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Biogenic amine production in Feta cheese

Konstantina Valsamaki, Alexandra Michaelidou, Anna Polychroniadou *

Department of Food Science & Technology, Faculty of Agriculture, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

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Abstract

The formation of biogenic amines during Feta cheese ripening was investigated for 4 months in relation to the parent amino acids. The total biogenic amines content in mature cheese (60 days) was 330 mg/kg and, after a 120-day storage, 617 mg/kg. Tyramine and putrescine were the main biogenic amines in mature samples (69.7% and 71.2% at 60 and 120 days, respectively), while tryptamine and phenylethylamine concentrations were very low all along ripening. Total amine content increased throughout ripening reaching, ~620 mg/kg at 120 days; the periods of major amine production were from 1 to 15 days and from 60 to 120 days. It appears that the low pH and high salt content of Feta cheese do not create favourable conditions for amino acid decarboxylation, keeping the level of biogenic amines relatively low.

Keywords: Biogenic amines; Free amino acids; Cheese ripening; Feta cheese

1. Introduction

Biogenic amines (BA) are low molecular weight organic bases possessing biological activity. Several of them play important roles in many human physiological functions (Halász, Baráth, Simon-Sarkadi & Holzapfel, 1994). However, the consumption of food containing high concentrations of these compounds may cause toxic effects for susceptible individuals. Biogenic amines are also of concern in relation to food spoilage.

After fish, cheese is the next most commonly implicated food associated with histamine poisoning (Stratton, Hutkins & Taylor, 1991). Tyramine and β-phenylethylamine have also been implicated in adverse reactions involving headache and hypertensive crisis (Rice, Eitenmiller & Koehler, 1976). Other BA found in cheese, such as tryptamine, putrescine and cadaverine, have been identified as potentiators of toxic effects because they can inhibit histamine-detoxifying enzymes such as MAO, DAO and HMT (Stratton et al., 1991).

Determination of the exact toxicity threshold of BA in individuals is extremely difficult, since the toxic dose is strongly dependent on the efficiency of the detoxification mechanisms of each individual (Halász et al., 1994). An intake of >40 mg BA per meal has been considered potentially toxic. As not all amines are equally toxic, histamine, tyramine and phenylethylamine are of concern (Shalabi, 1996). Spanjer and van Roode (1991) suggested that the sum of tyramine, histamine, putrescine and cadaverine should not exceed of 900 mg/kg cheese. For the moment, no legal upper limit for BA in cheese has been established. In the European Union and the United States such a limit is established only for histamine in fish.

Biogenic amines are mainly generated by the enzymic decarboxylation of amino acids by micro-organisms. The amine-producing abilities of various bacteria differ widely; thus, no direct correlation could be found between the histamine and tyramine contents and the total bacterial count (Halász et al., 1994). Among decarboxylase-positive micro-organisms many strains of *Enterobacteriaceae* and certain lactobacilli, pediococci and enterococci are particularly active (Halász et al., 1994). In cheeses containing high levels of free amino acids, amines can accumulate rapidly even with low levels of decarboxylating lactobacilli (Joosten & Northolt, 1989). It appears that amine-producing micro-organisms are adventitious organisms rather than part of the starter culture population (Joosten & Stadhouders, 1987; Voight & Eitenmiller, 1977). Many factors such as bacterial density, synergistic effects between microorganisms, level of proteolysis (availability of

* Corresponding author. Fax: +30-31-99-8789.
E-mail address: annapoly@agro.auth.gr (A. Polychroniadou).

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substrate), pH, salt-in-moisture level and ripening and storage temperatures are found to have limiting effects on the build-up of amines (Edwards & Sandine, 1981; Joosten & van Boekel, 1988; Schneller, Good & Jenny, 1997; Stratton at al., 1991). However, the BA concentration in cheese may reach hazardous levels only if more than one of these factors are favourable; cheeses with comparable microbiological profiles may differ enormously in their BA content.

Once BA are formed, it is difficult to destroy them by pasteurization or cooking. Therefore, BA formation should be controlled by strict use of good hygiene in both raw material and manufacturing environment, with corresponding inhibition of spoiling microorganisms (Silla Santos, 1996).

