Influence of Starter and Nonstarter on the Formation of Biogenic Amine in Goat Cheese During Ripening

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ABSTRACT

Two commercial starters were investigated for their potential ability to decarboxylate amino acids during goat cheese ripening. Two batches of goat cheese were produced with identical pasteurized milk but different starter cultures. One of them contained *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* and the other *Lactococcus lactis* subsp. *lactis*. The amine contents, microbial counts, proteolysis-related parameters, pH, total solids and salt content were studied in raw materials and cheeses. In raw materials, polyamines were the prevailing amines, whereas the main amines in cheeses were putrescine, tryptamine and, in particular, tyramine (94.59 mg/kg). Aerobic mesophilic microorganisms and *Lactococcus* counts increased throughout ripening, while *Enterobacteriaceae* were no longer detectable in cheese after 30 days of ripening. Amine concentration rose during cheese ripening in both batches. Moreover, the decarboxylase activity of microorganisms isolated from samples during cheese ripening was assayed and discussed.

(**Key words:** biogenic amine, cheese, starter, high performance liquid chromatography)

Abbreviation key: AG = agmatine, CA = cadaverine, DO = dopamine, FAA = free amino acids, HI = histamine, OC = octopamine, PHE = β -phenylethylamine, PU = putrescine, SD = spermidine, SE = serotonin, SM = spermine, TN = total nitrogen, TR = tryptamine, TY = tyramine, WSN = water soluble nitrogen.

INTRODUCTION

Cheese, like other fermented foods, usually contains a high concentration of biogenic amines, which result

from the amino acid decarboxylase activity of microorganisms (Leuschner and Hammes, 1998). These amines have been studied for their potential risk for human health, since they can cause “cheese syndrome” and histamine intoxication related to tyramine and histamine, respectively. The adverse effects of these biogenic amines include emesis, respiratory distress, heart palpitations, headache, hyper or hypotension, and hypertensive crisis due to the interaction with monoamine oxidase inhibitor drugs (MAOI) (Hernández-Jover et al., 1997; González de Llano et al., 1998). These reactions can be potentiated by other biogenic amines, such as putrescine, cadaverine, spermine and spermidine (Stratton et al., 1991). Furthermore, diamines (putrescine and cadaverine) can react with nitrites to form carcinogenic nitrosamines (Scanlan, 1983).

On the other hand, owing to their microbial origin, biogenic amines can be formed by microbial contamination. In this sense, their presence in food may be regarded as a useful indicator of the quality or hygienic degree of the raw material employed in food (Hernández-Jover et al., 1997; Schneller et al., 1997). However, they can also be produced by the decarboxylase activity of certain fermentative microorganisms (Stratton et al., 1991). Furthermore, the proteolytic activity of starters increases free amino acids (FAA) and may enhance biogenic amine accumulation when microorganisms show decarboxylase activity (Leuschner et al., 1999).

The production of biogenic amines in cheese has often been linked to non-starter lactic acid bacteria and *Enterobacteriaceae* (Joosten and Northolt, 1987; Petridis and Steinhart, 1996). Several authors (Joosten and Northolt, 1987; Petridis and Steinhart, 1996; Roig-Sagués et al., 1997) have reported a decarboxylase-positive ability in lactobacilli isolates, which produce histamine, tyramine, putrescine and cadaverine.

In enterococci, many isolates may decarboxylate amino acids to the corresponding amines, especially tyrosine to tyramine (Joosten and Northolt, 1987;

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Leuschner et al., 1999). Many coliform bacteria can form putrescine and cadaverine by the decarboxylation of ornithine and lysine, respectively (Stratton et al., 1991; Petridis and Steinhart, 1996; Sharaf et al., 1997). In standard conditions, however, Joosten and Northolt (1987) found that these bacteria grow slowly and die quickly. Therefore, most of the cases in which large amounts of amines are produced in cheese have been attributed to lactic acid bacteria with decarboxylating activity.

