig. 1(a); Table 1). This could be explained by the higher moisture of the low-fat cheeses (Katsiari et al., 2002).

Values found for butyric acid (Table 1) agree with those reported by Katsiari, Voutsinas, Alichanidis, and Roussis (2000) and by Kandarakis, Moatsou, Georgala, Kamarinides, and Anifantakis (2001) for Feta. An increase in concentration during ripening was observed for butyric acid (Fig. 1(b)). Similar results were reported for Feta cheese by Georgala et al. (2005) and for Afuega'l Pitu by de Llano et al. (1996). The full-fat cheese had a higher ($P < 0.05$) butyric acid content than all low-fat cheeses (Fig. 1(b); Table 1). This was expected as butyric acid is mainly produced by the lipolytic acti- vity of cheese microflora on milk fat, which was lower in the low-fat cheeses. However, the butyric acid content of cheeses C and D, made using an adjunct culture, was higher than that of the low-fat control (cheese B); it seems that the adjunct culture had a higher than the starter lipolytic activity, which increased the rate of fat hydrolysis (Kondyli, Katsiari, Masouras, & Voutsinas, 2002).

Acetic acid is considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria. It contributes greatly to the final flavour of Feta cheese (Abd El-Salam & Alichanidis, 2004; Kandarakis et al., 2001; Kondyli et al., 2002). Acetic acid content increased significantly ($P < 0.05$) during ripening. This is in agreement with the results reported by Mallatou, Pappa, and Massouras (2003) and Georgala et al. (2005) for Feta cheese. Treatments found to not affect ($P > 0.05$) the acetic acid content. Ardö (1993), Kondyli et al. (2002), and Kondyli, Katsiari, Masouras, and Voutsinas (2003), working with semi-hard cheeses, Feta and Kefalograviera, respectively, did not find significant ($P > 0.05$) differences between full-fat and low-fat cheeses. However, it is note worthy that values for acetic acid in experimental cheeses C and D increased substantially after 20 d of ripening compared to the control cheeses in which no increase was observed (Fig. 1(c)). This increase could be due to the higher amount of free amino acids in cheeses C and D (Michaelidou et al., 2003), which might have served as precursors for the formation of acetic acid.

Propionic acid is not a typical OA of Feta but its content was increased ($P < 0.05$) in all cheeses after 2 d (Table 1). Acetoin showed a similar trend (Table 1, Fig. 1(j)). Bouzas, Kantt, Bodyfelt, and Torres (1991, 1993), Lues and Botha (1998) and Lues and Bekker (2002), working with Cheddar cheese, reported similar results for propionic acid. Cheeses with adjunct culture (C and D) showed a more marked increase ($P < 0.05$) of propionic acid (and acetoin) content than the two controls (Fig. 1(d)). It seems that the microorganisms of the adjunct culture have the appropriate enzymatic system for lactate fermentation into propionate. Additionally, the increased acetoin content of cheeses C and D indicates a higher potential of the adjunct culture for acetoin production, probably through the glycolytic pathway.

Concentration of pyruvic acid showed a dramatic drop in 2 d, followed by a more moderate but still significant ($P < 0.05$) decrease from 2 to 20 d. Then it remained the same for the rest of the experiment (Table 1, Fig. 1(e)). A similar trend was observed for orotic acid (Fig. 1(f)). As both acids participate in various metabolic pathways, they were probably consumed by the microorganisms mainly during the period of their maximum propagation and activity (0–2 d). An important decrease of pyruvic acid at the beginning of the ripening period was also reported by Califano and Bevilacqua (1999) and Akalin et al. (2002) for Mozzarella and white brined cheese, respectively. Also, a decrease in orotic acid content was reported by Bouzas et al. (1991). The effect of treatment on pyruvic and orotic acids content was weak (Table 1) but low-fat control cheese (B) had a significantly higher concentration ($P < 0.05$).