Cheese represents an ideal environment for amine production but amine concentration varies widely and depends on such factors as cheese variety, age and microflora (Joosten & van Boekel, 1988). Large variation has been also observed within each variety, especially in raw milk cheeses (Lavanchy & Sieber, 1993; Stratton et al., 1991).

Feta belongs to a particular group of cheeses which are matured and stored in brine. No information is available on the occurrence of BA in this cheese variety. Furthermore, sheep and goat milk, having received a thermal treatment below pasteurization conditions, is often used in small cheese plants for Feta cheese production; thus, indigenous milk flora may be present as well as starter microorganisms. A contamination from the cheese plant environment is also possible. It is therefore interesting, for both scientific and for public health purposes to investigate the formation of BA during Feta cheese ripening and to determine their concentration range in mature Feta. The aim of the present study was to derive this information. The evolution of the free amino acid content of the cheese was also investigated because these compounds are precursors of the BA.

2. Materials and methods

2.1. Cheese samples

Three batches of Feta cheese were manufactured using the traditional method described by Michaelidou, Alichanidis, Urlaub, Polychroniadou and Zerfiridis (1998). Cheese milk was a mixture of 70% sheep milk and 30% goat milk, which was standardized to have a casein to fat ratio of 0.8 and treated at 65°C for 15 min. Yoghurt was used as a starter culture together with the mesophilic homozymotic culture, Hansen R-703, Type 0 (Hansen's Laboratory, Copenhagen, Denmark). From each batch, samples were collected at 1, 3, 15, 60, 90 and 120 days after cheese making.

2.2. Chemical analyses

Dry matter (DM) and NaCl content were determined according to IDF standard methods (4A:1982 and 17A:1972, respectively). Moisture content was calculated by difference (100-DM) and expressed as % (w/w) of cheese; sodium chloride was expressed as salt-in-moisture. Total nitrogen (TN) was determined by the Kjeldahl method (Association of Official Analytical Chemists [AOAC] 1990) and expressed as % (w/w) of DM content. The pH was measured potentiometrically in a cheese slurry made by 10 g grated cheese dispersed in 10 ml H_2O. All analyses were carried out in duplicate.

2.3. Assessment of proteolysis

2.3.1. Nitrogen fractions

The water-soluble fraction of cheese was prepared according to Kuchroo and Fox (1982) but a cheese to H_2O ratio of 1:5 was used. The soluble-in 12% TCA-fraction was obtained by mixing equal volumes of water-soluble fraction and 24% (w/w) trichloroacetic acid (TCA) solution, followed by filtration through a white ribbon filter paper (Schleicher & Schuell, Dassel, Germany). The nitrogen content of both fractions (WSN and TCA-SN, respectively) was determined by the Kjeldahl method (AOAC, 1990) and expressed as % of the TN. All analyses were carried out in duplicate.

2.3.2. Caseins and peptides

α_{s1}-Casein (α_{s1}-CN) and β-casein (β-CN) were analyzed by polyacrylamide gel electrophoresis (PAGE) according to Andrews (1983); the proteins were stained according to Blakesley and Boezi (1977), and the absorbance at 590 nm of the zones measured by an optical scanning densitometer (RTF Transdyne General Corp, Ann Arbor, MI). Peptides in the water-soluble fraction were separated by reversed-phase high-performance liquid chromatography (RP-HPLC) as described by Michaelidou, Alichanidis, Urlaub, Polychroniadou and Zerfiridis (1998).

2.3.3. Free amino acids and biogenic amines

Free amino acids (FAA) and biogenic amines (BA) in the cheese samples were simultaneously determined by RP-HPLC as dabsyl-derivatives according to Krause, Bockhardt, Neckerman, Henle and Klostermeyer (1995).

2.4. Microbiological analyses

Total aerobic count, coliforms and lactic acid bacteria were enumerated as described by Hatzikamari, Litopoulou-Tzanetaki and Tzanetakis (1999).