From a toxicological point of view, it would be useful to produce cheeses with low amounts of amines to avoid potential adverse reactions. Thus, Leuschner and Hammes (1998) studied the addition of microbial isolates that degrade biogenic amines in order to prevent the presence of hazardous levels of amines in the final product. Likewise, Joosten and Nuñez (1996) reported that the use of bacteriocin-producing starters prevented the formation of histamine in cheese. More information is needed, however, to determine the effects of these treatments on organoleptic properties and their possible technological consequences.

This research examined the effect of two commercial starter cultures (*Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis*) on the formation of biogenic amines and on the characteristics of the microorganisms present in the cheese during the manufacturing process. The experiment was designed to determine whether the amounts of biogenic amines produced or the main amines formed differ between the selected starters.

MATERIALS AND METHODS

Cheese Making

Goat cheeses were manufactured from pasteurized (72°C, 15 s) milk in the Pilot Plant of the Unit of Food Technology-CeRTA at the Autonomous University of Barcelona following a standard protocol. Cheese manufacture was performed in triplicate. All cheeses were prepared from the same raw materials but with different starter cultures. Each production was divided into two batches. The starter in batch A was *Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris* (MAO 16, Texel, Larbus S.A., Madrid, Spain) and for batch B, we used *Lactococcus lactis* subsp. *lactis* (R-058, Abiasa, Pontevedra, Spain). These starters were selected as they are normally used in goat cheese manufacture.

Milk was heated to 32°C and a 2% lactic starter was inoculated. Thereafter, 35% (w/w) calcium chloride at 0.025% (v/w) and calf rennet at 0.02% (v/w), containing 780 mg chymosin/l (Renifor-15/E, Lamirsa, Barcelona, Spain), were added. The cheese making procedure in-

cluded curdling, cutting of the curd, draining off the whey, and pressing in a pneumatic press at 0.35 kPa for 1 h and 0.7 kPa for 13 h at 14°C. The resulting cheeses were then salted in brine (19% NaCl solution) for 6 h. Regular ripening conditions were $14 \pm 1^\circ\text{C}$ and $85 \pm 2\%$ relative humidity.

Sampling

Duplicate analyses were performed on the milk, rennet, curd, whey and cheeses on d 1, 7, 14, 30, 60, and 90 of ripening. Biogenic amine, microbial counts, total nitrogen (TN), water-soluble nitrogen (WSN), WSN/TN, FAA, pH, and salt were determined in 108 samples.

Biogenic Amine Determination

Tyramine (TY), putrescine (PU), cadaverine (CA), histamine (HI), tryptamine (TR), β -phenylethylamine (PHE), agmatine (AG), spermidine (SD), spermine (SM), octopamine (OC), dopamine (DO), and serotonin (SE) were assessed as described by Novella et al. (2000). Results are expressed on a dry weight basis.

Bacteriological Analysis

Ten grams of samples were placed in sterile Stomacher bags and homogenized for 2 min in 90 ml of buffered peptone water (Oxoid Ltd. Basingstoke, Hampshire, UK) with 1% Tween 80 (Liofilchem, Roseto degli Abruzzi, TE, Italy), using Stomacher Lab-blender 400 (Seward Medical, London, UK). Serial decimal dilutions were prepared in the same diluent. Total aerobic mesophilic microorganisms were enumerated on Plate Count Agar (PCA, Liofilchem) at 30°C for 48 h, lactococci on M17 agar plates (Oxoid) supplemented with lactose (Scharlau Microbiology, Barcelona, Spain) and incubated for 48 h at 30°C, lactobacilli on Rogosa agar (Oxoid) and incubated at 30°C for 5 days in 10% CO₂, enterococci on Kanamycin Aesculin Azide agar (KAA, Oxoid) at 37°C for 48h, *Enterobacteriaceae* on Violet Red Bile Glucose agar (VRBG, Biokar Diagnostics, Beauvais, France) and incubated with a double layer for 24 h at 37°C, *Micrococcaceae* on Manitol Salt Agar (MSA, Liofilchem) at 30°C for 48 h, and molds and yeasts on Rose-Bengal Chloramphenicol Agar (Scharlau) at 20°C for 5 days. After the counts, at least five colonies were taken from those plates containing 30 to 300 colonies. These isolates were later evaluated for their ability to produce biogenic amines.