Succinic acid content showed a rapid decrease at 2 d but then it remained stable throughout the ripening period ($P > 0.05$). Certain strains of *Lactobacilli* produce or consume succinic acid (Ocando, Granados, Basanta, Gutierrez, & Cabrera, 1993). It is assumed that the catabolic reactions were prevalent in the first 2-day period of cheese ripening (Table 1, Fig. 1(g)). No significant differences ($P > 0.05$) were found between treatments.

Citrate in milk is metabolized by many lactic acid bacteria into flavour components, such as acetate, acetaldehyde and diacetyl (Hugenholz, 1993). Although the decreasing rate of citric content during ripening was low (Table 1, Fig. 1(h)), differences were significant ($P < 0.05$). An analogous trend was observed for uric acid content (Table 1, Fig. 1(i)). Similar results were reported for citric acid in Cheddar (Lues & Botha, 1998) and for uric acid in Reggianito (Hough et al., 1996). The level of both acids was significantly ($P < 0.05$) higher in all low-fat cheeses, probably because of the higher moisture content.

4. Conclusions

The main OA of all Feta and Feta-type cheeses, throughout ripening, were lactic, citric and acetic acids. The effect of ripening time was significant ($P < 0.05$) for all OA but treatments did not affect acetic, succinic and uric acids. Cheeses with lower fat content were found to contain significantly ($P < 0.05$) more lactic and citric

acids but less butyric acid than the full-fat control. The addition of the adjunct culture had a positive effect on butyric acid, propionic acid and acetoin contents. Treatment had only weak effect ($P > 0.05$) on orotic and pyruvic acids. The amount of the adjunct culture added seems not to influence the OA level. It is concluded that the use of the adjunct culture could enhance the production of OA in low-fat Feta-type cheeses, eventually giving a positive effect on their sensory properties.

Acknowledgements

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

References

Abd El-Salam, M. H., & Alichanidis, E. (2004). Cheese varieties ripened in brine (3rd ed.. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.). *Cheese: chemistry, physics and microbiology* (Vol. 2, pp. 227–249). Amsterdam: Elsevier.

Akalin, A., Gönc, S., & Akbas, Y. (2002). Variation in organic acids content during ripening of pickled white cheese. *Journal of Dairy Science, 85*(7), 1670–1676.

Ardö, Y. (1993). Characterizing ripening in low-fat, semi-hard, round-eyed cheese made with undefined mesophilic DL-starter. *International Dairy Journal, 3*(4–6), 345–357.

Bevilacqua, A. E., & Califano, A. N. (1992). Changes in organic acids during ripening of Port Salut Argentino cheese. *Food Chemistry, 43*(5), 345–349.

Bouzas, J., Kantt, C. A., Bodyfelt, F., & Torres, J. A. (1991). Simultaneous determination of sugars and organic acids in Cheddar cheese by high-performance liquid chromatography. *Journal of Food Science, 56*(1), 276–278.

Bouzas, J., Kantt, C. A., Bodyfelt, F., & Torres, J. A. (1993). Time and temperature influence on chemical aging indicators for a commercial Cheddar cheese. *Journal of Food Science, 58*(6), 1307–1312.

Califano, A. N., & Bevilacqua, A. E. (1999). Freezing low-moisture Mozzarella cheese: changes in organic acid content. *Food Chemistry, 64*(2), 193–198.

Careri, M., Spagnoli, S., Panari, G., Zannoni, M., & Barbieri, G. (1996). Chemical parameters of the non-volatile fraction of ripened Parmigiano–Reggiano cheese. *International Dairy Journal, 6*(2), 147–155.

de Llano, D. G., Rodriguez, A., & Cuesta, P. (1996). Effect of lactic starter cultures on the organic acid composition of milk and cheese during ripening-analysis by HPLC. *Journal of Applied Bacteriology, 80*(5), 570–576.

Fedio, W. M., Ozimek, L., & Wolfe, F. H. (1994). Gas production during the storage of Swiss cheese. *Milchwissenschaft, 49*(1), 3–8.