2.5. Statistical analysis

The effect of time of ripening on all parameters of proteolysis and on total FAA and BA content of the cheese was assessed by analysis of variance (ANOVA) using the SPSS 6.1 for Windows software (SPSS, 1994). When a significant difference ($P < 0.05$) was observed, means were compared by Duncan's test. Correlations between various parameters were also investigated using the Table Curve for Windows software (Jandel Scientific, San Rafael, CA).

3. Results and discussion

3.1. General proteolysis

The evolution of the various parameters of proteolysis during the ripening of the experimental cheeses is presented in Table 1. It is obvious that during the first 15-day period, when the cheese was kept at 16°C, proteolysis was very intense but, later, the rate of casein and peptide degradation became slower. Changes in WSN%TN, TCA-SN%TN, residual α_{s1}-CN and water-soluble peptides, as estimated by the total area of the RP-HPLC chromatogram fitted well with the logarithmic model $y = a + b$ ln [age]; the coefficient of determination, R^2 was 0.9252, 0.9800, 0.9633 and 0.9852, respectively. However, the slope was not the same for all parameters.

Based on previous work of van den Berg and Extercate (1993) and Michaelidou et al. (1998), we could assume that the water-soluble peptides were mainly produced from the α_{s1}-CN through the action of chymosin, which seems to be very active in the environment of Feta cheese (high moisture, low pH, high salt content), while high plasmin activity would be improbable in that environment. In fact, the degradation of β-CN—which is mainly hydrolysed by plasmin (Visser & de Groot-Moster, 1977)—was very slow; 73.6% of the residual β-CN was found in the cheese following a 120-day ripening. Furthermore, it seems that also the proteinases and peptidases of the starter culture were very active at the temperature of the ripening room (16°C) where the cheese was kept for 15 days. Their action resulted in the additional production of low molecular weight protein fragments (small peptides and free amino acids), a fact proved by the substantial increase of TCA-SN and FAA levels. At the end of that period 72.4% of the WSN was soluble in 12% TCA.

3.2. Free amino acids

For 90 days, the changes in total FAA content followed a pattern similar to that of the other parameters of proteolysis, but a further substantial increase was observed from 90 to 120 days. Taking as reference the FAA content of the curd at the first day of ripening, FAA contents at 15, 90 and 120 days were, respectively, 4.45, 5.63 and 7.29 times higher. Alichanidis, Anifantakis, Polychroniadou and Nanou (1984) found similar levels of FAA. It is worthy of note that FAA content at 1 days was significantly different from zero. Bütikofer and Fuchs (1997) attributed this fact to an accelerated amino acid release at the day of manufacture, when starter cultures are incubated for more than 30 min at temperatures favourable for their development and activity.

Since FAA release is attributed to the action of microbial peptidases (Visser, 1993) we could assume that aminopeptidases of the starter micro-organisms were responsible for the massive production of FAA

Table 1
Effect of ripening time on composition, pH, salt and proteolysis parameters of Feta cheese[a]

	Age (days)[b]						
	1	3	15	30	60	90	120
Moisture%	62.19a	57.57b	53.93b,c	54.47b,c	51.27c	54.20b,c	53.50b,c
pH	6.13a	5.19b	4.45c	4.42c	4.41c	4.52c	4.44c
Salt[c]	–	5.26	–	–	5.82	–	–
TN (%DM)[c]	2.30a	2.44a,b	2.78c,g	2.66b,c,d,e	2.82e,g	2.53a,c,f	2.69b,c,e,f
Residual α_{s1}-CN[c]	100a	92.5a	53.6b	49.4b,c	41.2c,d	39.2c,d	35.4d
Residual β-CN%	100a	96.1a,b	93.2b,c	88.6c,d	84.7d	79.8e	73.6f
WSN%TN[c]	5.19a	9.37b	12.3b,c	12.1b,c	13.0c	14.6c	14.21c
TCA-SN%TN[c]	2.52a	4.39a	8.90b	9.63b,c	10.1b,c	11.4c	11.8c
Peptides[c]	2.15a	3.39b	4.89c	5.29d	5.86d,e	5.96d,e	6.17e
FAA (mg/kg)[c]	546a	1016a	2430b	2498b,c	2922c,d	3073d	3978e

[a] Values are means from 3 batches ×2 replications.