Amino Acid Decarboxylase Activity

The potential decarboxylation capacity of lactic starter cultures and isolates obtained from samples

throughout ripening was studied following Bover-Cid and Holzapfel (1999).

Physicochemical Analysis

TS contents were determined in milk and cheese whey by IDF (1987) and IDF (1970) standards, respectively. Samples of cheeses were analyzed for TS (IDF, 1982). Water-soluble fractions of cheeses were prepared as described by Kuchroo and Fox (1982), and the pH 4.6-soluble nitrogen (WSN) was prepared from water-soluble fractions. TN and WSN were determined by the Kjeldahl method (IDF, 1993). Total FAA were assessed on the WSF of cheeses by the cadmium-ninhydrin method of Folkertsma and Fox (1992). WSN/TN, WSN, and FAA were used as proteolysis indexes. The pH of a cheese/distilled water (1:1) slurry was measured. Salt was determined with chloride analysis (Corning 926 Salt Analyzer; Corning Medical and Scientific Glass Works, Medfield, MA).

Statistical Analysis

Biogenic amines were evaluated by the Wilcoxon test for non-parametric data, since the values of biogenic amines in cheese were not normally distributed. To determine correlations between time of ripening, biogenic amines, microbial counts, proteolysis indexes and pH, linear regression analyses were applied. Statistical analyses were performed with SPSS (SPSS Inc., Chicago IL, USA). All results are expressed as the average values from samples corresponding to the three productions, with their standard deviation.

RESULTS AND DISCUSSION

Raw Materials

The biogenic amine levels and microbial counts in milk were low (Table 1); this agrees with studies by ourselves and others (Bardócz, 1993; Petridis and Steinhart, 1996; Novella-Rodríguez, et al., 2000). The prevailing amine in all samples of milk was SD, followed by SM. The microorganism counts were lower than reported elsewhere (Pouillet et al., 1991; Pérez-Elortondo et al., 1993; Trujillo et al., 1999), which may explain the absence or low levels of the amines. In addition, Bardócz (1995) has reported that polyamines are naturally found in foods, and so do not come from bacterial metabolism. The biogenic amine content of rennet was relatively high, but its contribution to the final content was negligible as a result of the dilution effect, because 20 mL of rennet was added to 100 kg of milk. Thus, Table 1 also includes the content of biogenic

Table 1. Biogenic amines (mg/Kg) and microbial counts (log CFU/g) in milk and rennet.

Sample	Pasteurized Milk	Rennet	Rennet contribution to biogenic amines in cheese ²
TY ¹	ND ³	69.30 (1.56)	13.86 (0.31) ⁵
PU	ND	37.07 (0.30)	7.41 (0.13)
CA	0.11 (0.01) ⁴	48.98 (0.60)	9.80 (0.12)
HI	ND	10.47 (1.14)	2.09 (0.23)
TR	ND	4.60 (0.53)	0.92 (0.11)
PHE	ND	5.16 (0.63)	1.03 (0.13)
AG	ND	2.43 (0.29)	0.49 (0.06)
SD	0.35 (0.07)	4.38 (0.60)	0.88 (0.12)
SM	0.96 (0.09)	4.83 (0.08)	0.97 (0.19)
PCA	2.27 (0.11)	2.07 (0.12)	
LAC	3.47 (0.32)	2.81 (0.32)	
ENT	ND	ND	
Molds	ND	1.52 (0.06)	

¹TY = Tyramine, PU = putrescine, CA = cadaverine, HI = histamine, TR = tryptamine, PHE = β -phenylethylamine, AG = agmatine, SD = spermidine, SM = spermine, PCA = aerobic mesophilic microorganisms, LAC = lactococci, ENT = Enterobacteriaceae.

²The dilution was 20 mL of rennet added to 100 kg of milk.

³ND = not detected.

⁴Mean (SD).

⁵The amines were expressed in $\mu\text{g/kg}$.

amines in rennet after application of the dilution factor, expressed by $\mu\text{g/L}$.