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

Georgala, A., Moschopoulou, E., Aktypis, A., Massouras, T., Zoidou, E., Kandarakis, I., et al. (2005). Evolution of lipolysis during ripening of traditional Feta cheese. *Food Chemistry, 93*(1), 73–80.

Hough, G., Califano, A. N., Bertola, N. C., Bevilacqua, A. E., Martinez, E., Vega, M. J., et al. (1996). Partial least square correlations between sensory and instrumental measurements of flavor and texture for Reggianito grating cheese. *Food Quality and Preference, 7*(1), 47–53.

Hugenholz (1993). Citrate metabolism in lactic acid bacteria. *FEMS Microbiology Review, 12*(1–3), 165–178.

Kandarakis, I., Moatsou, G., Georgala, A. I. K., Kamarinides, S., & Anifantakis, E. (2001). Effect of draining temperature on the biochemical characteristics of Feta cheese. *Food Chemistry, 72*(3), 369–378.

Katsiari, M. C., & Voutsinas, L. P. (1994). Manufacture of low-fat Feta cheese. *Food Chemistry, 49*(1), 53–60.

Katsiari, M. C., Voutsinas, L. P., Alichanidis, E., & Roussis, I. G. (2000). Lipolysis in reduced sodium Feta cheese made by partial substitution of NaCl by KCl. *International Dairy Journal, 10*(5–6), 369–373.

Katsiari, M. C., Voutsinas, L. P., Kondyli, E., & Alichanidis, E. (2002). Flavour enhancement of low-fat Feta-type cheese using a commercial adjunct culture. *Food Chemistry, 79*(2), 193–198.

Kondyli, E., Katsiari, M. C., Masouras, T., & Voutsinas, L. P. (2002). Free fatty acids and volatile compounds of low-fat Feta-type cheese made with a commercial adjunct culture. *Food Chemistry, 79*(2), 199–205.

Kondyli, E., Katsiari, M. C., Masouras, T., & Voutsinas, L. P. (2003). Free fatty acids and volatile compounds in low-fat Kefalograviera-type cheese made with commercial adjunct cultures. *International Dairy Journal, 13*(1), 47–54.

Lues, J. F. R., & Bekker, A. C. M. (2002). Mathematical expressions for organic acids in early ripening of a Cheddar cheese. *Journal of Food Composition and Analysis, 15*(1), 11–17.

Lues, J. F. R., & Botha, W. C. (1998). Relationship amongst South African processed, young and matured Cheddar cheese pertaining to organic acid content and non-starter population. *Food Research International, 31*(6–7), 449–457.

Mallatou, H., Pappa, H., & Massouras, T. (2003). Changes in free fatty acids during ripening of Teleme cheese made with ewes', goats', cows' or a mixture of ewes' and goats' milk. *International Dairy Journal, 13*(2–3), 211–219.

Marsili, R. T., Ostapenko, H., Simmons, R. E., & Green, E. D. (1981). High performance liquid chromatographic determination of organic acids in dairy products. *Journal of Food Science, 46*(1), 52–57.

Michaelidou, A., Katsiari, M. C., Kondyli, E., Voutsinas, L. P., & Alichanidis, E. (2003). Effect of commercial adjunct culture on proteolysis in low-fat Feta-type cheese. *International Dairy Journal, 13*(2–3), 179–189.

Ocando, A. F., Granados, A., Basanta, Y., Gutierrez, B., & Cabrera, L. (1993). Organic acids of low molecular weight produced by lactobacilli and enterococci isolated from Palmita-type Venezuelan cheeses. *Food Microbiology, 10*(1), 1–7.

Papadakis, E. N., & Polychroniadou, A. (2005). Application of a microwave-assisted extraction method for the extraction of organic acids from Greek cheeses and sheep milk yogurt and subsequent analysis by ion-exclusion liquid chromatography. *International Dairy Journal, 15*(2), 165–172.

Skeie, S., Lindberg, C., & Narvhus, J. (2001). Development of amino acids and organic acids in Norvegia, influence of milk treatment and adjunct Lactobacillus. *International Dairy Journal, 11*(4–7), 399–411.