[b] Means in the same row bearing different letters differ significantly ($P < 0.05$).

[c] DM = dry matter; TN = total N; Salt = NaCl% of the aqueous phase of cheese; CN = casein; WSN%TN = water-soluble N%TN; TCA-SN%TN = 12% TCA-soluble N%TN; Peptides = total area of chromatogram ($\times 10^{-7}$) from the RP-HPLC analysis of the water-soluble fraction; FAA = free amino acids.

during the first 15 days of ripening. Lactococcal peptidases are intracellular and their action indicates cell lysis. Wilkinson, Guinee, O'Callaghan and Fox (1994) found that a positive correlation existed between the release of amino acids in cheese and the extent of cell lysis of the starters. In the present experiment, the high salt content and the low pH of the curd may create favourable conditions for cell lysis. Thus, we suppose that the high rate of FAA production during the first two weeks resulted from the cell lysis of the starter micro-organisms. According to Chapot-Chartier, Deniel, Rousseau, Vassal and Gripon (1994) and Boutrou et al. (1998), a high cell lysis and aminopeptidase activity was also observed for some bacterial strains as early as the first day of ripening of the Saint Paulin-type cheese.

The role of the non-starter lactic acid bacteria (NSLAB) in the FAA production cannot be ignored. Although no detailed microbiological analyses were carried out, it is probable that the thermal treatment of cheese milk allowed part of the indigenous flora to survive. Furthermore, the technology of Feta cheese (dry salting and further manipulations during 3–4 days) permits the curd surface to be contaminated by micro-organisms of the cheese plant environment. Earlier publication showed that high lactic acid bacteria and lactobacilli counts are present in curd, which increase significantly ($P < 0.05$) during a 15-day ripening (Tzanetakis & Litopoulou-Tzanetaki, 1992). The presence of this native flora is usually desirable since it improves the characteristic flavour of Feta cheese. It is, therefore, possible that NSLAB contributed to the formation of FAA up to 15 days, together with the population of the starter micro-organisms. The high temperature of the ripening room favoured their development and proteolytic activity.

During the period between 15 and 90 days, the change in FAA content was not as large as previously, although still significant ($P < 0.05$); an increase of 26% was observed in 75 days. As the cheese was kept at 4°C, an attenuation of the enzyme activity was expected. Also, it is known that amino acids participate in many biochemical reactions and numerous compounds are issued from their catabolism. However, a factor of major importance for the composition of the water-soluble fraction of Feta cheese — including the FAA content — is its ripening and storage in brine. Unpublished results obtained in our laboratory showed that a dynamic equilibrium exists between the aqueous phase of the cheese and the brine surrounding the cheese blocks and that many low molecular weight compounds migrate from cheese towards the brine. FAA are among these compounds. Therefore, the FAA content, as determined by HPLC, was underestimated and did not represent the true level of amino acids released during cheese ripening.

Although the exchange of compounds between cheese and brine continues up to the end of cheese storage, a more significant increase of FAA content was observed from 90 to 120 days. This could be explained by a massive starter and NSLAB cell lysis; the cheese mass, rich in peptides after 3 months of ripening, offered an abundance of the substrate for the action of the peptidases released.

3.3. Biogenic amines

The total BA content of Feta cheese at 120 days was 617 mg/kg. The biogenic amines determined were tyramine (TYA), putrescine (PUT), histamine (HIA), cadaverine (CAD), tryptamine (TRA) and phenylethylamine (PHA). TYA and PUT were the major amines in ripe cheese (69.7% and 71.2% at 60 and 120 days, respectively), while TRA and PHA concentrations were very low at all ripening periods. Similar results were found by Joosten (1988a) for Edam and Gouda. However, the amount of BA build-up was obviously higher than that found in the experimental cheeses because of the dynamic equilibrium existing between cheese and brine (see Free amino acids, Section 3.2). It is also noteworthy that a large variation in the BA content was observed between samples of the same age but from different batches, making an analysis of variance unreliable. A much larger variation was observed when 15 Feta samples, obtained randomly from commercial sources, were analysed; the range of total BA content was from 2.96 to 1339 mg/kg (average = 390 mg/kg; median = 193 mg/kg).