Curd and Whey

The contents of biogenic amines were higher in curd than in whey (Table 2), which indicates that a substan-

Table 2. Biogenic amines (mg/kg) and microbial counts (log CFU/g) in curd and whey.

Sample	Batch A		Batch B	
	Curd	Whey	Curd	Whey
TY ¹	2.00 (0.06) ²	0.65 (0.02)	2.01 (0.06)	0.67 (0.06)
PU	0.89 (0.03)	0.31 (0.01)	0.86 (0.03)	0.32 (0.02)
CA	0.67 (0.02)	0.22 (0.02)	0.64 (0.07)	0.25 (0.01)
HI	0.98 (0.06)	0.28 (0.04)	0.68 (0.07)	0.32 (0.02)
TR	1.32 (0.01)	ND ³	ND	ND
AG	0.28 (0.01)	0.21 (0.01)	0.25 (0.01)	0.16 (0.01)
SD	1.77 (0.06)	0.24 (0.01)	1.18 (0.02)	0.13 (0.01)
SM	2.23 (0.01)	ND	2.53 (0.08)	ND
PCA	7.98 (0.38)	...	8.18 (0.21)	...
LAC	7.66 (0.60)	...	8.60 (0.50)	...
LAB	3.28 (0.03)	...	2.48 (0.06)	...
ENC	3.19 (0.36)	...	4.59 (0.01)	...
ENT	3.47 (0.30)	...	3.73 (0.32)	...
MSA	2.56 (0.12)	...	2.73 (0.22)	...
Molds	1.22 (0.01)	...	1.70 (0.06)	...
Yeast	1.92 (0.01)	...	2.07 (0.06)	...

¹TY = tyramine, PU = putrescine, CA = cadaverine, HI = histamine, TR = tryptamine, AG = agmatine, SD = spermidine, SM = spermine, PCA = aerobic mesophilic microorganisms, LAC = lactococci, LAB = lactobacilli, ENC = enterococci, ENT = Enterobacteriaceae, MSA = Microcococaceae.

²Mean (SD).

³ND = not detected.

Table 3. Microbial counts, mean (standard deviation), (log CFU/g) in cheese from both batches.

Sample (day)	PCA ¹	LAC	LAB	ENC	ENT	MSA	Molds	Yeast
1 d	9.82 (0.33) ² 9.64 (0.58)	9.69 (0.36) 9.64 (0.61)	4.50 (0.18) 3.86 (0.32)	3.98 (0.51) 5.30 (0.07)	5.41 (0.30) 4.77 (0.45)	2.96 (0.26) 3.72 (0.07)	1.77 (0.01) 2.00 (0.10)	2.00 (0.01) 2.26 (0.39)
7 d	9.84 (0.67) 9.86 (1.92)	9.81 (0.87) 9.91 (1.80)	7.15 (0.19) 6.78 (0.03)	6.41 (0.16) 5.96 (0.25)	2.85 (0.31) 2.78 (0.87)	3.62 (0.26) 5.49 (0.32)	2.20 (0.26) 2.84 (0.12)	2.62 (0.17) 4.55 (0.24)
14 d	9.76 (1.39) 9.76 (0.82)	9.78 (1.05) 9.68 (0.91)	7.93 (0.16) 7.66 (0.14)	7.19 (0.13) 6.86 (0.07)	1.91 (0.10) 1.90 (0.23)	2.12 (0.17) 2.91 (0.37)	2.86 (0.13) 2.07 (0.39)	2.65 (0.69) 2.83 (0.29)
30 d	8.59 (0.70) 8.66 (0.40)	8.27 (0.65) 8.82 (1.26)	8.05 (0.06) 7.94 (0.67)	7.99 (0.21) 7.72 (0.08)	ND ³ 0.70 (0.17)	2.98 (0.23) 3.30 (0.15)	1.52 (0.76) ND	2.88 (0.22) 3.40 (0.09)
60 d	8.02 (0.41) 8.12 (1.00)	7.96 (0.18) 7.96 (0.88)	7.89 (0.80) 8.03 (1.12)	7.78 (0.04) 7.38 (0.08)	ND ND ^b	1.88 (0.82) 3.05 (0.15)	2.28 (0.01) 2.51 (0.86)	1.62 (0.76) 2.73 (0.17)
90 d	7.47 (0.43) 8.15 (0.10)	7.45 (0.66) 7.54 (0.44)	7.51 (0.59) 7.63 (0.77)	6.94 (0.49) 6.59 (0.18)	ND ND	1.88 (0.80) 2.92 (0.68)	2.24 (0.66) 2.65 (0.19)	1.52 (0.01) 1.22 (0.09)