The evolution of the individual BA content is presented in Table 2. Fig. 1 shows the changes in the total amount of BA in comparison with the changes in the total amount of their precursor amino acids (PAA): tyrosine, lysine, histidine, tryptophan, phenylalanine and ornithine.

The results show that the level of BA was significantly increased from 3 to 15 days, changed slightly from 15 to 60 days and almost doubled from 60 to 120 days. From 1 to 60 days the increase fitted to the equation: [amines] = 92.2 × ln [age]-16.5 ($r = 0.960$) but after 60 days the increase was linear: [amines] = 52.6 + 4.78 x [age]

Table 2
Biogenic amines[a] in Feta cheese during ripening

Amines (mg/kg)	Age (days)						
	1	3	15	30	60	90	120
Tyramine	0.00	5.12	140	158	152	204	246
Histamine	2.40	0.00	25.9	38.2	47.0	76.4	84.6
Putrescine	1.62	2.51	36.2	53.6	77.7	153	193
Cadaverine	0.27	6.77	63.0	67.9	44	60.2	82.8
Tryptamine	4.39	6.24	4.01	3.27	2.18	3.53	5.74
Phenylalanine	3.51	0.77	3.32	3.02	7.04	3.77	4.94
Total	12.2	21.4	273	324	330	501	617

[a] Average values.

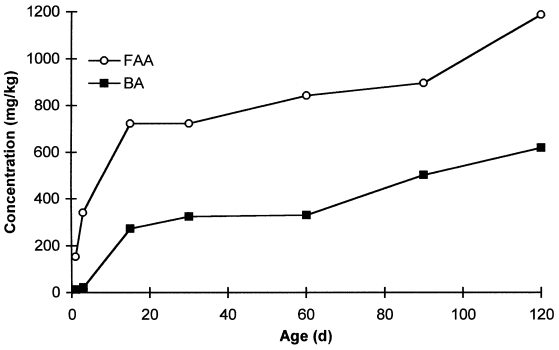

Fig. 1. Changes in total biogenic amines and their precursor amino acid content during ripening of Feta cheese. FAA = free amino acids; BA = biogenic amines.

($r = 0.989$). This trend was different from that reported by Schneller et al. (1997) who found that total BA level remained more or less stable after 3 months, even in cheeses with high BA content (> 3000 mg/kg).

The total PAA content presented a similar trend but two things may be pointed out: (a) At 1 days the individual BA content was from negligible to nil while their parent amino acids were present in concentrations significantly different from zero. This is also visible in Fig. 1; the intercept for total BA is close to zero, while that of the PAA is much higher. This fact was not unexpected since BA are generated from the decarboxylation of amino acids and their appearance follows the liberation of them. (b) From 60 to 90 days the concentration of PAA was almost stable while the BA content increased. This fact indicates that the release of PAA in that period of ripening was slower than their conversion to BA.

As the main factor for BA production in cheese is the presence of microorganisms with high decarboxylation activity, several studies have been published on this matter (e.g. Joosten & Northolt, 1987; Schneller et al., 1997). According to Voigt and Eitenmiller (1977) and Joosten and Stadhouders (1987) the micro-organisms showing the higher decarboxylation activity do not belong to the starter culture. Similar results are reported by Voigt and Eitenmiller (1978). Further study revealed the role of some NSLAB, such as salt-tolerant lactobacilli and *Enterobacteriaceae,* for the accumulation of BA in Gouda cheese (Joosten & Northolt, 1987). The importance of NSLAB in BA production was also shown during an inter-laboratory experiment: when raw milk semi-hard cheeses were manufactured in different countries from local milk, the levels of individual amines and their total amount differed significantly ($P < 0.05$) between cheeses, although the same technology and the same starters were used (Polychroniadou, unpublished results).