¹PCA = aerobic mesophilic microorganisms, LAC = lactococci, LAB = lactobacilli, ENC = enterococci, ENT = Enterobacteriaceae, MSA = Micrococaceae.

²Batch A (standard font), Batch B (italics).

³ND = not detected.

tial proportion of the biogenic amines were retained in the curd. Thus, the draining process involved in the manufacture of cheese does not reduce the amine contents. The main amines in curd were SM and TY, while TY prevailed in whey and SM was not detected. Owing to low levels of amines in curd, raw materials were not considered the main origin of the relatively high concentration of amines in cheese. The Wilcoxon test revealed that the differences in the content of all amines between the two batches were not significant ($P > 0.05$). Microorganism counts in curd were between 2–4 log units higher than in milk, perhaps because of the concentration effect due to water loss.

Cheese

Microbial counts were, in general, slightly higher in batch B than in batch A (Table 3). The aerobic mesophilic and lactococci microorganism counts increased from the start of cheese making to 14 days of ripening, after which they decreased. The lactobacilli and enterococci counts increased during the first month and then decreased. *Enterobacteriaceae* decreased throughout ripening, and *Micrococaceae* decreased from the first month, owing to the conditions of the medium (high concentration of salt, low pH and starter occurrence). In fact, *Enterobacteriaceae* disappeared after 30 days of ripening. The population of molds and yeast did not show a clear evolution throughout ripening. Similar microbial profiles during ripening have been described elsewhere (Pérez-Elortondo et al., 1993; Schneller et al., 1997). Statistical differences ($P < 0.05$) were found only in the case of *Micrococaceae* and yeast between the two batches by the Wilcoxon test. Neither *Micrococaceae* nor yeast is usually associated with biogenic amine production.

WSN/TN and FAA increased during ripening, showing a positive correlation with ripening time ($P < 0.05$) (Table 4). pH values showed an initial decrease, followed by a slight increase, probably due to lactic acid production. This phenomenon may be attributed to the use of lactic acid by the fermentative metabolism of microorganisms and to the formation of basic compounds from proteolysis, as reported by Pouillet et al. (1991) and Pérez-Elortondo et al. (1993). TS and salt values rose gradually during ripening. Cheese proteolysis-related parameters, pH, moisture, and salt were similar in both batches, and so they were not significantly affected ($P > 0.05$) by the starter culture used.

The values of biogenic amines increased progressively throughout ripening, reaching the maximum, in general, at 90 days (Figure 1). Biogenic amines usually increase throughout the ripening period (Martelli et al., 1993; Schneller et al., 1997; Ordoñez et al., 1997; Pinho et al., 2001). In fact, the increase in the free amino acid content (proteolysis) and the decarboxylative activity of bacterial enzymes may favor the formation of these compounds. Cheeses from the same pasteurized milk but produced with distinct starters cultures differed in their biogenic amine concentration. The lowest biogenic amine content was found in cheese from batch A. We would like to point out that higher biogenic amine contents in cheese from batch B were expected, since the bacterial counts in batch B were slightly higher than in batch A. The sum of all biogenic amines significantly differed ($P < 0.05$) between the two batches, as revealed by the Wilcoxon test.

According to linear regression ($P < 0.05$), TY, PU, CA, HI, and TR were produced during ripening. TY showed the highest level, followed by PU in both batches, although their contents were significantly lower ($P < 0.05$) in cheese from batch A. The increase in these amines

Table 4. Composition of cheeses, mean (SD), of both batches.

Cheese (day)	NT ¹	WSN	WSN/TN (%)	FAA	pH	TS	S
1 d	2.99 (0.30) ²	0.31 (0.01)	10.52 (1.15)	0.24 (0.06)	5.01 (0.03) ^a	49.90 (0.35)	1.05 (0.51)
7 d	2.67 (0.10)	0.30 (0.01)	11.33 (0.51)	0.24 (0.02)	4.98 (0.03)	49.47 (0.69)	0.76 (0.07)
	3.15 (0.25)	0.35 (0.02)	11.09 (0.71)	0.29 (0.03)	4.73 (0.07)	53.02 (1.71)	0.80 (0.04)
14 d	3.49 (0.14)	0.47 (0.04)	13.66 (2.08)	0.40 (0.03)	4.77 (0.04)	56.65 (0.86)	1.09 (0.17)
	3.50 (0.15)	0.48 (0.03)	13.73 (1.30)	0.36 (0.05)	4.77 (0.09)	57.18 (0.37)	0.98 (0.04)
30 d	3.82 (0.11)	0.59 (0.04)	15.54 (0.93)	0.54 (0.05)	4.86 (0.03)	64.52 (0.90)	1.48 (0.14)
	3.88 (0.22)	0.59 (0.05)	15.14 (1.85)	0.56 (0.06)	4.87 (0.05)	65.29 (1.20)	1.51 (0.09)
60 d	4.40 (0.06)	0.78 (0.11)	17.70 (3.00)	1.52 (0.09)	4.98 (0.08)	72.24 (1.13)	1.68 (0.13)
	4.35 (0.26)	0.76 (0.13)	17.55 (4.36)	1.63 (0.05)	4.89 (0.04)	73.57 (1.20)	1.71 (0.07)
90 d	4.49 (0.18)	0.85 (0.03)	19.01 (1.35)	2.39 (0.05)	4.94 (0.03)	76.98 (1.08)	1.81 (0.14)
	4.60 (0.20)	0.89 (0.02)	19.47 (1.09)	2.78 (0.03)	4.91 (0.03)	77.28 (0.45)	1.91 (0.07)

¹TN = total nitrogen, WSN = water soluble nitrogen, FAA = free amino acids, mg Leu/g, S = salt.

²Batch A (standard font), Batch B (italics).

was observed on d 7 of ripening, immediately after the pH drop and when *Lactobacillus* counts started to increase. Amine production has been associated with pro-

tektive mechanisms of microorganisms against an acidic environment (Eitenmiller et al., 1978). However, no significant correlation ($P > 0.05$) was found between pH and lactobacilli and the levels of TY and PU. In contrast, WSN/TN (Figure 2) and the levels of these amines were positively correlated, suggesting that extensive proteolysis, before or during the fermentation/ripening process, is one of the critical factors of the formation of these amines throughout cheese making. Likewise, a positive correlation was also found between TY and PU and FAA ($r > 0.95$ and > 0.94 , respectively, $P < 0.05$). Salt level was also a significant factor in the formation of amines. Thus, a low salt concentration seems to enhance the formation of biogenic amines (Joosten, 1988). Furthermore, lactobacilli showed the highest correlation with amines (Figure 3). The TY and PU contents observed in this study were similar or lower than those reported elsewhere in various cheeses, like Gorgonzola (Martelli et al., 1993), Idiazábal cheese (Ordoñez et al., 1997) and Manchego cheese (Fernández-García et al., 2000).

Batch A.

Batch B.

Figure 1. Changes in biogenic amine contents during ripening period of cheese from both batches.

Figure 2. Correlation between tyramine (TY) and putrescine (PU) contents with WSN/TN (%) during ripening.

Figure 3. Correlation between tyramine (TY) and putrescine (PU) contents with lactobacilli (LAB) during ripening.