The BA formation in this experiment cannot be linked to specific species because of the lack of detailed microbiological analyses. Coliforms were present both in cheese milk and curd but in lower counts than those reported by Joosten and Northholt (1987). Lactobacilli were the major group (about 76 and 81% of the total count in curd and in 15-days cheese, respectively) but no isolation and identification of any strain was performed. However, earlier study (Tzanetakis & Litopoulou-Tzanetaki, 1992) showed the presence of high counts of NSLAB, especially *Lactobacillus plantarum,* in Feta cheese. This permits the assumption that NSLAB also made a major contribution to the decarboxylation of the FAA in the experimental cheeses. Strains of *L. plantarum* were reported among cheese isolates producing amines (Edwards & Sandine,1981; Sumner, Speckhard, Somers & Taylor, 1985).

On the other hand, environmental conditions as pH, salt-in-moisture (Table 1) and temperature (5°C after 15 days) of experimental cheeses were not favourable for BA production (Edwards & Sandine, 1981; Joosten & van Boekel, 1988). In some cases, substrate availability might also be a limiting factor because proteolysis was rather moderate. In general, BA content of Feta cheese was found to be low: in experimental cheeses and, also, in commercial samples of Feta, total BA concentration was never found higher than 1339 mg/kg.

3.3.1. Tyramine

From 15 to 120 days TYA was the major BA in Feta (246 mg/kg at 120 days). Its level increased during ripening; at 120 days it was within the concentrations found by Voigt, Eitenmiller, Koehler and Hamdy (1974) in various cheeses and Schneller et al. (1997) in semi-soft cheeses but lower than the level reported by Koehler and Eitenmiller (1978) for the same cheese category. The predominant place of TYA is also reported by Voigt et al. (1974) for 'sharp' Cheddar, by Joosten (1988a) for Dutch-type cheeses, by Bütikofer, Fuchs, Hurni, and Bosset (1990) for different cheeses, by Lavanchy and Sieber (1993) for Raclette, Appenzel and Tilsit, by Ordóñez, Ibáñez, Torre and Barcina (1997) for Idiázabal and by Durlu-Özkaya, Alichanidis, Litopoulou-Tzanetaki & Tunail (1999) for Beyaz cheese, a Turkish brine-ripened cheese. Tyramine was also found in several cheeses, such as Camembert, Colby, Parmesan, Romano, Roquefort and Danish blue cheese (Stratton et al., 1991). Heat treatment or bactofugation of the milk used for Emmental production had little effect on TYA content of the cheese (Krause, Bockhard & Klostermeyer, 1997).

Joosten and Northolt (1987) reported that some strains of *L. brevis* can produce high amounts of TYA. About 10% of the isolates from another Feta cheese was found to belong to this species (Tzanetakis & Litopoulou-Tzanetaki, 1992) but the presence of *L. brevis* in the experimental cheeses cannot be confirmed. Also, a strain of *L. delbrueckii* subsp. *bulgaricus* was found (Chander, Batish, Babu & Singh, 1989) to have in

vitro decarboxylating activity on tyrosine (optimum pH, 5; optimum temperature, 37°C). The presence of some strain(s) of this micro-organism in the experimental cheeses is certain, since yoghurt was part of the starter culture. However, the environmental conditions of the cheese (pH, temperature, NaCl) were different from those reported by Chander et al. (1989) and do not seem favourable for amine production by *L. delbrueckii* subsp. *bulgaricus*.

The rate of TYA build-up was similar to that described for the total BA concentration. This is not surprising since TYA constituted 46 and 40% of the total BA content at 60 and 120 days, respectively. The relationship between TYA and its precursor amino acid tyrosine (Tyr) was studied by following the Tyr/TYA ratio throughout ripening (Fig. 2a). It can be seen that this ratio decreased dramatically from 3 to 15 days showing that, in that period, the rate of Tyr release was much lower than the rate of its decarboxylation. After 15 days, the Tyr/TYA ratio showed little change, increasing slightly after 60 days of ripening.