The behavior of CA, HI, TR, and PHE differed from that of TY and PU throughout ripening (Figure 1). In general, these amines increased only slightly during ripening, especially TR. Furthermore, in this case, their contents were not significantly different between batches ($P > 0.05$) according to the Wilcoxon test, and a significant correlation was only found between HI and TR and WSN/TN and FAA. These results differ from those obtained by Ordoñez et al., (1997), who reported that HI, PHE and TH follow a declining trend and that the levels of CA were higher. Likewise, Schneller et al., (1997) found higher contents of CA and similar levels of HI and PHE. Moreover, Fernández-García et al., (2000) found greater contents of HI. The low levels of CA may be due to the fact that the *Enterobacteriaceae* disappeared after 30 days of ripening. Several authors (Stratton et al., 1991; Petridis and Steinhart, 1996; Sharaf et al., 1997) have reported the decarboxylase-positive ability of *Enterobacteriaceae* yielding the formation of CA.

TY and HI are especially relevant, since they are a potential health hazard. However, no cheese sample would be hazardous given normal consumption. Vind et al. (1991) reported that the removal of foods with levels of HI higher than 20 mg/kg could be advisable. The TY and HI levels of all cheese samples were always lower than this value.

Polyamines (AG, SD and SM) presented similar levels and trends during ripening in the two batches (Table 5). SM and AG increased slightly, while SD increased during the first step and decreased thereafter. A declining trend for SD was also observed by Ordoñez et al., (1997) in Idiazábal cheese.

As for the other minor amines, OC was not found in any sample, the contents of DO were very low (< 0.5 mg/kg), and SE was detected in only a few samples ($n = 3$).

Table 5. Polyamines contents, mean (SD), (mg/kg) in cheeses from both batches.

Sample	AG ¹	SD	SM
1 d	0.96 (0.33) ² 0.78 (0.18)	1.34 (0.95) 1.44 (0.50)	2.71 (0.45) 2.95 (0.34)
7 d	1.25 (0.52) 0.99 (0.26)	8.78 (0.01) 6.98 (2.35)	2.63 (0.20) 2.83 (0.27)
14 d	0.45 (0.01) 0.54 (0.01)	4.20 (0.01) 3.34 (0.01)	4.16 (0.91) 4.17 (0.57)
30 d	3.00 (0.81) 3.66 (6.82)	3.38 (0.11) 4.25 (3.95)	3.61 (0.66) 3.38 (0.16)
60 d	5.76 (1.63) 6.31 (0.77)	2.00 (0.13) 1.58 (0.14)	3.97 (1.19) 3.88 (1.45)
90 d	5.15 (1.48) 5.25 (2.70)	1.29 (0.94) 1.39 (0.10)	4.99 (0.14) 5.28 (0.53)

¹AG = agmatine, SD = spermidine, SM = spermine.

²Batch A (standard font), Batch B (italics).

Biogenic Amine Production by Starter and Nonstarter Bacteria

Initially, we evaluated the amino acid decarboxylative ability of starters to determine whether they play a key role in the production of biogenic amines in cheese. Thus, we tested two commercial starters (*Lactococcus lactis* subsp. *lactis*, plus *Lactococcus lactis* subsp. *cremoris*, and *Lactococcus lactis* subsp. *lactis*) following Bover-Cid and Holzapfel (1999) and found that (at least in vitro) they do not decarboxylate amino acids or produce amines. Therefore, the biogenic amine contents found in cheese should mostly be attributed to the non-starter bacteria present in cheeses.

Special emphasis was done to monitor the microorganisms that can produce biogenic amines, mainly lactobacilli, enterococci and *Enterobacteriaceae*. However, the capacity to form amines is strain-dependent rather than species-dependent, which explains why a particular group of microorganisms showed large differences in amine production. The correlation between isolated microorganisms and biogenic amines is summarized in Table 6. In general, the isolates from batch B were stronger amine producers than those from batch A. All 84 isolates obtained from Rogosa Agar were found to be TY formers, 29 were PU formers, and 16 were CA and HI formers. Moreover, the content of TY varied from 0.87 to 884.89 mg/kg, while that of PU ranged from 0.13 to 34.54 mg/kg. The production of CA and HI was very low (< 5 mg/kg). The ability of lactobacilli to form amines in cheese has been described by Joosten and Northolt (1987), and Petridis and Steinhart (1996). Although certain lactobacilli show high histidine decarboxylase activity (Joosten and Northolt, 1989), no significant HI production was observed in the isolates tested. Likewise, no significant correlation ($P > 0.05$) was found between the amine production capacity of

Table 6. The formation of biogenic amine by nonstarter isolates from Rogosa Agar, KAA and VRBG plates.

Amine	n	Rogosa Agar isolates n = 84	n	KAA isolates n = 76	n	VRBG isolates n = 42
TY ¹	41	0.87–678.06 ^{2,3}	36	9.60–583.44	—	—
	43	<i>7.04–884.89</i>	40	<i>9.70–629.84</i>	—	—
PU	14	0.13–26.41	—	—	19	0.82–126.55
	15	<i>0.12–34.54</i>	—	—	23	<i>0.53–266.07</i>
CA	7	0.11–4.11	—	—	19	2.21–653.28
	9	<i>0.12–4.27</i>	—	—	23	<i>1.84–622.15</i>
HI	9	0.10–0.47	—	—	—	—
	7	<i>0.20–0.39</i>	—	—	—	—
PHE	—	—	36	0.12–71.67	...	—
	—	—	40	<i>0.13–110.29</i>	—	—

¹TY = tyramine, PU = putrescine, CA = cadaverine, HI = histamine, PHE = β -phenylethylamine.

²Range (minimum-maximum), (mg/kg).

³Batch A (standard font), Batch B (italics).

microorganisms and the ripening time of the corresponding sample.

All the isolates from KAA tested (n = 76) showed amino acid decarboxylative activity, and TY and PHE were detected, especially TY; this agrees with Joosten and Northolt (1987), and Leuschner et al. (1999), who also described TY production by enterococci in cheese but only if the density of enterococci was 10⁷ cfu/g or higher (Joosten and Northolt, 1987). Moreover, when the density of enterococci was higher than 2 10⁹ cfu/g, Joosten and Northolt (1987) reported accumulation of PHE, owing to the high tyrosine decarboxylase activity, which also decarboxylates phenylalanine. In this study, PHE was detected in samples of isolates with lower counts (10⁷ cfu/g). Sharaf et al (1997) also detected formation of TY and PHE with lower enterococci counts (10⁶ cfu/g). Our study reveals samples with high levels of TY show high levels of PHE, and thus the highest contents of these amines were found in the same sample. Although several authors (Tham 1988; Tham et al., 1990) have described the formation of HI by enterococci in goat cheeses, we did not find this amine production. No significant correlation ($P > 0.05$) was found between these amines and ripening time, however.

High amounts of PU and CA were produced by the isolates from VRBG tested (n = 42). All samples contained these amines, especially PU. HI formation by *Enterobacteriaceae* was not detected, although large levels of HI in cheese (Sharaf et al., 1997; Marino et al., 2000), sausages (Roig-Sagués et al., 1996) and fish (Beutling, 1996) by coliform bacteria have been described. A rapid decrease in the number of coliform bacteria during cheese making may explain the low levels of CA, as suggested by Joosten and Northolt (1987). No significant correlation ($P > 0.05$) was found

between these amines and the ripening time of the sample in which the amine was studied.

The presence of amines in cheese is attributable to the simultaneous decarboxylative activity of the isolates. However, this capacity may vary in vivo. Moreover, other factors (pH, salt, and temperature) can also contribute to the final content of biogenic amines in cheese.

In conclusion, the lowest biogenic amine content was found in cheese from batch A, with the starter culture (*Lactococcus lactis* subsp. *lactis* plus *Lactococcus lactis* subsp. *cremoris*) normally used for the manufacture of cheese. On the other hand, the nonstarter bacteria present in cheese are important for biogenic amine formation. Thus, those isolated from Rogosa Agar and KAA seem to contribute essentially to the concentration of TY in cheese, whereas the decarboxylative properties of the isolates from VRBG may account for PU and CA production.

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