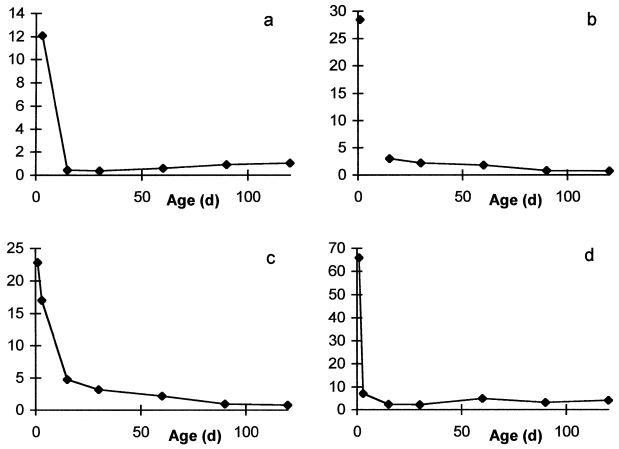

Fig. 2. Ratio of parent amino acids to the corresponding biogenic amines during ripening of Feta cheese. [a] Tyrosine/Tyramine; [b] Histidine/Histamine; [c] Ornithine/Putrescine; [d] Lysine /Cadaverine.

3.3.2. Histamine

Histamine concentration in mature Feta cheese (60 days) was found to be about 47 mg/kg and increased to 84.6 mg/kg at 120 days. This level is much lower than that reported for Swiss cheese (Stratton, Hutkins, Sumner & Taylor, 1992; Taylor, Keefe, Windham & Howell, 1982), Cheddar (Voigt et al., 1974), Emmental and Bergkäse (Krause et al., 1997) and some Dutch-type cheeses (Joosten, 1988b). Histamine production draws the attention of many researchers because the most frequent foodborne intoxications caused by BA involve this amine. As symptoms of clinical illness have been associated with consumption of 100–180 mg HIA (Taylor et al., 1982) and an average portion of Feta cheese is 65 g, it seems rather improbable that a Feta cheese could cause intoxication, except for cheeses with extremely high contamination and/or stored for a long period at high temperatures.

The trend of HIA accumulation observed in Feta was different from that found in Gouda (Joosten & van Boekel, 1988). In that study, histidine decarboxylase activity reached its maximum after about one month and decreased later: the 3-month-old cheese sample had only about 40% of the maximum enzyme activity. In our experiment, a substantial increase of HIA content was observed after 60 days and the increase did not stop up to the end of the storage period.

Strains of mesophilic and thermophilic lactobacilli are found to possess histamine decarboxylase activity, e.g. *L. buchneri* (Joosten & Northolt, 1987; Sumner et al., 1985) and *L. delbrueckii* subsp. *bulgaricus* (Chander et al., 1989). Although lactobacilli are found to be the major group of Feta cheese bacteria (Tzanetakis & Litopoulou-Tzanetaki, 1992) and *L. delbrueckii* subsp. *bulgaricus* was used in this study as part of the starter culture, no information can be given on the strain(s) present in the experimental cheeses. However, pH and salt-in-moisture of these cheeses probably had a negative effect on histidine (His) decarboxylation; according to Joosten (1988b) and Chander et al. (1989), high pH (>5) and temperature (>14°C) and low salt-in-moisture (<5.0%) favours HIA production. Only temperature had an obvious positive effect during the first ripening period (1–15 days), but HIA production remained at a low level.

The relation of thermophilic lactobacilli to high free His content (Joosten, 1988b) was not proved in the experimental cheeses. Percentage of free His on total FAA did not exceed that of paracasein. It was found that the His/HIA ratio decreased quickly from 3 to 15 days and continued to decrease gradually during the rest of the ripening period (Fig. 2b), showing that the rate of His decarboxylation was always higher than the rate of its release. Free His content was low (about 70 mg/kg) and remained nearly stable during ripening, decreasing slightly after 60 days. Thus, according to Joosten and van Boekel (1988), substrate availability could be a limiting factor for HIA production in the experimental cheeses.

3.3.3. Putrescine and cadaverine

Both PUT and CAD have less pharmacological activity than the aromatic amines but they are probably potentiators of their toxicity (Joosten, 1988a). Furthermore, they may be indices of extended protein degradation and spoilage.

Enterobacteriaceae are possibly responsible for PUT and CAD build-up, especially decarboxylase-positive strains with low death rate (Joosten & Northolt, 1987; Schneller et al., 1997). In this experiment the average

coliform count at 3 days was 2×10^5 cfu/g but their number decreased throughout ripening and did not exceed 1.2×10 cfu/g at 60 days. Some mixtures of lactobacilli were also found to produce CAD (besides TYA and HIA) and salt-tolerant lactobacilli caused massive formation of CAD and, to a less extent, PUT (Joosten & Northolt, 1987).

For similar counts of coliforms and lactobacilli (as assumed from the lactic acid bacteria counts and previous results of Tzanetakis & Litopoulou-Tzanetaki, 1992) the level of CAD observed was much lower than that reported by Schneller et al. (1997). Values reported by Joosten and Northolt (1987), Halász et al. (1994), Lavanchy and Sieber (1993), Krause et al. (1997) and Durlu-Özkaya et al. (1999) were also higher. Taking into account the high concentration of free lysine (Lys) in the experimental cheeses (> 150 mg/kg after 15 days), the low CAD content may be explained either by a lack of strains with lysine-decarboxylating activity or, most probably, by the inhibition of this activity by the unfavourable environment of Feta cheese. The Lys/CAD ratio followed a pattern similar to that of Tyr/TYA (Fig. 2d) but its absolute value was much higher because of the high Lys content of the cheese.

Putrescine content was quite high (77.7 and 193 mg/kg at 60 and 120 days, respectively). Similar PUT levels were found by Joosten and Northolt (1987) when mixtures of lactobacilli were used, but Schneller et al. (1997) found lower values. Joosten (1988b) reports that PUT formation ceased after 30 days and Schneller et al. observed a decrease in PUT content after 3 months of ripening. On the other hand, in our experiment an important increase of PUT concentration was found after 60 days.

Although the final (120 days) PUT level was comparable to that of TYA, the rate of its accumulation was much slower. Substantial amounts of this amine were measured only after 60 days, although its parent amino acid, ornithine (Orn), was found in large quantities (> 160 mg/kg) as early as 15 days. This late production had also an influence on the Orn/PUT ratio (Fig. 2c): the quick decrease observed in the case of the previously mentioned amines (Tyr/TYA, His/HIA and Lys/CAD) was absent. Instead, a gradual decrease of Orn/PUT ratio occurred throughout ripening and its value did not reach the minimum up to 90 days.

This observation could be explained by the fact that caseins do not contain ornithine. This amino acid is a degradation product of arginine which, depending on the micro-organisms involved, may be converted to ornithine, citrulline or agmatine. This special situation may cause some delay in PUT build-up since ornithine production occurs gradually, following arginine release, and its subsequent decarboxylation is retarded. A late manifestation of the ornithine-decarboxylase activity might also be possible.

4. Conclusions

All BA reported to be formed in cheese (TYA, HIA, PUT, CAD, TRA and PHA) were found in the experimental Feta cheese samples; TYA and PUT were the main BA in mature samples. The periods of major amine production were 1–15 days and 60–120 days. This may be explained by the elevated temperature of ripening up to 15 days and the substrate (FAA) abundance after 60 days, respectively. However, the total BA content was relatively low. It seems that the characteristic features of Feta cheese (low pH, high salt content, ripening and storage in brine, not extended proteolysis) did not create an environment favourable for BA accumulation. Although the traditional technology applied permitted the presence of bacterial species, eventually having decarboxylating properties, BA content did not reach a level similar to that reported for cheeses suspected for outbreaks of food poisoning (Joosten & van Boekel, 1988; Rice et al., 1976). However, heavy contamination is avoidable by strict use of good hygiene in both raw material and manufacturing environment. Additionally, low temperatures should be applied for cheese storage, to inhibit any important decarboxylase activity.

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